Effects of the pharmacological interventions and a structured education in the management of polycystic ovary syndrome (PCOS)

Mohammed Altigani Abdalla

MBBS, M.Sc. Clinical Endocrinology, M.Sc. Diabetes

A thesis submitted for the degree of Doctor of Philosophy (Ph.D.)

To the Faculty of Medicine

The University of Hull and The University of York

Hull York Medical School

March 2022

Abstract

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder that affects women of reproductive age and is associated with an array of metabolic disorders. Insulin resistance, increased body weight, dyslipidaemia and excess androgen are the main drive for PCOS symptoms and the associated health risks. Lifestyle modifications remain the first-line intervention to treat PCOS. However, there are various pharmacological options available as second-line treatment.

Methods

The first study was a systematic review and meta-analysis that evaluated the effect of the different pharmacological interventions on the lipid profiles, C-reactive protein (CRP), anthropometric indices, insulin resistance and the biochemical hyperandrogenaemia in women with PCOS. The second study was a feasibility pilot study of developing and implementing an evidence-based structured education for women with PCOS. The study has two parts; the first part was a patient's perspectives survey where 320 women were surveyed to establish the need for developing an education programme. The second part was implementing and piloting the evidence-based structured education.

Results

In the systematic review and meta-analysis, pharmacological interventions including Metformin, Atorvastatin, Saxagliptin, Rosiglitazone and Pioglitazone of various dosage, frequencies and duration were associated with a significant reduction in the mean total cholesterol (TC), triglycerides (TGs), low-density lipoprotein cholesterol (LDL-C) and CRP. However, no significant effect was found on the high-density lipoprotein cholesterol (HDL-C). There was also a significant reduction in the mean fasting blood glucose (FBG), fasting insulin (FI) and the homeostatic model assessment of insulin resistance (HOMA-IR) when Metformin alone or combined with Acarbose, Pioglitazone and exenatide in various dosage, frequencies and duration was assessed. There was, however, no significant effect on the homeostatic model of the Beta-cell (HOMA-B).

There was also a significant reduction in the mean body weight, body mass index (BMI), waist circumference (WC) and the waist to hip ratio (WHR) when Metformin, Orlistat and Sitagliptin of various dosage, frequencies and duration were compared with placebo. In contrast, regardless of the duration, dosage, and frequencies, Rosiglitazone and Pioglitazone were associated with a significant increase in body weight, BMI, and WC. The study also showed a significant increase in the ovulation rate, pregnancy rate and live birth rate when clomiphene citrate (CC) and letrozole alone or added to Metformin of various dosage, duration and frequencies were used. There was also a significant reduction in the mean total testosterone (TT), free testosterone (FT), dehydroepiandrosterone sulphate (DHEAS) and an increase in the sex hormone-binding globulin (SHBG) when metformin, dexamethasone, oral contraceptives pills (OCP), finasteride and Flutamide of various dosage, frequencies and duration were used.

In the second study, there was a lack of knowledge about PCOS among women living with the condition. There was also a need for developing and implementing an evidence-based structured education for women living with PCOS. A single exposure to a structured education did not increased knowledge but provided valuable skills for women with PCOS.

Conclusions

This research work demonstrated a significant effect of the various pharmacological interventions used in PCOS management. The work also supports the concept of developing, implementing and integrating an evidence-based structured education in the management of women with PCOS.

List of publications from this research work

1) **Abdalla, M. A.,** Shah, N., Deshmukh, H., Sahebkar, A., Ostlundh, L., Al-Rifai, R. H., . . . Sathyapalan, T. (2021a). Effect of pharmacological interventions on lipid profiles and c-reactive protein in polycystic ovary syndrome: A systematic review and meta-analysis. *Clin Endocrinol (Oxf)*. doi:10.1111/cen.14636.

2) **Abdalla, M. A.,** Shah, N., Deshmukh, H., Sahebkar, A., Ostlundh, L., Al-Rifai, R. H., . . . Sathyapalan, T. (2021b). Impact of pharmacological interventions on insulin resistance in women with polycystic ovary syndrome: A systematic review and meta-analysis of randomised controlled trials. *Clin Endocrinol (Oxf)*. doi:10.1111/cen.14623.

3) **Abdalla, M.A**, Shah N, Deshmukh H, Sahebkar A, Ostlundh L, Al-Rifai RH, et al. Impact of pharmacological interventions on anthropometric indices in women with polycystic ovary syndrome: A systematic review and meta-analysis of randomised controlled trials. Clin Endocrinol (Oxf). 2021. doi: 10.1111/cen.14663.

4) **Abdalla, M.A**, Shah N, Deshmukh H, Sahebkar A, Ostlundh L, Al-Rifai RH, et al. Impact of pharmacological interventions on biochemical hyperandrogenemia in women with polycystic ovary syndrome: a systematic review and meta-analysis of randomised controlled trials. Arch Gynecol Obstet. 2022.

5) **Abdalla, M.A,** Deshmukh, H., Atkin, S. L., & Sathyapalan, T. (2020). miRNAs as a novel clinical biomarker and therapeutic targets in polycystic ovary syndrome (PCOS): A review. *Life Sci, 259,* 118174. doi:10.1016/j.lfs.2020.118174.

6) **Abdalla, M. A.**, Deshmukh, H., Atkin, S., & Sathyapalan, T. (2020). A review of therapeutic options for managing the metabolic aspects of polycystic ovary syndrome. *Ther Adv Endocrinol Metab, 11*, 2042018820938305. doi:10.1177/2042018820938305.

7) **Abdalla, M.A.,** Deshmukh, H., Atkin, S., & Sathyapalan, T. (2021). The potential role of incretin-based therapies for polycystic ovary syndrome: A narrative review of the current evidence. *Ther Adv Endocrinol Metab, 12*, 2042018821989238. doi:10.1177/2042018821989238.

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LIST OF ABBREVIATIONS

PCOS	Polycystic ovary syndrome
PCO	Polycystic ovaries
ICSH	Interstitial Cell-Stimulating Hormone
LH	Luteinizing hormone
FSH	Follicular stimulating hormone
RIA	Radioimmunoassay
T2DM	Type 2 diabetes mellitus
CVD	Cardiovascular disease
NAFLD	Non-Alcoholic Fatty Liver Disease
ALT	Alanine aminotransferase enzyme
QoL	Quality of life
ASRM	American Society of Reproductive Medicine
FAI	Free androgen index
SHBG	Sex hormone-binding globulin
CYP11A1	Cytochrome P450 family 11 subfamily A 1
CYP21	P450 21-hydroxylase
17-OHP	17 hydroxyprogesterone
SNP	Single nucleotide polymorphism
PAI-1	Plasminogen activator inhibitor-1 (PAI
AGD	Anogenital distance
HPO	Hypothalamic-pituitary ovarian

GnRH	Gonadotrophin releasing hormone
A4	Androstenedione
DHEAS	Dehydroepiandrosterone sulphate
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
PI3K	Phosphatidylinositol-3-kinase
GLUT-4	Glucose transporter-4
PDK-1,2	3-phosphoinositide-dependent protein kinase 1 and 2
MAPK-ERK	Mitogen-activated protein kinase-ERK
GSK3	Glycogen synthesis kinase 3
PTP1B	Protein tyrosine phosphate 1 B
CRP	C-reactive protein
IL-6	Interlukin-6
TNF-α	Tumour necrosing factor
CAC	Coronary artery calcium
HDL-C	High-density lipoprotein cholesterol
BMI	Body mass index
LDL-C	Low-density lipoprotein cholesterol
AF	Atrial fibrillation
OGTT	Oral glucose tolerance test
HbA1C	Haemoglobin A1C
CC	Clomiphene Citrate
HMG	Human menopausal gonadotropin

GDM	Gestational diabetes mellitus
AMH	Anti-Mullerian hormone
IR	Insulin resistance
FFAs	Free fatty acids
HOMA-IR	Homeostatic model assessment for insulin resistance
FSIGTT	Frequently sampled intravenous glucose tolerance test
QUICK	Quantitative insulin sensitivity check index
WHO	World Health Organisation
IGT	Impaired glucose tolerance
NIDDM	Non-insulin dependent DM
WC	Waist circumference
WHR	Waist to hip circumference
VEGF	Vascular endothelial growth factors
TC	Total cholesterol
VLDL	Very low-density lipoprotein
Apo A-1	Apoprotein A-1
AKR1C3	Androgen-activating enzyme Aldo-ketoreductase type 1 C3
SR-B1	Scavenger receptor B1
ROS	Reactive oxygen species
NADPH	Nicotinamide adenine nucleotide phosphate
AGEs	Advanced glycation end-products

Acknowledgements

First and foremost, I would like to express my sincere gratitude to my amazing supervisor, Professor Thozhukat Sathyapalan. This work would not have been completed without his outstanding leadership, guidance, trust, understanding and encouragement. I also sincerely thank my co-supervisor, Dr Ireneous Soyiri, for his intellectual and thoughtful input and support throughout this journey.

I am grateful to Dr Najeeb Shah for his contribution to screening, selecting and assessing for risk of bias in the included studies in the systematic review and meta-analysis, which formed the core of my thesis.

My utmost gratitude goes to Linda Östlundh for conducting a systematic literature search in the electronic databases.

I would also like to extend my deepest gratitude to Dr Maria Papageorgiou to obtain funds from the British dietetic association (BDA) for the living with PCOS study.

I would also like to thank Dr Dayo Adeleke for providing help with statistical analysis.

I would like to express my appreciation to all my collaborators for their invaluable expert opinion and inputs; Professor Stephen L. Atkin, Dr Rami H. Al-Rifai, Dr Amirhossein Sahebkar and Dr Harshal Deshmukh.

Allow me to thank the Centre for Academic Diabetes, Endocrinology, and Metabolism staff. Individually, I wish to express my appreciation to Gill Byne, Lucy Newton, Carrie Willby, Zoe Sugdon and finally Lisa Baldwin for their assistance and guidance all the way.

I am deeply beholden to all the participants whose trust and willingness made the work possible.

Finally, and unaccountably, I would like to thank my amazing wife and my lovely daughter and son for their continued support throughout this journey; this would not have been completed without it.

Author's declaration

I confirm that this work is original and that if any passage(s) or diagram(s) have been copied from academic papers, books, the internet or any other sources, these are identified by the use of quotation marks and the reference(s) is thoroughly cited. I certify that, other than where indicated, this is my work and does not breach the regulations of HYMS, the University of Hull or the University of York regarding plagiarism or academic conduct in examinations. I have read the HYMS Code of Practice on Academic Misconduct, and state that this piece of work is my own and does not contain any unacknowledged work from any other sources. I confirm that any patient information obtained to produce this piece of work has been appropriately anonymised. Any ethical requirement has been met.

1 Chapter 1: Introduction

1.1 The Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects women of reproductive age, and it is the leading cause of infertility and other endocrinopathies (1).

1.1.1 History and Overview

The history of PCOS perhaps began around the early 17th century when an Italian Scientist Antonio Vallisneri (Figure 1-1, 1-A), described "a married, young, peasant, infertile women with a whitish surface, lumpy and shiny ovaries in size of pigeon eggs"(2). In 1844, PCOS was initially described in the medical literature as cystic oophoritis (3). However, it was not until 1935 that Irving Stein (Figure 1-1, 1-B) and Michael Leventhal (Figure 1-1,1-C) worked as gynaecologists at Michael Rees hospital and north-western university medical school, Chicago, Illinois, USA. Although they first reported the association between the clinical feature "amenorrhoea, infertility and hirsutism" and polycystic ovaries (PCO), they described the histological and macroscopic features of PCO (4). Therefore, it was initially labelled Stein-Leventhal syndrome, later referred to as a polycystic ovary syndrome.

1-A 1-B 1-C

Figure 1-1: Fig.1-A; photo of Antonio Vallisneri (1661-1730), Fig.1-B; photo of Dr Irving Stein (1887-1976), Fig.1-C; photo of Dr Michael Leventhal (1901-1971). Fig.1-A & Fig.1-B reprinted with permission of the Hektoen International Journal of Medical Humanities. Excerpts from the book All our lives: a centennial history of Michael Reese Hospital and Medical Center, 1881-1981. Gordon S, ed. Chicago: The Hospital and Medical Center; 1981.

In 1953, scientists suggested using cortisone therapy to treat sclerocystic ovaries and hirsutism (5, 6). In 1958, McArthur et al. and his colleagues reported changes in the urinary excretion of interstitial cell-stimulating hormone (ICSH), later known as luteinising hormone (LH), and follicular stimulating hormone (FSH) in women with the disease of the reproductive tract (i.e. PCOS) compared to average menstruating women. Women with PCOS demonstrated relatively low urinary excretion of LH compared to FSH. Moreover, the mid-cycle peak of FSH and ICSH was associated with ovulation and menstruation (7).

By 1960, with the arrival of the radioimmunoassay (RIA), scientists were able to measure hormone levels in women with PCOS, later confirming that PCOS was correlated with increased ovarian androgen production and abnormal LH secretion (8). The focus has shifted from PCOS as a reproductive disorder to primarily an endocrine disease by then.

In 1961, D Ferriman and DJ Gallwey invented a method for semiquantitative assessment of body hair growth in women using five grading scores based on densities and areas involved

in 11 sites of the body. This score is suitable for assessing clinical problems associated with hirsutism in women with PCOS (9).

By 1970, sequential measurements of LH and FSH using specific immunoassay were made. As a result, an inordinately and consistently high LH concentration with disproportionately low FSH was observed in women with PCOS. These findings have supported the concept that a defect of hypothalamic-pituitary regulation of gonadotropin secretion might be related to the abnormal androgen production and ovulatory disorder in women with PCOS (10).

By the early 1980s, the most remarkable scientific breakthrough was discovering the relationship between insulin resistance (IR) and PCOS. Hyperinsulinemia and hyperandrogenism are more prevalent in women with PCOS compared to control. Insulin stimulates ovarian androgen production by acting via insulin growth factor receptors as an intermediary in ovarian dysfunction (11).

In the early 1990s, Reaven et al. hypothesised the causal effect of IR on central adiposity (apple shaped-male pattern obesity), impaired glucose tolerance, diabetes and hypertension. These symptoms were initially coined syndrome X, known today as metabolic syndrome (12).

By the 21ST century, PCOS was conceived more like a metabolic disorder with a cluster of cardiometabolic risk factors including (IR, impaired glucose tolerance, hypertension and dyslipidaemia) that linked to an increased risk of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (13).

PCOS is the most common endocrine disorder in women of reproductive age. The syndrome is heterogeneous and characterised by ovulatory dysfunction, hyperandrogenism and polycystic ovarian morphology. PCOS is also associated with various metabolic

disarrangements, including insulin resistance, fasting hyperinsulinemia, impaired glucose tolerance, metabolic dyslipidaemia, metabolic syndrome, obesity, increased risk of progression to type 2 diabetes mellitus (T2DM), hypertension and high risk of cardiovascular disease (CVD). Additionally, there is also a considerable risk of endometrial, ovarian and breast cancer (14). There is also a significant increase in depression, anxiety, eating disorders, mood swings, sexual problems and social maladaptation, which collectively reduce health-related quality of life (QoL) in women with PCOS (15).

1.1.2 Prevalence of PCOS

PCOS is a heterogeneous endocrine disorder common among women of reproductive age with worldwide prevalence. Although several epidemiological studies have investigated the exact prevalence of PCOS, there are variations in their results. These discrepancies were influenced by race and ethnicity, study populations and phenotypes (16). A racial difference in the PCOS prevalence reflects the differences in the genetic and environmental predisposition (17). The diagnosis of PCOS was initially standardised after an expert conference in April 1990 sponsored partly by the National Institute of Child Health and Human Disease (NICHD) of the National Institute of Health (NIH), which adopted the following diagnostic criteria “in order of importance"; **i)** Hyperandrogenism and/or Hyperandrogenaemia, ii**)** oligoovulation and/or anovulation, iii**)** exclusion of other endocrine disorders such as congenital adrenal hyperplasia (CAH), Cushing’s syndrome and hyperprolactinemia (29).

Another expert conference was established in Rotterdam, the Netherlands, in May 2003, sponsored partly by the American Society of Reproductive Medicine (ASRM) and the European Society for Human Reproductive and Embryology (ESHRE), known today as “the

Rotterdam 2003 criteria". According to the Rotterdam criteria, PCOS should be diagnosed by exclusion of related disorders and presence of at least two of the following three features; **i)** oligo/anovulation, **ii)** clinical and /or biochemical evidence of hyperandrogenism, or **iii)** polycystic ovaries (30, 31). Polycystic ovaries refer to the presence of at least 12 or more ovarian follicles measuring 2-9 mm in diameter with a total volume of 10 cm^3, as defined by transvaginal ultrasound (29). A similar diagnostic approach was adopted by the androgen excess and polycystic ovary syndrome society (AE-PCOS) in 2006, which proposed the following diagnostic criteria; **i)** hyperandrogenism: hirsutism and/or hyperandrogenaemia, and **ii)** ovarian dysfunction: oligo-anovulation and/or polycystic ovaries, and **iii)** exclusion of other androgen excess disorders (32).

According to the national institute of health (NIH), the Rotterdam and the androgen excess (AE) society diagnostic criteria, the overall reported prevalence of PCOS is 6 % and 10 %, respectively (16). Of the 7,233 women from the United Kingdom, PCOS was diagnosed based on the Rotterdam criteria in 2.27 % in 2014 (18). Conversely, a study of 230 women aged 18-25 years in Oxford, UK, suggested the prevalence of PCOS in this age group could be as low as 8 % or as high as 26 % based on criteria of diagnosis used (19). In 400 unselected, consecutive, premenopausal women (aged 18-45 years) who undertook pre-employment health checks at the University of Alabama, USA, PCOS was diagnosed in 8 % and 4.8 % in black Afro-Americans and white Caucasians, respectively (20). Moreover, in a population of 369 consecutive, unselective women of the south-eastern USA, PCOS was diagnosed using NIH criteria in 4.7 % in white and 3.4 % in black populations (21). Two major South European studies present an approximate prevalence of PCOS in those populations. Data from a total of 154 unselected Caucasian Spanish women demonstrate a 6.5 % prevalence of PCOS using NIH/NICHD 1990 endocrine criteria (22). This data was compared to a prevalence of 6.7 % reported in a Greek

study that involved 192 women living on the Greek Island of Lesbos (23). In a retrospective birth cohort Australian study of 728 women aged 27-34 years, the prevalence of PCOS was 8.7 % using the NIH 1990 criteria; however, under the Rotterdam 2003 criteria, the prevalence was significantly higher at around 11.9 %. Furthermore, of the women with PCOS, 68-69 % had no prior PCOS diagnosis, reflecting the population of women with PCOS remain undiagnosed (24). Another cross-sectional study of 248 indigenous Australian women aged 15-44 years reported a prevalence of 15.3 % using the NIH 1990 criteria (25). PCOS is also prevalent in Asia. The community-based prevalence of PCOS studied by random cohort selection using the Rotterdam criteria illustrates the rates to be 6.3 % in a random sample of 3,030 Sri Lankan women aged 15-39 (26), 2.2 % in a population of 915 women in Southern China (27) to 5.7 % in Thailand (28).

1.1.3 Diagnosis and phenotypes of PCOS

The 2003 Rotterdam criteria have expanded the NIH 1990 criteria. Therefore, the proportion of patients who fit the diagnostic criteria of PCOS has increased dramatically after establishing two new phenotypes of the PCOS in addition to the previously established phenotypes, which were; hyperandrogenism + oligo/anovulation + polycystic ovaries (phenotype-1, full-blown PCOS), hyperandrogenism + oligo/anovulation (phenotype-2,classic PCOS) (33). Newly added phenotypes were as follows; patients with clinical and/or biochemical hyperandrogenism, polycystic ovaries but normal ovulatory function (phenotype-3, ovulatory PCOS), and patients who have ovulatory dysfunction, polycystic ovaries but no features of androgen excess (phenotype-4, mild PCOS) (29). However, even though both the NIH 1990 and the Rotterdam 2003 agree on those two phenotypes, the Rotterdam 2003 defined additional two phenotypes, including patients with ovulatory dysfunction and polycystic ovaries, and women

with hirsutism and/or hyperandrogenism and polycystic ovaries (34). Both criteria are currently used for the clinical diagnosis and clinical research purposes of PCOS.

1.1.4 Morphology of PCO

PCOS was initially named for the ovary's pathological feature in women with hyperandrogenism and menstrual irregularities. However, the definition was varied over the years. Stein and Leventhal first described the macroscopic appearance as bilaterally enlarged and globular ovaries, with glistening and smooth capsules, similar to an oyster shell (4). After the technological advances and the introduction of gynaecological transvaginal ultrasound in the early 1970s, PCOS diagnosis was made using ultrasound instead of histology (35). The criteria used today for diagnosing PCOS is the Rotterdam criteria (30). However, there is evidence that polycystic ovaries are observable in 20-30 % of women of reproductive age with similar features to those observed in patients with PCOS (34). Images of typical ultrasound scans of the PCOS ovary are presented in Figures 1-2-A &B.

Fig 1-2-A

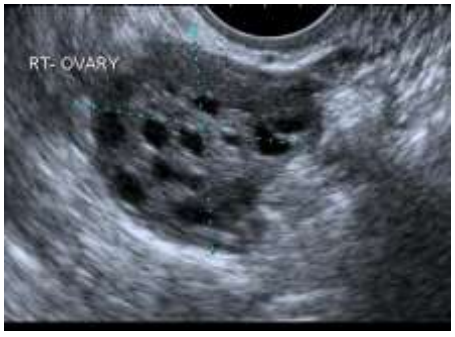


Fig 1-2-B

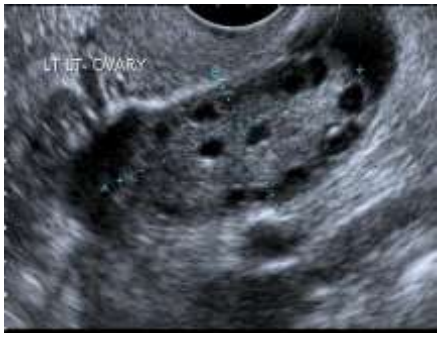


Figure 1-2: Typical images of transvaginal ultrasound of PCOS ovary. They are printed with the kind permission of Professor Sathyapalan.

1.1.5 Clinical manifestations of PCOS

The clinical manifestation of PCOS is variable. Patients might be symptomatic or have various dermatologic, gynaecologic and metabolic presentations (Figure 1-3). Women with PCOS commonly present with a constellation of oligo/amenorrhoea or infertility, signs of hyperandrogenism and polycystic ovarian morphology. Ovulatory dysfunction refers to irregular menses defined as a menstrual interval of more than 35 days, absence of menses for more than six months, a cycle lasting between 21-35 days and amenorrhea is defined as an absence of menstrual bleeding for more than 6-12 months. Hyperandrogenaemia is caused by excessive androgen production by ovaries and adrenal glands and can be clinically verified by the presence of acne, hirsutism (terminally distributed hair in a male pattern fashion), androgenic alopecia. The Ferriman-Gallwey scoring system is commonly used to assess the extent of hirsutism; it assesses the density of terminal hair at 11 sites in the human body, including; upper lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, arm, forearm, thighs and lower legs. For each area, a score of zero (absence of terminal hair) to 4 (extreme terminal hair growth) will be assigned. However, there is considerable variability about the extent of hirsutism as it is relatively subjective with various inter-rater variabilities.

The biochemical hyperandrogenism is determined by the baseline measurement of total serum testosterone, salivary testosterone, serum androstenedione, salivary androstenedione and the calculated free androgen index (FAI). FAI is a ratio used to assess abnormal androgen status calculated as the total androgen levels divided by sex hormone-binding globulin (SHBG) level multiplying by 100 as constant.

$$\boldsymbol{FAI = \frac{(total\ androgen)\times 100}{(SHBG)}}$$

PCOS is also associated with insulin resistance, diabetes, hypertension, obesity and lipid disorders. However, there is considerable heterogeneity of PCOS symptoms and signs among women with PCOS. Available evidence suggests that mild evidence of insulin resistance and ovarian dysfunction are more prevalent amongst hyperandrogenic ovulatory women with PCOS than anovulatory women with PCOS. Moreover, oligoovulatory women with PCOS have a relatively low risk of long-term metabolic disorders and insulin resistance. Even though weight gain is associated with severe PCOS symptoms, a modest weight loss as little as 5% of total body weight may improve the reproductive hormonal profile, restore ovulation and improve insulin sensitivity (36). Generally, these classical PCOS features are varied, with approximately 60-80 % having hyperandrogenism (20, 37), 75 % have clinically evident menstrual disturbance (38), about 60 % found to have hirsutism (39, 40), acne affects around 15-25 % of PCOS patients (20, 41, 42) and 75 % had ultrasound detected polycystic ovarian morphology (38).

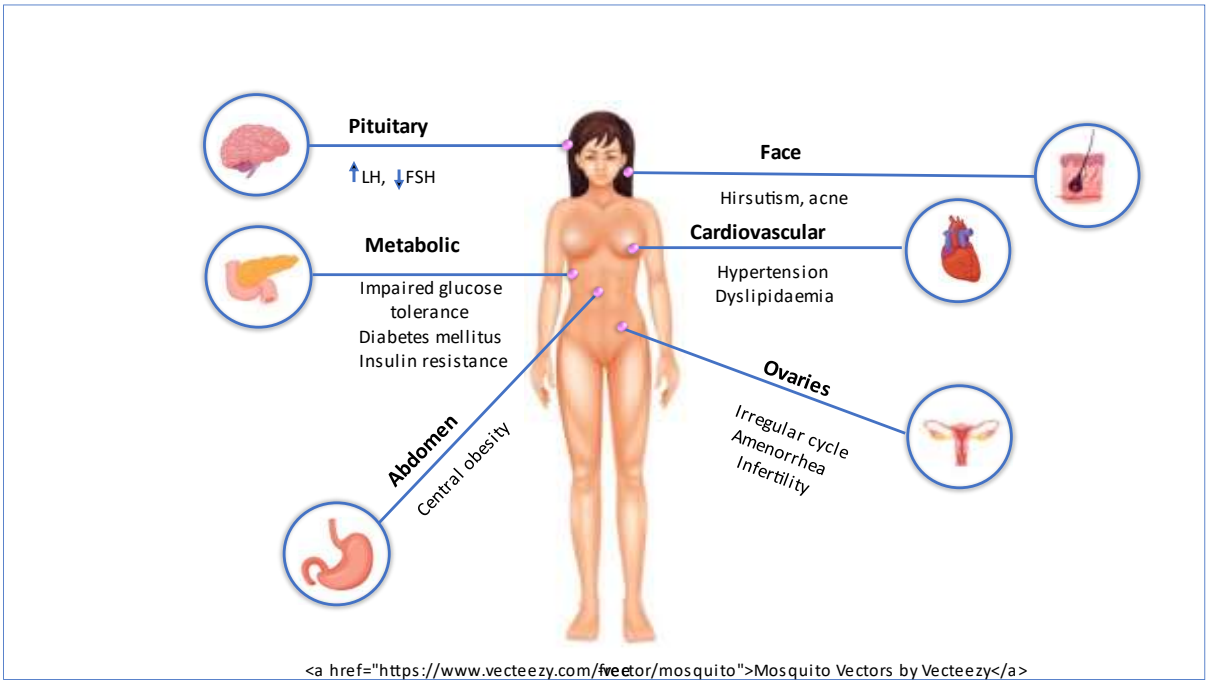


Figure 1-3: Illustration of the characteristics of clinical manifestations of PCOS-printed from www.vecteezy.com/free-vector/mosquito

1.2 Aetiology and pathophysiology of PCOS

1.2.1 Genetic factors

There is evidence of genetic basis in the causes of PCOS where either gene-to-gene interaction or gene-to-environment interaction were postulated (43). Twin studies of monozygotic or dizygotic twins highlighted genetic involvement in nearly 70% of the PCOS variance (44, 45). Most of the genes directly or indirectly affect the ovaries are related to the genetic causes of PCOS. Figure 1-4. Cytochrome P450 family 11 subfamily A 1 (CYP11A1) is a protein-coding gene involved in steroidogenesis by mediating the conversion of cholesterol to progesterone (46). Polymorphism in CYP11A1 has been reported as an associated factor for PCOS (47). There is a hypothesis that active mutation in the LH receptor gene could cause an increase in the production of androgen hormone in PCOS. This is mainly in PCOS women with normal serum LH and elevated androgen levels (48, 49). The polymorphism in the coding site of SHBG has been postulated to cause PCOS. A study identified a missense mutation in P156L of the SHBG in 482 women with PCOS, ovarian dysfunction and hirsutism (50). Recently, AC/T single nucleotide polymorphism (SNP) in the tyrosine kinase domain of the insulin receptor gene has been identified (51). A Greek study of women with PCOS has recently established 4G5G polymorphism in promotor gene of the plasminogen activator inhibitor-1 (52).

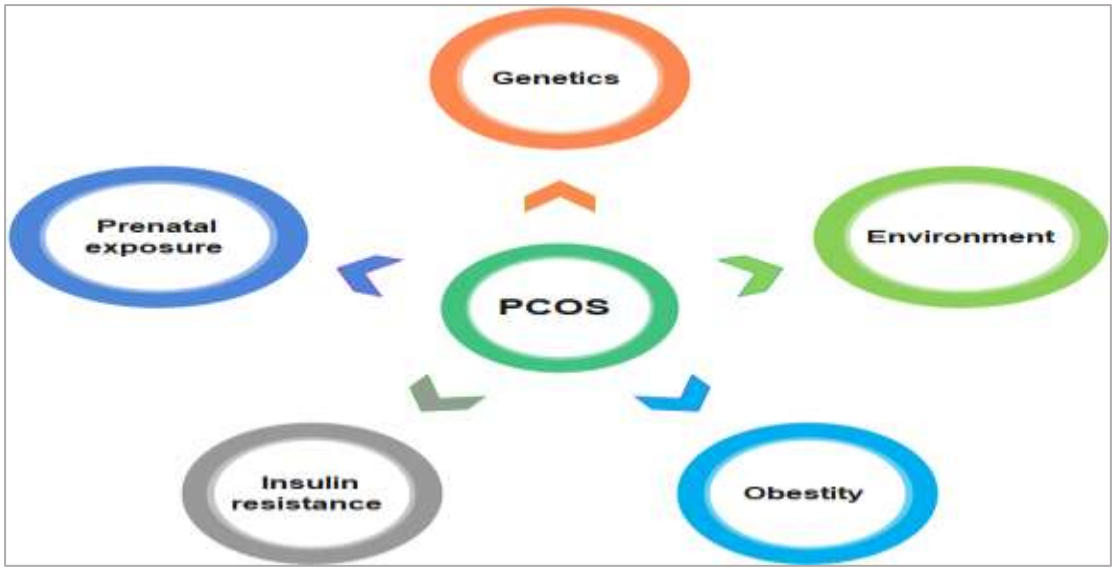


Figure 1-4: potential PCOS aetiology

1.2.2 Environmental factors

Several environmental factors, including acute and prolonged exposure to endocrine disruptors and geographical and socio-economical factors, are related to the defect in hormonal homeostasis. However, the timing of the exposure to such factors is crucial. For instance, early exposure to the endocrine disruptors during childhood increases their effects in later life (53). This is because they mimic the action of the endogenous hormone and interfere with its function (54).

1.2.3 Prenatal exposure

There is a theory that prenatal exposure to excess androgen hormones has been linked to PCOS development. However, this theory has its limitation as the mechanism of prenatal androgen exposure causing PCOS remains elusive. A potential mechanism is the effect of excess androgen in priming the hypothalamic-pituitary system long before birth (55). Women with PCOS have higher androgen levels compared to women without PCOS. A recent study has reported an increased anogenital distance (AGD), a marker for prenatal androgen

exposure in offspring of women with PCOS (56). It measures the distance between the anal orifice and the genital tubercle. It is usually longer in women with PCOS, indicating positive androgen exposure (57).

1.2.4 Hypothalamic-pituitary ovarian axis

Hypothalamic-pituitary ovarian (HPO) axis imbalance is an essential underlying pathology for PCOS. Increased levels of the luteinising hormone (LH), increased amplitude of LH, and high LH to FSH (follicular stimulating hormone) ratio are features of PCOS. The hypothalamic neurons produce a gonadotrophin-releasing hormone (GnRH), which travels to the pituitary gland results in pulsatile LH and FSH secretion. Increased GnRH secretion leads to an increase in the pulsating release of LH and reduces FSH. Subsequently, a high level of LH affects ovarian androgen secretion, oocyte development and folliculogenesis (58). In contrast, the high sex hormone can modulate the release of GnRH through negative feedback. This feedback is lost in women with PCOS (59).

1.2.5 Ovarian and adrenal androgen excess

PCOS is characterised by excess ovarian and adrenal androgen production. In women, androgens including testosterone, androstenedione (A4), dehydroepiandrosterone sulphate (DHEAS), dehydroepiandrosterone (DHEA), 17-hydroxyprogesterone (17-OHP), and dihydrotestosterone (DHT) are mainly produced by the ovaries and the adrenal gland, with a minute amount being produced by the peripheral tissue via the action of the aromatase enzyme (60). Testosterone and dihydrotestosterone (DHT) are the most bioactive androgens; however, over 60% of testosterone is bound to sex hormone-binding globulin (SHBG) and approximately 30% bound to albumin, resulting in 1% being biologically active testosterone that exerts its effects (61). Testosterone can be transformed into oestradiol by the action of

the aromatase enzyme and synergise their action to control female reproductive functions (62). An excessive amount of oestradiol leads to disturbance in the hypothalamic-pituitary-gonadal adrenal axis, which affects GnRH secretion (63). This, in turn, will affect the release of LH and FSH and alter the ratio of LH to FSH (64). Excessive LH increases ovarian androgen production, and the low FSH drives anovulation (65). Hyperandrogenism is also an independent risk factor for long-term health consequences associated with PCOS, including obesity and type 2 diabetes (66). Around 30% of women with PCOS have excess adrenal androgen, which is biochemically presented as elevated DHEAS, a precursor of androgen and oestrogen.

1.2.6 Sex hormone-binding globulin production

The liver produces sex hormone-binding globulin (SHBG), a transporter for sex hormones. It has a high affinity for bioactive testosterone and reduces its bioavailability (67). On the other hand, increased body weight is the main feature of PCOS, with over 80% of women with PCOS being either overweight or obese (68). Overweight and obese women with PCOS have a low level of SHBG compared with normal-weight women with PCOS. This low SHBG is correlated negatively with hyperandrogenaemia, insulin resistance and increased body weight (69). Insulin regulates SHBG and is believed that it reduces the hepatic production of SHBG. Therefore, an increase in serum insulin as a compensatory effect for reducing insulin sensitivity reduces the SHBG (70). A low level of SHBG observed in PCOS also promotes dyslipidaemia in women with PCOS.

1.2.7 Insulin resistance and hyperinsulinemia

Insulin is an anabolic hormone secreted by the β-cells of the pancreas. It functions on the body cells by binding to its receptors. Insulin receptors are scattered among various tissues,

including skeletal muscles, liver, ovaries and adipose tissues. Insulin receptors are hetero-tetramer consisting of two α and β subunits connected by disulfides bonds. The α dimers are located extracellularly and contain the binding domains, while the β dimers extend through the cell membrane and bear tyrosine kinase activity. The insulin molecule binding with its receptor activates the tyrosine kinase of the β subunit and leads to autophosphorylation. Subsequently, this will lead to the phosphorylation of insulin-receptor substrate (IRS), which acts as a docking site for the signalling molecule to initiate insulin signal transduction. This signalling molecule commonly contains the Src homology 2 (SH2) domain, such as the phosphatidylinositol-3-kinase (PI3K). The activation of PI3K translocates glucose transporter-4 (GLUT-4) to the cell membrane and facilitates insulin-dependent glucose uptake. This is mediated by the action of PI3K activation of phospholipids and phosphatidylinositol 4,5-bisphosphonate, which leads to the activation of 3-phosphoinositide-dependent protein kinase 1, 2 (PDK-1,2) (71).

Insulin also stimulates the growth and differentiation of various cells. This mitogenic action is mediated by activating the mitogen-activated protein kinase-ERK (MAPK-ERK) pathway. The activation of MAPK-ERK stimulates a series of cascade serine/threonine kinase leading to the stimulation of MAPK-ERK and subsequently initiating cell growth and differentiation (72). Insulin also regulates protein synthesis and degradation through the mammalian Target of Rapamycin (m TOR) which PI3K also activates. Furthermore, insulin stimulates the glycogen synthesis kinase 3 (GSK3) inhibition via its action on PI3K and Atk/PKB and increases glycogen synthesis (73). The insulin signal can be discontinued by dephosphorylation of the signalling molecules such as protein tyrosine phosphate 1 B (PTP1B) (74).

1.2.8 Increased body weight

Increased body weight and obesity have a significant effect on the pathology of PCOS, and the majority (80%) of women with PCOS are either overweight or obese (75). Furthermore, weight gain is associated with worsening PCOS symptoms and increased risk of cardiovascular disease (CVD) and type 2 diabetes. Moreover, obesity exacerbates the reproductive and metabolic features of PCOS, pregnancy complications and impairs insulin resistance. Increased body weight is also linked to the resistance to various pharmacological interventions in PCOS. For instance, most obese women with PCOS are resistant to clomiphene citrate, hindering its efficacy (76,77). At the same time, weight loss has a remarkable effect by improving fertility, PCOS symptoms and metabolic disturbances. It has been reported that weight loss as low as 5% of the total body weight could improve fertility and PCOS symptoms (78). The exact mechanism of obesity and increased weight gain in PCOS is relatively straightforward. An interaction between the various environmental factors and genetics is the biggest contributor (79). There was a significant finding regarding this environmental-genetics link; for instance, heavier sisters have irregular cycles and high androgen levels than unaffected sisters with lower body weight (80).

Interestingly, increased body weight and the quantity and quality of food has also been reported to interact with different genes (81). In response to excess food intake, there is a proliferation in adipocytes, leading to increased body weight, particularly around the abdominal area referred to as central or abdominal obesity. This is usually associated with proinflammatory cytokines release (82). Increased visceral adiposity also increases these markers, including c-reactive protein (CRP), inflammatory cytokines, oxidative stress and interleukins (83). These products play a pivotal role in creating chronic inflammation and accelerating endothelial dysfunction. For example, Interleukin-6 (IL-6) and tumour necrosing

factor (TNF-α) produced by the adipocytes enhances the hepatic release of CRP, a significant risk factor for CVD, which is elevated in women with PCOS (84). Central adiposity is also associated with dysregulation of androgen hormones in PCOS. Obese women with PCOS have significantly low SHBG levels with an increased androgen production (85).

1.3 Consequences of PCOS

1.3.1 Cardiovascular risk in PCOS

1.3.1.1 Factors of cardiovascular risk in PCOS

Women with PCOS have a higher prevalence of cardiovascular disease (CVD) risk factors than the general population, which remains the leading cause of death in women (86). These factors could be divided broadly into modifiable risk factors such as obesity, insulin resistance, metabolic syndrome, dyslipidaemia, T2DM, physical inactivity, psychosocial factors and hypertension and non-modifiable risk factors (87). These potentially modifiable risk factors were accounted for up to 94% of the population's risk of myocardial infarction (MI) in women (88). On the other hand, non-modifiable risk factors exist such as gender, age and family history of CVD. However, recently, there has been growing solid evidence of the association between PCOS and other markers for cardiovascular risk, such as increased markers for chronic inflammation, coagulation, homocysteine, fibrinogen, plasminogen activator inhibitor-1 (PAI-1), oxidative stress, arterial stiffness and endothelial dysfunction (89, 90). In addition to these markers, several clinical measurements for atherosclerosis have been recognised. For instance, women with PCOS over 45 years have been found to have higher carotid intima-media thickness (IMT) and higher coronary artery calcium levels (CAC)(91). Furthermore, high androgen and low SHBG have independently been linked to increased risk of CVD in both pre and postmenopausal women with PCOS (92). Figure 1-5.

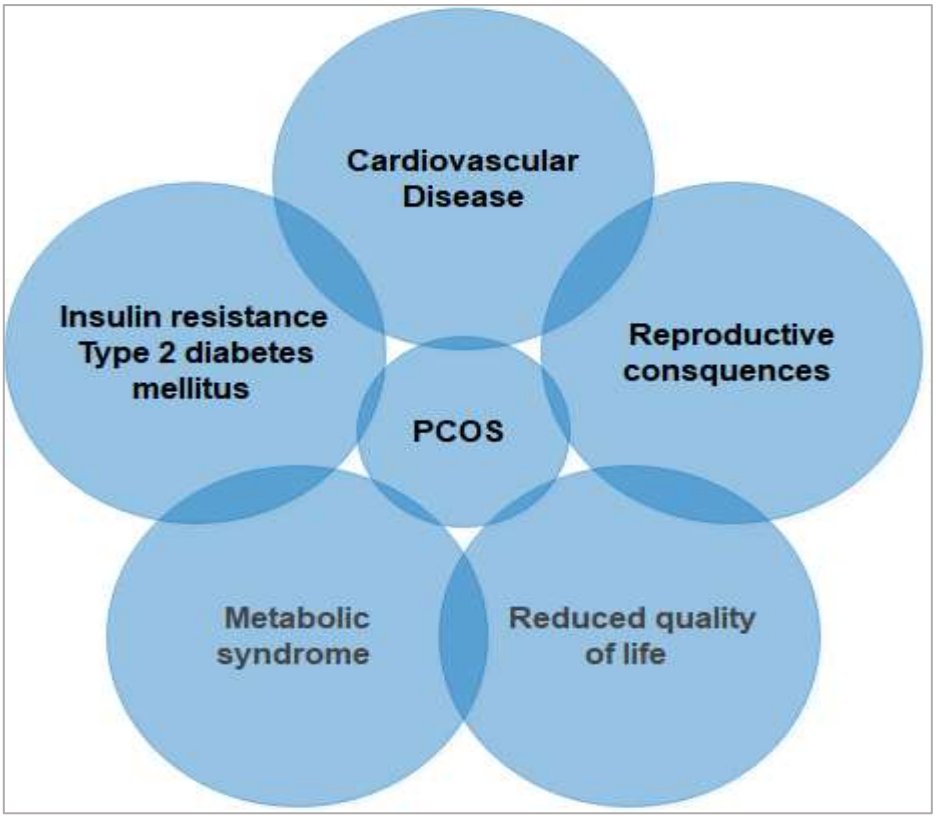


Figure 1-5: Consequences of PCOS

1.3.1.2 Cardiovascular disease (CVD) in PCOS

Talbott et al. reported that women with PCOS have a higher coronary artery and aortic calcification prevalence than controls. After adjustment for age and BMI, PCOS was a strong predictor for coronary artery calcification. Low serum levels of high-density lipoprotein cholesterol (HDL-C) and high insulin level were associated with coronary artery calcification, while total testosterone was independently associated with an increased risk of aortic calcification in PCOS when adjusted for age and BMI (93). Women with PCOS usually have a significantly higher low-density lipoprotein cholesterol (LDL-C), total cholesterol and testosterone. Collectively, the high LDL-C and cholesterol level have been reported as a predictor for high CAC levels when adjusted for BMI (94).

The Coronary Artery Risk Development in Young Adults (CARDIA) study, was a multi-centre population-based study of young women with PCOS who had an irregular period and evidence

of high androgen levels. In this study, women were divided into groups; irregular period and hyperandrogenism group, isolated oligomenorrhoea group and isolated hyperandrogenism. In general, women with PCOS had a significant high risk of coronary artery calcification (10.6%) and increased internal carotid intima (IMT) measurements (95). Moreover, a low HDL-C level was also reported as a strong predictor for carotid IMT in PCOS (96).

Recently, a Danish study identified a cohort of patients who received assisted reproductive technology (ART) between 1994 and 2015. A total record of 60,574 of women was retrieved from the Danish national registry. Of the 60,574 women, 10.2% (6,149) women with PCOS were identified using the International Classification of Disease (ICD-10). After a median follow-up of 8.9 years, around 4.8% (2,925) have developed CVD. After adjusting for smoking, BMI and alcohol, the risk was still higher for PCOS women. There was also evidence of greater CVD risk in younger women below the age of 30 years, while there was no evidence for CVD in women with PCOS above 50 years (97). However, 138 (0.2%) developed atrial fibrillation (AF). The results were significant compared with women who received ART for non-ovulatory female factor infertility (98). Women with PCOS have higher BMI and insulin resistance which could potentially justify the association between AF and PCOS. Women with PCOS are also at greater risk of T2DM due to insulin resistance, and T2DM is another risk for AF.

Shroff et al. studied 24 young and obese women with PCOS, and they were compared with 24 healthy cohorts in a case-control study. In eight (33%) out of the 24 women with PCOS, there was a significantly higher level of CAC compared with only two (8%) in the healthy controls (99). These findings highlight the significant risk of subclinical atherosclerosis in women with PCOS and the importance of rigorous screening for CVD in these cohorts.

A meta-analysis of nine studies examining the association between PCOS and stroke has concluded that women with PCOS are at a slightly higher risk of developing stroke than women without stroke. However, the risk was slightly attenuated as some of it was explained by the increased BMI of the patients (100).

1.3.1.3 Cardiovascular mortality and morbidity in PCOS

Although there is accumulating evidence of increased CVD risk and high CVD risk factors in women with PCOS, the exact effects of PCOS on CVD morbidity and mortality remain uncertain. In a retrospective cohort follow-up study of 319 women with PCOS and 1060 age-matched controls, even though the risk of diabetes, hypertension, hypercholesterolemia and hypertriglyceridemia was significantly higher in women with PCOS, there was no difference in cardiovascular mortality and morbidity (101). Similarly, a recent population study of 219034 women diagnosed with PCOS evaluated the risk of major cardiovascular events (MACE) and CVD mortality. It was reported an increased risk of MI and angina in women with PCOS with no significant difference in the CVD mortality between PCOS (*n*= 68) and the control group (*n*=63)(102). Furthermore, a study examining 10-years mortality in postmenopausal women with clinical features of PCOS reported no significant difference in the cumulative 10-years mortality in women with PCOS (28%) compared with 27% in women without PCOS (103).

A recent systematic review and meta-analysis of literature examined the risk of CV and cerebrovascular events in PCOS. The pooled estimates effect showed an increased risk of MI, stroke and ischemic heart disease in women with PCOS with no significant difference in the overall mortality and CVD related deaths (104).

Nonetheless, metabolic disorders such as insulin resistance, T2DM, lipid disorders, hyperandrogenism, and increased BMI in women with PCOS are major risk factors for CVD, data regarding PCOS-related CVD mortality and morbidity are inconsistent.

1.3.1.4 Cardiovascular risk reduction in PCOS

Since lifetime risks for CVD are significantly higher in women with PCOS and the majority are preventable, women with PCOS should be regularly assessed for CVD risks. A panel of experts in PCOS and CVD from the Androgen Excess and PCOS (AE-PCOS) Society have reviewed the literature and presented the following recommendations. It is agreed that women with PCOS who are obese, cigarette smokers and those with impaired glucose tolerance, dyslipidaemia, T2DM, subclinical CVD and metabolic syndrome are at greater risk of CVD. Thus, assessing serum lipid, serum glucose, waist circumference, BMI and blood pressure is recommended for all women with PCOS. In contrast, an oral glucose tolerance test (OGTT) should be preserved for those with a family history of T2DM, personal history of gestational diabetes, and advanced age. Haemoglobin A1c (HbA1c) should be considered for those unwilling to undergo an OGTT. Assessment for mood disorder was recommended for all women with PCOS. Lifestyle interventions, including dietary management and physical activity, was also recommended for the primary prevention of CVD, aiming at reducing the serum levels of LDL-C and non-HDL-C. Insulin-sensitisers and other medications should be added if the lipid disorders and other risk factors persist (105, 106).

The recommendations from the international evidence-based guideline for the assessment and management of PCOS-2018 acknowledged the need for regular monitoring of weight changes and excess weight at least at each visit or biannually. In addition, all women with PCOS should be assessed for CVD risk, and blood pressure should be measured annually (107).

1.3.2 Reproductive consequences in PCOS

1.3.2.1 Infertility

Ovulatory disturbances are the key diagnostic feature for PCOS, resulting in subfertility, infertility and other adverse pregnancy outcomes. PCOS constitutes around 70-80% of anovulatory infertility, with two-thirds of women with PCOS not ovulating regularly and seeking fertility treatment (108,109). First-line fertility treatment in PCOS is centred around modifiable lifestyle factors, including excess body weight, which exacerbates infertility and hinders the response to fertility treatment (109). There is strong evidence that weight loss of 5-10% of the original body weight will, over six months, reduce central obesity and hyperandrogenaemia and increase ovulation rate regardless of the BMI (110). Preconception counselling and the administration of folic acid are also recommended.

Moreover, smoking, alcohol consumption, recreational and prescribed drugs, untreated sexually transmitted diseases (STDs), vitamin D supplementation and nutritional status are all identified as modifiable preconception risk factors (107). Psychological factors such as depression and anxiety can also affect relationships and sexual intimacy among women with PCOS, impairing quality of life (111, 112). Therefore, care for mental health problems should be optimised in women with PCOS, improving adherence to infertility treatment. The recommended first-line treatment for ovulation induction is the oral administration of clomiphene citrate (CC) (113). CC is a selective estrogen receptors modulator with anti-androgen properties; it competes with estrogen for its receptor in the hypothalamic-pituitary region and terminates the negative feedback mechanism (114). The initial starting dose of CC is 50 mg per day for five days, starting between the second and fifth day of the menstrual cycle, and may be increased up to 150 mg a day (115,116). CC is an effective option with an

average ovulation rate of up to 80%, successful conception rate of up to 22%, and overall pregnancy rate of up to 70% per six cycles (117,118). However, CC resistance and failure is well known, with around 15% of PCOS patients not responding to the maximum dose of CC (150 mg/day). These patients are usually obese with insulin resistance which increases ovarian androgen production causing premature follicular atresia and subsequently anovulation. This justifies the use of insulin sensitising drugs such as metformin in ovulation induction (119-121). With CC, multiple pregnancies and ovarian hyperstimulation syndrome (OHSS) are very low (114).

Second-line treatment for induction of ovulation in PCOS is injectable gonadotropin therapy, including recombinant FSH (r FSH) or human menopausal gonadotropin (HMG), particularly in those who failed to respond to the first-line therapy (122). However, exogenous gonadotropin is associated with a higher incidence of multiple pregnancies and overstimulation. Therefore, close monitoring of the follicular development using a frequent ultrasound scan is required. Moreover, the traditional step-up regimen can be replaced by either a low-dose step-up or step-down regimen (123, 124).

Laparoscopic ovarian surgery is considered second-line therapy for infertility in women with PCOS. However, because the technique is highly invasive and requires anaesthesia and high cost, this option should be reserved for CC-resistant women with PCOS or those undergoing laparoscopy for unrelated reasons (125). There are various methodologies for performing the procedure, and one of these is ovarian drilling (126, 127). Monopolar electrocautery is applied to the ovarian capsules to create punctures in the ovaries. The exact mechanism of how the technique works is by ameliorating the endocrine effect of PCOS by reducing androgen production (128).

When the above methods have failed to achieve the desired effects, ART including in-vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) in male factor infertility (motile sperms < 5 million) should be considered as a third-line therapy (129). Furthermore, as where there is a high risk of OHSS, controlled ovarian stimulation should be used by administering a low dose of gonadotropin. Finally, the pituitary can be suppressed with a GnRH agonist (130).

1.3.2.2 Pregnancy complications in PCOS

Women with PCOS are at high risk of developing adverse pregnancy outcomes, including early pregnancy loss and ectopic pregnancy compared with women without PCOS (131). Excess androgen levels and insulin resistance were the main reasons for these complications. Additionally, infants born to mothers with PCOS are at greater risk of prenatal mortality (132).

1.3.2.2.1 Early pregnancy loss

Women with PCOS have a three times higher risk of miscarriage in the early months of pregnancy than women without PCOS (132, 133). Insulin resistance, endometrial dysfunction and impaired fibrinolysis, and hyperhomocysteinemia were the most reported aetiologies (134, 135). Some research has shown that treatment with metformin reduces the risk of early pregnancy loss in women with PCOS. However, this evidence is not conclusive (136).

1.3.2.2.2 Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) is a common complication in pregnancy, and it is higher in women with PCOS, particularly in the first trimester. During pregnancy, the elevated levels of hormones that antagonise the insulin effect and the insufficient β-cell function to counteract these hormones exacerbate the insulin resistance in the peripheral tissues, followed by hyperinsulinemia (137, 138). A cross-sectional study of 468 patients underwent ART half of them have PCOS and found that the incidence of GDM was significantly higher in

pregnant women with PCOS (44.4%) compared with non-PCOS pregnant women (29.9%) (139). A similar result was also reported in a study by Bals-Pratsch et al., which included 107 women with PCOS undergoing ART and found a significantly higher rate (40.6%) of GDM at the first week of pregnancy in women with PCOS compared to women without PCOS (26.1%)(140). There is evidence that continuing metformin throughout the pregnancy improves insulin resistance and reduces the incidence of GDM in women with PCOS (141). However, data from PregMet2-study, a large randomised controlled trial (RCT) of metformin in pregnant women with PCOS, showed that even though starting metformin treatment from the first trimester until delivery was associated with a reduction of miscarriages and preterm birth, it did not prevent the development of GDM (142).

1.3.2.2.3 Pre-eclampsia

Pre-eclampsia, a sudden increase in blood pressure after the 20th week of pregnancy, is significantly higher in obese women with PCOS than pregnant women without PCOS (143). A retrospective cohort study of 1023 pregnant women with PCOS and 1023 pregnant women without PCOS found that pregnant women with PCOS had a significantly higher incidence of pre-eclampsia (4.9% versus 3.0%), gestational hypertension (5.8% versus 3.6%) and pregnancy-induced hypertension (10.8% versus 6.6%) compared with pregnant women without PCOS (144). A recent population-based study of 9.1 million pregnant women (PCOS = 14,882 , non-PCOS = 9,081906) used data collected during 11 years between 2004 and 2014. It reported that pregnant women with PCOS were more likely to develop pregnancy-induced hypertension, pre-eclampsia and GDM than women without PCOS (145).

1.3.2.2.4 Preterm birth

The delivery of newborns before 37 weeks of pregnancy is significantly higher in women with PCOS (146). Pregnancy-induced hypertension, GDM and poor maternal health are among the most common causes for preterm birth (147). A retrospective cohort study of 908 pregnant women with PCOS had 12.9% preterm birth compared with 7.4% in women without PCOS (148). Recently, a Swedish population-based study that included over a million women with and without PCOS found that pregnant women with PCOS had a significantly higher incidence of preterm birth than women without PCOS (6.7% versus 4.8%, respectively) (149). In addition, there is emerging evidence of the association of high anti-Mullerian hormone (AMH) and the increased risk of preterm birth (150, 151). Thus, AMH could be utilised to predict preterm birth in women with PCOS, particularly in the third trimester (152).

1.3.3 Insulin resistance

1.3.3.1 Definition of insulin resistance

Insulin resistance (IR) is defined as the suboptimal response to insulin stimulation in the target tissues, principally the liver, adipose tissue and skeletal muscles (153).The impaired sensitivity to the insulin action hinders the glucose disposal into the target tissues, leading to a compensatory increase in the production of insulin from the β-cells and subsequent hyperinsulinemia (154).

1.3.3.2 Insulin action on glucose metabolism

Insulin mediates its anabolic function and maintains glucose homeostasis by acting on its target tissues. Skeletal muscles account for up to 75% of the insulin-mediated glucose uptake (155). Adipose tissue is a metabolically active organ and plays a significant role in maintaining energy balance, metabolism and providing essential hormones for the human body (156).

Adipose tissue has a vital role in glucose and lipid homeostasis; science has determined that insulin action closely guarded lipolysis (153). However, adipose tissue only contributes a much smaller fraction to glucose disposal than skeletal muscle (157, 158).

1.3.3.3 Glucose transporters

Glucose is the primary energy source for the vast majority of the tissues in the human body. Thus, maintaining glucose homeostasis requires a complex regulatory mechanism. However, glucose cannot cross the cell membrane by simple diffusion due to its polarity. Therefore, the glucose entry is mediated by a large family of transporter proteins known as glucose transporters (159). At present, there are three families of glucose transporters. The largest is the Glucose Transporters (GLUTs). Fourteen members of GLUTs have been identified in the human body, with only four (GLUTs 1-4) being the most investigated (160).

1.3.3.4 Insulin action on lipid metabolism

Adipose tissue functions as a storage facility for the excess energy in the form of lipid until it is needed. In the postprandial state, insulin switches the metabolism from fatty acid to glucose in the peripheral tissues. It increases the glucose uptake by muscles and adipose tissues and inhibits the rate of glycogen breakdown (161). It also decreases the rate of lipolysis in the adipose tissue and hence lowers the level of FFA. Insulin also increases the uptake of the triglycerides from the blood by the muscles and adipose tissue and, therefore, reduces the circulating rate of the FFA in the plasma (162).

1.3.3.5 Insulin resistance in PCOS

Insulin resistance is a cornerstone in PCOS pathology, with approximately 65-80% of women with PCOS having some degree of insulin resistance regardless of their body weight (72). However, the severity of insulin resistance is positively correlated with increased age and

body weight, and around 80% of women with PCOS are either overweight or obese (75, 163). Recently, some evidence showed that insulin resistance appears independent of obesity and is related to PCOS (164). The association between insulin resistance and hyperandrogenism was first described in 1921 by Archard and Theirs, who coined the phenomena as “diabetes of bearded women” or Archard Theirs syndrome (165). In a study of 45 women with PCOS and 35 age-matched controls, hyperandrogenism and increased body weight were the significant predictors for insulin resistance and CVD development related to PCOS (166). Although increased body weight is strongly associated with insulin resistance, data showed that normal-weight women with PCOS have an increased risk of insulin resistance compared with BMI and age-matched women without PCOS (167). A decrease in the whole-body insulin-mediated glucose disposal and insulin sensitivity in women with PCOS was also reported compared with matched controls. Eighteen women with PCOS and 18 matched controls were examined using the modified frequent intravenous glucose tolerance test (m IVGTT). Women with PCOS had a higher homeostatic model assessment for insulin resistance (HOMA-IR), lower sensitivity and deposition index (168). A meta-analysis of eight studies showed that obese women with PCOS had higher HOMA-IR than non-obese PCOS and non-PCOS obese women (169). Interestingly, the peri-muscular adipose tissue is a reliable predictor for the whole-body insulin sensitivity index (WBISI) and HOMA-IR in women with PCOS when 30 obese women with PCOS were matched with 38 women without PCOS (170).

1.3.3.6 The pathophysiology of insulin resistance in PCOS

The pathology of insulin resistance in PCOS is a complex and multifactorial process with the hypothesis that the defect in insulin secretion, insulin action and clearance could play the central role.

1.3.3.6.1 Defect in the insulin signalling pathway

PCOS is correlated with molecular defects in the insulin signalling pathways at the post-receptor level in the adipose tissue, muscles and ovaries. Adipose tissue isolated from women with PCOS showed a defect in receptor kinase and the glucose transport with a rightward shift in the insulin-dose response curve of the glucose transport stimulation (171, 172). There was also a decrease in the maximal insulin-dependent glucose transport in adipose tissue of women with PCOS compared with controls (173). However, Lystedt et al. reported a normal maximal insulin-dose response curve with diminished insulin-stimulated glucose transport in adipose tissue isolated from women with PCOS (174). These findings suggested the possibility of a defect in the insulin signal at the receptor level between the tyrosine kinase and glucose transport. However, in a study by Ciaraldi et al., there was no apparent defect in the kinase activity, but there was a decrease in the insulin-stimulated autophosphorylation (171). Also, a significant decrease in the tyrosine phosphorylation of IRS-1 and 2 was found in insulin-resistant women with PCOS compared to healthy controls (175-177).

Furthermore, GLUT4, the most abundant insulin-dependent glucose transporter, was also found to be decreased in the adipose tissue of women with PCOS, signalling a decrease in the glucose uptake (178). There was also a decrease in hepatic glucose synthesis in obese women with PCOS, amplifying the reduction of insulin sensitivity (179). Data also showed that in around 50% of the cultured fibroblast of women with PCOS, there was a decrease in the insulin-mediated tyrosine autophosphorylation of the β-subunit of the insulin receptor, defect in GSK-3 phosphorylation and the IRS-1 mediated PI3K activation (180-182).

In skeletal muscle, both in-vitro and in vivo studies showed inconsistent findings on the effect of PCOS in glucose uptake. However, an in vivo study reported a significant decrease in the

IRS-1 activated PI3K activity with a significant increase in IRS-2 insulin-mediated activity (183). Corbould et al. studied the effect on the insulin signalling pathway in cultured skeletal muscle of insulin-resistant women with PCOS (184). Even though he did not find any decrease in the insulin-mediated glucose uptake or the insulin-stimulated phosphorylation of the β-subunit of the IR, the IRS-1 activity was increased by 35%. However, after adjusting for the IRS-1, there was a significant reduction in the IRS-1 and 2 insulin-mediated PI3K activity (184). Moreover, he also found normal abundancy of GLUT4 with an increased abundance of GLUT1 correlating with the increase in glucose uptake (184).

LDL-C is the primary source for cholesterol in the ovaries, the substrate for steroid synthesis. It is usually transported to the ovarian cells via the LDL-C receptor pathway (185). It has been found that LH, FSH and insulin stimulate the LDL receptor gene in the cultured cells from the ovarian tissue (186). The steroid hormone synthesis requires the steroid acute regulatory protein (StAR), a step-limiting protein in cholesterol transport to the mitochondria. Steroidogenesis is partly controlled by StAR and CYP17 genes, which are regulated by the synergetic action of the LH and insulin. Insulin exerts its action by activating the MAP-kinase and PI3K pathways (187). PI3K activity was the main pathway for insulin to act on theca cells by activating the 17-α hydroxylase (188). Nelson et al. showed a significant reduction in the MEK1/2 and ERK1/2 phosphorylation in the theca cells of women with PCOS compared with controls (189). The reduction of MEK and ERK correlated with the increase in androgen production through the insulin-dependent mechanism. Insulin was also shown to augment the FSH-mediated aromatase activity in the follicular granulosa cells (190).

1.3.3.6.2 Defect in the pancreatic β-cells function

The relationship between PCOS, insulin resistance, androgen and beta cells dysfunction is closely related to the pathology of PCOS (191). For example, a study of 64 lean women with PCOS and 20 healthy women examined the insulin sensitivity using OGTT showed that women with PCOS demonstrated an increased HOMA-IR, insulinogenic index and beta-cell function, indicating insulin hypersecretion due to diminishing in insulin response (192). Similar results were also reported when 100 women with PCOS and 100 BMI and aged-matched controls were examined for insulin resistance which showed high indices for insulin resistance (193).

1.3.3.6.3 Defect in insulin clearance

A high insulin level can result from increased insulin secretion and the reduction of insulin elimination. Decreased insulin clearance, the main feature of insulin resistance, is receptor-mediated. Insulin resistance is characterised by the significant reduction in the number and the function of insulin receptors which justify hyperinsulinemia (194). However, it is not clear that women with PCOS have impaired insulin clearance. Therefore, more studies are needed.

1.3.3.7 Evaluation of insulin resistance in PCOS

The hyperinsulinaemic-euglycemic clamp technique, which is considered a gold standard and the frequently sampled intravenous glucose tolerance test (FSIGTT) are the most reliable methods to quantify insulin sensitivity (195). In the hyperinsulinaemic-glycaemic clamp, a continuous infusion of supra-physiological insulin is used to maintain insulin-dependent glucose disposal. Meanwhile, the plasma glucose level is maintained at a constant fasting level by administering 20% dextrose infusion. Then the plasma glucose concentration is measured at 5-minute intervals for 20-30 minutes. When a steady state is reached, the glucose infusion

rate equals the glucose utilised by the body tissues in response to hyperinsulinemia; this measures the whole-body insulin sensitivity (196).

The other most widely used method to evaluate insulin sensitivity is the frequently sampled intravenous glucose tolerance test (FSIGTT). With this procedure, a bolus of 300 mg/kg glucose is rapidly administered intravenously and followed by frequent measurements of the plasma glucose and insulin levels for the next 3 hours. The resulting changes in the glucose and insulin levels then fit for a non-linear modelling algorithm to produce indices of the changes in plasma glucose levels in response to the insulin levels (195). However, this technique does not provide a steady state of glucose level in response to insulin. Moreover, these techniques are expensive, labour intensive, time-consuming and not suited for population-based studies. Thus, several simpler measurements have been proposed to measure insulin sensitivity (197).

In contrast to the aforestated dynamic methods, steady-state measurements of insulin sensitivity are used. The most widely used is the homeostatic model assessment of insulin resistance (HOMA-IR), which is calculated as (fasting insulin level [pmol/L]X fasting glucose [mmol/L])/22.5. A value of 1.00 is considered normal HOMA-IR, and a higher value indicates a severe state of insulin resistance. A similar steady-state measurement, known as the quantitative insulin sensitivity check index (QUICKI), is calculated as 1/(Log fasting glucose) + (Log fasting insulin)(198, 199).

Even though there are several methods to assess insulin sensitivity, its reliability is based on many factors such as age, body weight, ethnicity and genetic variability, which could limit its generalisability. However, HOMA-IR is reliable and easy to use, and OGTT is the best method to measure glucose tolerance and insulin resistance in women with PCOS (195).

1.3.3.8 The relationship between insulin resistance and hyperandrogenaemia

The core aetiology and the primary endocrine characteristics for PCOS are insulin resistance and hyperandrogenaemia. Hyperandrogenaemia remains the main feature of PCOS, with around 70-80% of women with PCOS showing the clinical manifestation of hyperandrogenism (200). Insulin resistance and hyperandrogenaemia are interplaying with each other in the development and the occurrence of PCOS. In women with PCOS, approximately 60% of the androgens are produced by the ovaries, while the remaining 40% is contributed by the adrenal glands (201). Insulin synergistically with LH stimulates the theca cells to increase androgen synthesis.

Consequently, this will reduce the hepatic production of SHBG, which increases the amount of free testosterone in the body (202). Interestingly, hyperinsulinemia also stimulates the pituitary release of LH increasing the LH/FSH ratio, consequently increasing the androgen levels (203). The adrenal glands produce dehydroepiandrosterone (DHEA) which is converted to dehydroepiandrosterone sulphate (DHEAS) by the action of sulfotransferase 2A1 (SULT2A1), which is abundant in the adrenal gland (204). DHEA is the precursor for steroid hormone in humans and contributes to 97% of the circulating DHEAS. Therefore, high DHEAS is a marker for excess adrenal androgen production (202). Elevated free testosterone is a sensitive indicator for excess androgen, raised in around 60% of women with PCOS. Hyperandrogenaemia is commonly calculated by measuring the total testosterone and the SHBG to estimate the FAI. Therefore, both FAI and free testosterone are sensitive modalities to assess hyperandrogenaemia.

1.3.4 Impaired glucose regulation in PCOS

1.3.4.1 Prevalence of impaired glucose in PCOS

Women with PCOS are insulin resistant and centrally obese, so they are at increased risk of impaired glucose tolerance (IGT). Populations with insulin resistance are also at increased risk for T2DM and CVD (205-207). Generally, there is a rise in the global prevalence of T2DM, and it is projected to continue to rise in the next few years (2.8% in 2000 to 4.4% in 2030)(208). A study of 244 women with PCOS aged 14-44 years were prospectively evaluated for T2DM using the World Health Organisation (WHO) criteria. IGT prevalence was 31.1% and 7.5% for T2DM (209). Similarly, in a prospective study of 252 Turkish women with PCOS and 117 healthy controls, the prevalence of IGT was 14.3% versus 8.5%, respectively. There was also a high prevalence of T2DM among women with PCOS (2%) compared to none (0%) in the control group (210). Moreover, a study of 122 women with PCOS evaluated glucose tolerance using the WHO criteria reported that IGT prevalence was as high as 35% compared to only 10% with T2DM (211). Even amongst non-obese women with PCOS, 10.3% have IGT, and 1.5% have T2DM (209). A systematic review and meta-analysis of 35 studies assessing the prevalence of IGT and T2DM in women with PCOS reported a high prevalence of IGT and T2DM in both BMI and non-BMI-matched women with PCOS (212). In a study of 102 Chinese women with PCOS, the prevalence of IGT was 20.5% and 1.9% for T2DM (213).

1.3.4.2 Prevalence of PCOS in women with T2DM

There is also a high prevalence of PCOS among women diagnosed with T2DM. In a study of 149 women with PCOS and 166 controls (214). It found that between 15 to 35% of the diabetes cases were attributed to PCOS (214). A cohort study of 14,135 women with PCOS estimated the prevalence of T2DM in women diagnosed with PCOS as high as 26.5% (215).

1.3.4.3 The progression to T2DM in PCOS

Women diagnosed with PCOS can suffer from the rapid conversion to T2DM. When 67 women with PCOS were followed for an average of 6.2 years in a prospective study, nine per cent (9%) of the women who were normoglycemic at the baseline developed IGT, and 8% were progressed directly to diabetes. Moreover, over half (54%) of the women with IGT at baseline progressed to T2DM (216). In a follow-up study of 84 women with PCOS and 45 healthy controls were followed for an average of 2.6 years. Eleven per cent (11%) of the women with PCOS who had normal glucose tolerance at the baseline converted to IGT with an annual incidence rate of 4.5%.

Moreover, 33.3% of those women with IGT converted to T2DM, increasing annually at a rate of 10.4%. Conversely, in the healthy control, only 2.3% of women's normal glucose tolerance at the baseline progressed to IGT with a rate of 0.9% a year (217). In a study, when 71 women with PCOS and 23 healthy controls were followed for 2-3 years, there was a high conversion rate (16% per year) from normal glucose tolerance to IGT and 2% to T2DM among women with PCOS (218).

1.3.4.4 Assessment of impaired glucose regulation in PCOS

According to WHO diagnostic criteria for the diagnosis of DM and intermediate hyperglycaemia. Impaired fasting glucose (IFG) is diagnosed when the fasting plasma glucose (FPG) is between 6.1 to 6.9 mmol/L, impaired glucose tolerance (IGT) when a venous plasma glucose more than or equal to 7.8 mmol/L but less than 11.1 mmol/L 2-hours after a 75 g of glucose load. Diabetes is diagnosed when the FPG is more than or equal to 7 mmol/L or the random plasma glucose of more than or equal to 11.1 mmol/L. The term impaired glucose regulation is a combination of IGT, IFG and DM. Table 1.

	Fasting		
	Normal	Impaired fasting glucose (IFG)	Diabetes Mellitus
(mmol/L)	< 6.1	≥ 6.1 and < 7.0	≥ 7.0
(mg/dL)	< 110	≥ 110 and < 126	≥ 126
	2-hour glucose tolerance test		
	Normal	Impaired glucose tolerance (IGT)	Diabetes Mellitus
(mmol/L)	< 7.8	≥ 7.8 and < 11.1	≥ 11.1
(mg/ dL)	< 125	≥ 140 and < 200	≥ 200

Table 1: World Health Organisation criteria for glucose regulations

1.3.5 Metabolic syndrome in PCOS

Metabolic syndrome is defined as the coexistence of several known cardiovascular risk factors, including insulin resistance, hypertension, obesity and dyslipidaemia (219). Women with PCOS are at an increased risk of developing metabolic syndrome, which also shares several features with PCOS. The prevalence of metabolic syndrome in PCOS is approximately 43-50% (20, 220). A systematic review and meta-analysis of forty-six studies (8,946 patients) assessing the prevalence of metabolic syndrome in PCOS estimated the prevalence of the metabolic syndrome to be 30% (221). A multi-centre study of 394 women with PCOS evaluated the prevalence and predictors of metabolic syndrome in PCOS. The majority of the participants met the National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) diagnostic criteria for metabolic syndrome. Three quarters of the women (80%) had waist circumference of > 88 cm, 66% had HDL-C < 50 mg/dl, 32% had triglycerides > 150 mg/dl and 21% had blood pressure > 130/85 mmHg. Overall, 33.4% of the women had metabolic syndrome (222). Azziz et al., in a prospective study of 129 women with PCOS and 177 controls, were studied to determine the prevalence of PCOS. Metabolic syndrome was reported in 34.9% of women with PCOS versus only 6.8% in the controls. The prevalence was significantly increased when adjusted for age and BMI with 47.3% versus 4.3% in PCOS and

controls group, respectively. The most identified components for metabolic syndrome among women with PCOS were increased BMI in 72.3%, low HDL-C in 63.7%, and high triglycerides in 46.8%. Overall, obese women with PCOS had a higher metabolic syndrome prevalence than obese controls (56% versus 38%)(223).

Moreover, a retrospective study of 106 women with PCOS reported the prevalence of metabolic syndrome was 43%, two-fold higher than reported in age-matched women in the general population (224). In a cross-sectional study of 49 adolescent girls with PCOS and 165 controls, 37% of adolescent with PCOS had metabolic syndrome, whereas only 5% of the controls had metabolic syndrome. The prevalence was even higher in obese girls with PCOS (63%) and overweight girls with PCOS (11%), whereas none of the girls with PCOS and normal BMI had metabolic syndrome. Adolescent girls with PCOS were 4.5 times more likely to have metabolic syndrome than age-matched controls when adjusted for BMI (225). Metabolic syndrome is associated with an increased CVD risk irrespective of waist circumference and free testosterone (226). In addition, women with PCOS and metabolic syndrome are at increased risk of developing cardiometabolic risk factors compared with age-matched controls (227). Thus, it warranted investigation and regular monitoring throughout their reproductive life.

1.3.5.1 Obesity and central adiposity

Obesity and adiposity are defined as abnormal or excess fat accumulation with detrimental health effects. There is an increase in the global prevalence of obesity which is also recognised as one of the critical healthcare problems in today's world (228). According to the WHO, in 2016, around 2 billion adults (≥18 years) were overweight, of those 650 million were considered obese. If these trends continue, it is estimated that by the year 2025, around 2.7

billion adults will be overweight, and 1 billion will become obese. Body mass index (BMI) is a simple measure of body weight in kilogram to height in metre square (kg/m^2). According to the WHO, normal healthy weight in adults is defined as BMI 18.5-24.9 kg/m^2, 25-29.9 kg/m^2 as overweight, 30-39.9 kg/m^2 as obese and BMI > 40 kg/m^2 is considered severely obese (229). Even though BMI is a simple, non-invasive and reliable technique with very low errors, it has limitations. For instance, BMI may not be sensitive to quantify fatness in lean, athletic and older people. There is also high age and racial variabilities (230).

Another means to measure the anthropometric indices are waist circumference (WC). Using anthropometric tape, waist circumference is measured at the midpoint between the rib cage and the iliac crest. It is an indirect (non-invasive) method to measure visceral adiposity. WC < 94 cm (37 inches) for adult males and < 80 cm (31.5 inches) for adult females associated with low risk of metabolic derangement, and WC ranges 94-102cm (37-40 inches) in men and 80-88 cm (31.5 -34.6 inches) in women considered as moderate risk, while WC ≥ 102 cm in men and ≥ 88 cm in women signify high risk (231). The regional distribution of fat can also be quantified by the waist to hip ratio (WHR). WHR of ≥ 0.90 for men and ≥ 0.85 for women indicates increased visceral fat (232, 233).

1.3.5.1.1 Obesity and central adiposity in PCOS

Women with PCOS are at an increased likelihood of having obesity and central adiposity. These are driving causes of worsening PCOS symptoms and risk factors for CVD (234). Obesity is associated with impaired glucose tolerance and insulin resistance. Hyperinsulinemia increases the thecal responsiveness to insulin to secrete androgen. Conversely, hyperandrogenaemia increases visceral fat deposition and dyslipidaemia (235). Insulin regulates glucose disposal by its action on the skeletal muscles and the liver. This action has

been reduced significantly in women with PCOS. Hepatic insulin resistance is characterised by reducing the hepatic response to insulin, reducing the endogenous glucose release and increasing the post-absorptive glucose production. These collectively lead to glucose intolerance and synergistically obesity in PCOS (75). A cross-sectional study of 76 women with PCOS and anovulatory cycles and 59 women with normal ovaries and regular cycles were studied. Obesity significantly affects the endocrine variables by reducing SHBG and increasing FAI and DHEAS in women with PCOS (236).

1.3.5.1.2 Effect of hyperandrogenism on fat distribution

There is a critical sex difference in the body fat distribution among males and females. Men are usually characterised by the android type of body fat distribution (accumulation around the abdomen), whereas women display gynecoid obesity (gluteal-femoral deposition)(237). These differences are shown to explain the differing metabolic profile and cardiovascular risk factors in men and women (238). In women with PCOS, increased androgen levels are associated with changes in the pattern of fat distribution with an increased visceral fat deposition and low SHBG (239). This alteration in the regional fat distribution has detrimental metabolic effects in women with PCOS; hence increased visceral adiposity is considered a risk for developing metabolic syndrome (240).

On the other hand, the molecular mechanism of increased visceral adiposity induced by the exposure to excess androgen remains undetermined. However, few studies indicated that increased androgen could directly increase the proliferation of the visceral preadipocytes via activating apolipoprotein B mRNA editing enzyme catalytic subunit 3B (APOBEC3b), cyclin A2 (CCNA2) and protein regulator of cytokinesis (PRC1)(241). Androgen exposure also reduced the gene expression of PPARy, C/EBPα and C/EBPβ and inhibited the stimulatory effect of

Bone Morphogenic Protein 4(BMP4) induced commitment of the adipose stem cells (ASC) to preadipocytes (242). It also limits adipocyte early differentiation reducing the fat cell storage capacity (243). There was also a proposed vicious cycle between adipose tissue and androgen, which could aggravate each other, particularly after some of the steroidogenic enzymes were expressed in the adipose tissues (66). Androgens also affect lipolytic regulations. Studies showed that excess androgen downregulates the expression of hormone-sensitive lipase (HSL) and β2-adrenergic receptor, reducing the catecholamine-induced lipolysis, particularly in the subcutaneous adipose tissue (244).

1.3.5.2 Hypertension in PCOS

There is an increased prevalence of hypertension in women with PCOS. Obesity, a prominent feature in PCOS, is a risk factor for high blood pressure. In a study of 37 PCOS women and 20 healthy controls, obese women with PCOS had significantly higher blood pressure than controls (29% versus 3%, respectively). Furthermore, there was a significantly high frequency of nocturnal non-dipper pattern in overweight and obese women with PCOS compared with overweight and obese controls (62% versus 25%, respectively)(245). However, another study that evaluated office ambulatory blood pressure and ambulatory blood pressure in women with PCOS reported that hypertension was more common in obese subjects irrespective of hyperandrogenism (246). Androgens, particularly high testosterone in women with PCOS, play a significant role in high blood pressure (247). Studies have shown that androgen modulates the vascular endothelial growth factors (VEGF), 20-hydroxyeicosatetraenoic acid (20-HETE) and matrix metalloproteinase-9 (MMP), which contribute to the pathophysiology of PCOS induced hypertension (248, 249). Sex hormone receptors are widely expressed across the vascular endothelial system as they play a vital role in regulating blood pressure. Androgen and estrogen receptors mediate their action by direct genomic and non-genomic

signalling pathways. This contributes to steroid receptors mediated alteration of the renin-angiotensin-aldosterone system (RAAS); therefore, it leads to the development of hypertension (250). Androgen also activates the nuclear factor kappa-B (NF-kB) pathway, leading to endothelial dysfunction and hypertension (251).

From a large cross-sectional study of 2,615 subjects from the Nordic ethnicity, 793 normal-weight women (BMI< 25kg/m^2), of which 512 were PCOS women and 281 age and BMI-matched controls. Women with PCOS had higher blood pressure than controls (11.1% versus 1.8%, respectively). When adjusted for age, WC and cholesterol, there was a strong association between high blood pressure and these parameters. The study was concluded that normal-weight women with PCOS have a higher blood pressure than the average population (252). In addition, insulin resistance, another main pathological and clinical feature of PCOS, was reported as an independent risk factor for exaggerated morning blood pressure surge in women with PCOS (253). Another cross-sectional study of 34 obese and non-obese girls with PCOS and age-matched controls. Obese girls with PCOS had significantly higher 24-hour mean blood pressure, heart rate, diastolic and nocturnal dip blood pressure than obese controls (254).

1.3.5.3 Dyslipidaemia in PCOS

Dyslipidaemia is a common abnormality in PCOS. It is characterised by low levels of high-density lipoprotein cholesterol (HDL-C), high levels of low-density lipoprotein cholesterol (LDL-C) and triglycerides (TGs) (72). However, this pattern is more prevalent in obese than non-obese women with PCOS, indicative of the negative effect of insulin resistance and hyperandrogenism on lipid metabolism (255).

1.3.5.3.1 Prevalence of dyslipidaemia in PCOS

Dyslipidaemia is one of the most typical metabolic abnormalities in PCOS. According to the National Cholesterol Education Programme (NCEP) guidelines, around 70% of women with PCOS have abnormal serum lipid profiles (256). Genetics and environment can all play a role in dyslipidaemia, but ethnicity may also be a factor. A retrosepctive study of 507 Chinese women with PCOS and 1246 controls matched for age and BMI found that 24.7% of women with PCOS had dyslipidaemia. Women with PCOS and insulin resistance had a significantly higher prevalence of dyslipidemia than those without (39.9% versus 15.3%, respectively) (257). A cross-sectional Indian study of 120 women with PCOS reported a higher prevalence of dyslipidaemia (93.3%) in women with PCOS (258). Another cross-sectional study of 106 American and 108 Italian women with PCOS evaluated the differences in dyslipidaemia between the two groups. There was a significantly higher level of dyslipidaemia amongst the Americans compared with the Italian women (259). A study of 140 women with PCOS and 31 age and BMI-matched controls reported the prevalence of dyslipidaemia as high as 76.1% in women with PCOS compared with 32.2% in the controls (260). Few studies showed that hypocaloric diets significantly affect the lipid abnormalities associated with PCOS. A study of 59 overweight and obese women with PCOS reported that hypocaloric diets significantly reduced triglycerides and total testosterone levels (261). Another study of overweight and obese women with PCOS was randomised to a high protein diet or a low protein diet for 12 weeks, followed by four weeks of weight maintenance. Changing the dietary composition has significantly improved the lipid profiles (262).

1.3.5.3.2 Types of dyslipidaemia in PCOS

Several patterns of lipid abnormalities were reported in women with PCOS, including low HDL-C, high LDL-C, total cholesterol (TC), TGs, very-low-density lipoprotein (VLDL), apolipoprotein-

B and lipoprotein-A (263,264). The HDL-C is a small, dense and protein-rich lipoprotein that constitutes various classes of lipoproteins with several different subclasses. These subclasses are heterogeneous in their composition, shape, size and function due to the differing proportions of the lipid, protein and nomenclature of the subclasses (265). HDL-C has two main subclasses (HDL_2-C and HDL_3-C); the former is less dense and relatively lipid-rich and contains twice as much cholesterol than the relatively dense protein-rich and cholesterol-poor HDL_3-C. However, HDL_2-C is the most cardio-protective of all HDL-C subclasses and decreased level of this subclass is associated with an increased risk of heart disease (266, 267). HDL protein can also be divided into several major subgroups, including apolipoproteins, enzymes, acute-phase response protein, lipid transfer protein, complement component and proteinase inhibitors which are the key functions for HDL-C (268). Several studies found that women with PCOS have significantly lower HDL-C and HDL_2-C than age-matched controls. Furthermore, even lean women with PCOS have shown reduced HDL-C levels and increased TGs (269, 270). It also reported that in addition to the low levels of HDL-C, women with PCOS have an increased level of LDL-C and TC, which was substantially higher than controls (269).

LDL-C particles are the primary carrier of cholesterol in the human body, and they are the key players in its transfer and metabolism. Broadly, LDL-C is a member of lipoprotein family which also include HDL-C and Chylomicrons, it is synthesised in the liver and derived from the intermediate-density lipoprotein (IDL) and very-low-density lipoprotein (VLDL). However, similar to HDL-C, LDL-C also has heterogenous subclasses with differing sizes, density, shapes, metabolic characteristics and atherogenicity (271). Including large (LDL-I), intermediate (LDL II), small (LDL III) and very small (LDL IV) LDL-Cs (272, 273). Small and very small LDL particles are atherogenic and strongly associated with CVD risk (256). Women with PCOS have significantly high levels of the small dense and atherogenic LDL particles or decreased mean

LDL-C particles (274). In overweight women with PCOS, insulin levels were inversely correlated with LDL sizes and positively correlated with the small atherogenic LDL particles, which signifies hyperinsulinemia's atherogenic effect (275).

In a cross-sectional study of obese and non-obese women with PCOS and obese and non-obese healthy controls, there were significant differences in the lipid profiles. These differences were mainly demonstrated among obese women with PCOS and controls regardless of PCOS presence (276).

1.3.5.3.2.1 Changes in the LDL particles

LDL particles are heterogeneous in their density, size and composition. Because of their small sizes they deserve much attention due to their association with increased CVD risk. A case-control study has shown that LDL particles increase the risk for coronary heart disease and ischemic heart disease irrespective of LDL, HDL, smoking, BMI, cholesterol and triglycerides concentration (277, 278). A follow-up study of 3684 patients with T2DM who received selective coronary angiography was followed for 5-years to determine the association between coronary heart disease and the small dense LDL (sdLDL-C). There was a significant rise in the level of sdLDL-C in the group with coronary heart disease compared with the non-coronary heart disease group. Therefore, sdLDL-C may be a potential biomarker to predict coronary heart disease in patients with T2DM (279). Recently, many studies have reported that measuring sdLDL-C could identify the undetectable risk of CVD in diabetic and normoglycemic nondiabetic individuals (280, 281). Dejager et al. showed that women with PCOS had significantly high levels of sdLDL-C when 31 women with PCOS were compared with 27 age and BMI-matched controls (274).

Moreover, the SHBG was an independent predictor for sdLDL-C, and this correlation was persisted after adjusting for confounding variables. Such findings modulate the effects of hyperinsulinemia and hyperandrogenaemia on LDL-C in young women with PCOS. However, Pirwany et al. demonstrated that even though there was a high level of sdLDL-C, testosterone and low SHBG amongst women with PCOS, only hepatic lipase, hyperinsulinemia and triglyceride but not androgen levels were strong predictors for sdLDL-C in women with PCOS (282). By contrast, a case-control study of 64 women with PCOS and 64 age-match controls failed to establish any differences in the absolute levels of LDL-C, mean LDL diameter and the atherogenic sdLDL in women with PCOS and healthy controls or between non-hyperandrogenic and hyperandrogenic PCOS subgroups (283). However, it must be noted that the study has included only non-obese subjects, which could be a potential confounding factor. However, a European study of young women with PCOS and healthy controls found that women with PCOS have higher levels of insulin, triglycerides and lower HDL-C than controls. Moreover, women with PCOS had significantly high sdLDL-C (type III and IV) due to the reduction in LDL subclass I, rendering it the second most common lipid derangement after decreased HDL-C in women with PCOS (272).

1.3.5.3.2.2 Changes in the HDL composition

Even though low HDL-C is the most prevalent lipid abnormality in PCOS, little is known about its composition (284). It comprises many subclasses that differ in their metabolic behaviour, sizes and pathophysiological significance. The heterogeneity of its particles is primarily due to the changes in its metabolic activity. Thus, HDL particles consistently lose their properties and acquire novel biological activities. Its metabolism is composed of a complex interplay between enzymes that control its synthesis and catabolism. The synthesis of HDL particles starts in the liver and partly the intestine. When there is excessive cholesterol in the blood, it is picked up

by HDL from the non-hepatic tissue and transported to the liver via a process known as reverse cholesterol transport (285). The circulating HDL particles are contain phospholipid, cholesterol and proteins. Each HDL particle contains apoprotein A-1(Apo A-1) and peroxygenase 1 (PON1), and when secreted from the liver, HDL is lipid-poor and contain a small amount of phospholipid and sphingomyelin (286). HDL and Apo A-1 have been shown to have a variety of functions, including antioxidant, anti-inflammatory, antithrombotic and antiapoptotic functions that prevent atherosclerosis (287). In obese women with PCOS, there was HDL modification by the depletion of lipid relative to protein. This modification reduces the ratio of HDL cholesterol and HDL phospholipid to Apo A-1, and PCOS was a major predictor for lipid-depleted HDL particles (284). Women with PCOS were also shown to have higher TGs to HDL-C ratio and lower ApoA-1 and HDL-C (288). In PCOS, the enhanced activity of the hepatic lipase enzyme induced by high insulin and androgen levels was also predicted to be the main drive behind the lipid-depleted HDL particles (289, 290).

1.3.5.4 The pathophysiology of lipid disorder in PCOS

Among the integrated risk factors observed in PCOS, obesity, insulin resistance, and hyperandrogenaemia have significant effects on dyslipidaemia in women with PCOS. Figure 1-6.

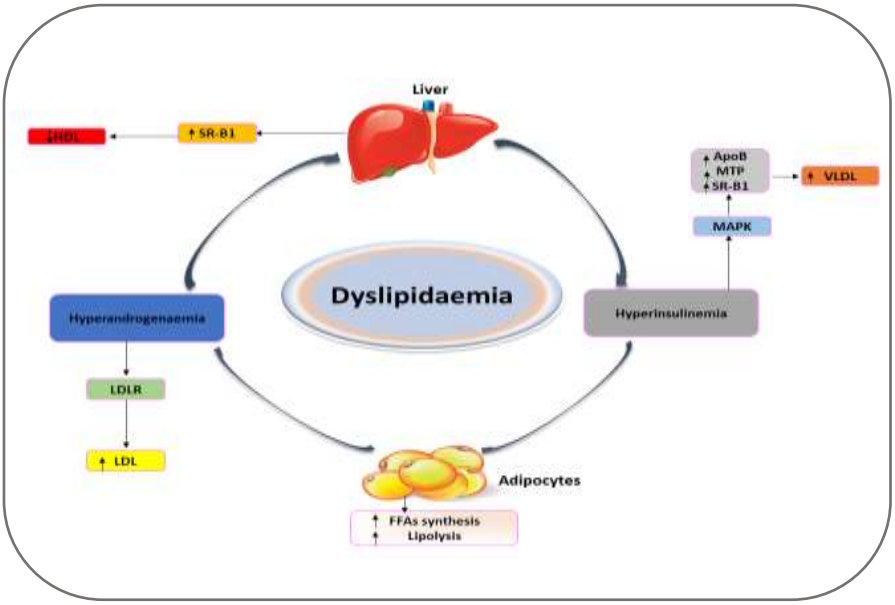


Figure 1-6: pathophysiology of dyslipidaemia in PCOS

1.3.5.4.1 Obesity

One of the main mechanisms underlying dyslipidaemia in obesity is the overproduction of VLDL particles and the lipoprotein lipase mediated lipolysis defect. Women with PCOS have an increased prevalence of central adiposity, which manifests as increased waist to hip ratio (WHR), rendering them more susceptible to dyslipidaemia. The hallmark of dyslipidaemia in obesity is the elevated TGs levels combined with high sdLDL and low HDL. Hypertriglyceridemia is the main driver for sdLDL and the delayed clearance of sdLDL (291). Elevated TGs also lead to elevated free fatty acid (FFA) in the liver and help produce VLDL, which competes with lipoprotein-lipase (LPL) and impairs the lipolysis of chylomicrons, increasing the delivery of TGs to the liver. Data from an in-vitro study identified a defect in the primary lipolysis in women with PCOS, especially protein-kinase A (PKA) hormone-sensitive lipase (HSL) complex activity (292). HDL metabolism is also strongly affected by obesity due to the increased chylomicrons and VLDL particles. This, increase the level of TGs-rich lipoprotein, which increases cholesterol esters-transferase-protein(CETP). CETP increases cholesterol ester exchange between the TGs, LDL, VLDL and HDL (293). Recently, a microarray gene expression analysis of adipose fat from omental adipose tissues of women with PCOS

identified the overexpression of PI3K receptor 1 (PI3KR1), a negative regulator of tyrosine kinase activity that affects the insulin signalling pathway and contributes to dyslipidaemia in PCOS (294).

1.3.5.4.2 Hyperandrogenism

The relationship between androgen and lipid metabolism are interconnected. One study reported that women with PCOS have increased intra-adipose tissue concentration of testosterone and DHT with increased expression of the androgen-activating enzyme Aldo-ketoreductase type 1 C3 (AKR1C3) (295). Similarly, in vitro study showed that insulin increases the expression and activity of AKR1C3 in the adipose tissues, whereas androgen enhances the de novo lipogenesis (296,297). Excessive androgen could also increase the proliferation of visceral preadipocytes by activating apolipoprotein B mRNA editing enzyme catalytic subunit 3B (APOBEC3b) and causes accumulation of lipid droplets (241). Androgen receptors are highly expressed in the adipose tissues of women with PCOS. Testosterone has been shown to induce androgen receptor-induced insulin resistance. However, this has occurred independently of PI3K activation. These findings demonstrate that androgen signalling via its receptors contributes to insulin resistance irrespective of PI3K activation (298). Testosterone has also been implicated to lower levels of HDL-C. The metabolism of HDL-C is a complex process involving enzymes, proteins and surface receptors. Testosterone can potentially target each level of this process. For instance, testosterone can upregulate two of the genes involved in the catabolism of HDL-C precisely, the scavenger receptor B1 (SR-B1) and the hepatic lipase. The SR-B1 mediates the selective uptake of HDL-C by the hepatocytes and steroidogenic cells and enhances the cholesterol transport from the peripheral tissues. Overexpression of SR-B1 is associated with reducing HDL-C levels and accelerating HDL-C clearance (299,300).

Hepatic lipase, an enzyme sensitive to androgen, can also reduce HDL-C. It catalyses the hydrolysis of phospholipid and on the surface of HDL resulting in conversion of HDL_2 to more lipid-denser HDL_3. HDL_3 is a good substrate for the liver, thereby increasing HDL clearance (301,302). However, a study conducted in women with PCOS failed to establish any correlation between hepatic lipase activity and testosterone (282). Androgens have also been found to reduce the catabolism of LDL-C, which is mediated by its receptors and mediate the estrogen receptor-mediated LDL receptor activity (303). Androgens also regulates the human lipoprotein lipase activity. In obese women with PCOS, there is a positive correlation between free testosterone and lipoprotein lipase and is negatively correlated with estradiol (304).

1.3.5.4.3 Insulin resistance

Insulin resistance is one of the main pathophysiological and clinical features of PCOS. Thus, women with PCOS are at increased risk of developing IFG and T2DM. Women with PCOS who have T2DM and IFG have a significantly higher risk (88%) of developing dyslipidaemia than women with PCOS and normal glucose tolerance (58%). Moreover, women with PCOS who have insulin resistance have a high risk of dyslipidaemia (81%) compared with normal insulin sensitivity women with PCOS (65%) (305). The hepatic production of apo-B containing VLDL seems to play a crucial role in linking excess TGs and insulin resistance. The insulin effect on microsomal triglyceride protein (MTP) expression favours the apo-B secretion and, subsequently, the VLDL production. It has been reported that insulin inhibits MTP, mediated by MAPK (306). In addition, women with PCOS have lower HDL-C, associated with insulin resistance; low HDL-C is a strong predictor for CVD (307). Moreover, in obese women with PCOS, there is significantly lower suppression of FFAs compared with controls indicating enhanced lipolysis. Conversely, lipolysis impairs insulin action on the adipose tissues (308).

1.3.5.4.4 Dyslipidaemia and oxidative stress and inflammation

Oxidative stress is characterised by an imbalance between the excessive production of the reactive oxygen species (ROS) such as superoxide anions, hydroxyl particles and hydrogen peroxide and the insufficient detoxification by antioxidants leading to the accumulation of ROS (309). HDL-C's impaired anti-inflammatory and antioxidant functions in women with PCOS leads to oxidative stress. Moreover, adipose tissues are the source of proinflammatory cytokines such as adipokines and can produce angiotensin II, which activate nicotinamide adenine nucleotide phosphate (NADPH)(310). NADPH is the primary pathway for ROS production in adipose tissues. Furthermore, the excess FFAs observed in women with PCOS stimulate NADPH and subsequently the production of ROS (311).

Adipokines secreted by the adipose tissues are associated with inflammation in women with PCOS, suggesting that inflammation is closely linked to dyslipidaemia (312). Several studies found that women with PCOS have a significantly higher c-reactive protein (CRP) levels and correlated positively with lipid levels (313). Furthermore, higher inflammatory markers, including interleukin-6 (IL-6) and tumour necrosing factor (TNF), also have been reported in women with PCOS (314, 315). These inflammatory markers are strongly associated with an increased risk of CVD and atherosclerosis (316).

1.3.5.5 C-reactive protein (CRP) in PCOS

C-reactive protein (CRP) is a protein produced by the liver in response to inflammation, and it is a sensitive inflammatory marker. Women with PCOS have significantly higher CRP levels than the average population, which is also a strong marker for CVD. A cross-sectional study of 116 women with PCOS and 94 BMI-matched controls compared CRP levels and CVD risk and reported that 36.8% of women with PCOS had higher CRP than 9.6% in the control group

(313). Furthermore, Verit et al., in a cross-sectional study of 52 normoinsulinemic PCOS women without metabolic syndrome and 48 controls found women with PCOS had a high level of CRP compared with control, and it was positively correlated with BMI, WHR, LDL and HDL (317). However, women have significantly higher CRP than controls regardless of body weight and BMI (318). Due to the significantly high level of CRP in women with PCOS, there is growing evidence hypothesising that CRP could be used as a biomarker for PCOS (319).

1.3.5.6 The risk of CVD in metabolic syndrome

Metabolic syndrome is associated with insulin resistance, obesity, endothelial dysfunction and dyslipidaemia, resulting in an increased risk of CVD and atherosclerosis (320). A cross-sectional study of 200 women with PCOS and 200 age-matched controls evaluated the cardiovascular risk profile. Women with PCOS had significantly higher risk factors, including higher BMI, WHR and hypertension compared with controls (321). Another study of 76 women with PCOS and 38-age matched controls showed that visceral adiposity index (VAI), HOMA-IR and insulin were significantly higher in women with PCOS than controls (322).

1.3.5.6.1 Surrogate CVD markers in PCOS

A meta-analysis of 130 data sets evaluated 7,174 and 5,076 CVD markers in 11 different outcomes in women with PCOS and controls. Women with PCOS showed significantly high levels of CRP, plasminogen activator inhibitor-1 (PAI-1), vascular endothelial growth factor (VEGF), advanced glycation end-products (AGEs) and endothelin-1 (ET-1), a marker for vascular activity compared with controls (323). Several studies evaluated the asymmetric dimethylarginine (ADMA), and nitric oxide (NO) in women with PCOS and controls found that ADMA was significantly higher in women with PCOS (324,325). Matrix metalloproteinase (MMPs) regulate important biological processes, including vascular remodelling and

angiogenesis and may be involved in the pathogenesis of CVD. Women with PCOS have significantly lower MMPs compared with controls (326). Intima-media thickness of the common carotid artery (CIMT) is a valid surrogate marker for atherosclerosis which is also reported to be significantly higher in women with PCOS (327). Recently, Endocan, a proteoglycan secreted by the vascular endothelium and associated with endothelial dysfunction has also been found to be increased in women with PCOS (328).

Moreover, Kallistatin, a secreted protein with anti-inflammatory, vasoactive and anti-oxidant properties, is increased and correlated positively with insulin resistance in women with PCOS (329). Copeptin, a surrogate marker for arginine vasopressin, is associated with an increased risk of CVD and is found to be high in women with PCOS (330). A study of 60 women with PCOS and 30 age-matched controls found that the serum level of copeptin was significantly higher in women with PCOS and positively correlated with BMI, WHR, Insulin and HOMA-IR compared with women without PCOS (331).

1.3.6 Risk of cancer in PCOS

Women with PCOS have a two-fold higher risk of developing endometrial cancer than the general population (332). The complexity of the relationship between PCOS and endometrial cancer has been recognised due to anovulation with prolonged exposure of the endometrium to the unopposed estrogen in the absence of sufficient progesterone (333). Endometrial responsiveness to the exogenous progesterone was also shown to be inherently lower in women with PCOS (334). Some women who received ovulation induction showed downregulation of progesterone response and increased cell proliferation which is the main driver for endometrial hyperplasia (335). Overexpression in the androgen receptors suggests a disordered androgen action in the endometrium (336). A high level of LH is a cardinal sign

in PCOS, and it also modulates the endometrial growth and subsequently endometrial hyperplasia (337). Most women with PCOS have insulin resistance, and the excess insulin enhances the theca cell androgen production by increasing free testosterone and reducing SHBG levels. It also amplifies the LH and IGF-1-mediated androgen production and enhances the activity of IGF-1, which accelerates endometrial growth (338).

Ovarian cancer is also increased by two to three-fold in women with PCOS. Of note, the risk was significantly higher in those not using oral contraceptives. This affirms the protective effects of oral contraceptives on endometrial and ovarian cancer (339).

1.4 Management of PCOS

Management strategies for PCOS include lifestyle modifications such as diet and physical activity and are the first-line treatment approach; however, they are reported to be minimally effective in reducing weight or treating PCOS related symptoms (340). Pharmacological options are also available; however, they are not explicitly approved for PCOS treatment as they have been primarily used to treat other conditions such as T2DM. The recent development of multiple new therapeutic agents for managing T2DM has broadened the options for patient-specific therapies in PCOS.

1.4.1 Lifestyle modification intervention

Over 50% of the women with PCOS are overweight or obese, with a higher propensity of central adiposity, increased body weight leads to impaired glucose tolerance and insulin sensitivity in this population (341). Increased visceral fat deposition is associated with increased severity of PCOS and plays a pivotal role in high serum androgen production and reduced serum levels of sex-hormone-binding globulin (SHBG) (342). Obese women with PCOS are more prone to an increased risk of metabolic abnormalities and severe

cardiovascular conditions compared to women without PCOS (343). Even though the aetiology of PCOS is not fully understood, PCOS has been strongly associated with obesity and insulin resistance (344). Given the links between obesity, insulin resistance and increased cardiometabolic risk factors, treating obesity is a priority, especially in obese women with PCOS.

In many cases, this can be achieved by modest weight loss (345), and lifestyle modification interventions, including dietary management and physical activity, are strongly recommended, particularly for those who are categorised as prediabetes, as this may delay the onset of T2DM (346). The recent international evidence-based guideline for the assessment and management of PCOS has emphasised the importance of physical activity and diet for managing PCOS-related symptoms and preventing its metabolic complications (347). Weight reduction can benefit obese women with PCOS through reduced adiposity, androgen levels, insulin levels, improved ovulatory function, increased fertility and a reduction in the overall risk of CVD (344, 345, 348). Given the strong link between obesity, insulin resistance and metabolic problems, a low glycaemic index diet would seem to be an attractive option for weight reduction; however, no one single diet has proven to be better than another though the trials to date have been small and of limited duration and heterogeneous in their design. A small study of women with PCOS assigned for a ketogenic, low-carbohydrate diet for six months reported significant improvement in their weight, hormonal profiles and fertility (349). Others have shown that a very modest reduction of carbohydrate intake from 55% to 41% of total energy reflected in favourable metabolic effects and a preferential decrease in fat mass among women with PCOS (350, 351).

A systematic review of relevant studies found that low carbohydrate diets improved the hormonal profile, reduced insulin levels and helped resume ovulation in women with PCOS (352). A survey of 14 women with PCOS administered a low ketogenic Mediterranean diet for 12 weeks reported a significant reduction in serum insulin, blood glucose level and average weight loss of 9.4 kg (353). Previous studies showed that lifestyle modifications could be associated with significant improvement in symptoms of PCOS. A study assessing the effect of isocaloric diets on insulin sensitivity and insulin levels has demonstrated that moderate carbohydrate intake reduced fasting and challenged insulin levels amongst women with PCOS (354). A recent meta-analysis assessed the effect of different dietary compositions on metabolic and reproductive outcomes in women with PCOS that showed there was more significant weight loss with monounsaturated fat, improved menstrual problems with a low glycaemic index diet and a more substantial reduction in insulin resistance (355). Physical activity usually acts as an adjunct to dietary intervention for PCOS management. A systematic review evaluating exercise intervention in PCOS reported that moderate-intensity regular aerobic exercise over a short period significantly improved menstrual regularity and ovulation and contributed to reduced weight and insulin resistance in young and obese women with PCOS (356). A recent systematic review and meta-analysis that examined the effects of lifestyle interventions, including exercise only or in combination with diet and behavioural therapy in women with PCOS, showed significant effects of exercise on the metabolic, anthropometric and cardiorespiratory outcomes (357).

1.4.1.1 Education

Disease-focused evidence-based education forms part of the broad spectrum of the management in many chronic conditions. It supports self-management to help patients living with long-term conditions such as diabetes and PCOS. Patients' education aims to improve

their knowledge, confidence and skills and enable them to take control of their condition and integrate effective self-management into their daily life. High-quality structured education can significantly improve the quality of life and the satisfaction of patients living with long-term conditions. Implementing and integrating a structured education has proven beneficial in managing diabetes and PCOS. Only one study by Mani et al. evaluated a structured education programme in women with PCOS. The study tested a single exposure to a group-based face-to-face structured education in 83 women with PCOS and 78 controls. The primary aim was to assess their physical activity level by evaluating the daily step count. The secondary aim was to assess the illness-perception, QoL and cardiovascular risk factors in women with PCOS for 12 months. Even though the study did not found any significant changes in the step-count, or biochemical and anthropometric outcomes the educational programme did improved the women's illness perception in two dimensions including understanding PCOS and sense of control. It also improved the QoL in three dimensions: emotion, fertility and general mental wellbeing (358). On the other hand, structured educational programmes have been tested and integrated into the management of diabetes. Several structured-education programmes have been running throughout the United Kingdom (UK). The most popular are Dose Adjustment For Normal Eating (DAFNE), which is for patients with type 1 diabetes mellitus (T1DM)(359), and Diabetes Education and Self-Management for Ongoing and Newly Diagnosed (DESMOND) for ongoing and newly diagnosed patients with T2DM (360). In 2002, a multidisciplinary team conducted an RCT in patients with T1DM and found significant improvement in the QoL sustained for up to 12 months and a fall in HbA1c of 1.0% in 6 months and 0.5% in 12 months. DAFNE's success has prompted its rollout to > 70 centres across the UK and Ireland. Four years later, the improvement in the QoL was maintained (359). Moreover, DESMOND is a multicentre-cluster RCT in 207 general practices and 13 primary

care sites in the UK; it followed 824 patients for three years after administering a structured group education programme in the community. The primary outcome was assessing HbA1c, and the secondary outcomes were blood pressure, body weight, lipid, physical activity, QoL, illness perception, depression, the emotional impact of diabetes and drug. As a result, there was a significant and sustained improvement in the illness perception at 12 months and at 3 years (360).

1.4.2 Pharmacological interventions

1.4.2.1 Insulin sensitising agents

The pathophysiology of PCOS includes defective insulin secretion and function (55). Hyperinsulinemia and insulin resistance contribute to the high level of androgens in PCOS (361). Insulin helps control ovarian function, and the ovaries are sensitive to high insulin levels (344). Theca cells produce high concentrations of androgens due to excess insulin, which contributes to the arrest of follicular maturation that predisposes to polycystic ovarian morphology, an indicator of PCOS (362). As well as its pivotal role in the pathology of PCOS, insulin resistance has a detrimental effect by predisposing PCOS patients to long-term health problems that include T2DM and CVD. Therefore, a therapeutic strategy to address insulin resistance, including pharmacotherapy and lifestyle intervention, is crucial in managing PCOS.

1.4.2.1.1 Metformin

Metformin is a member of the biguanide family with proven safety and efficacy. It is the single most studied insulin sensitiser in PCOS. Metformin has been used in the management of T2DM for a long time, and it is one of the insulin sensitising agents commonly used in the treatment of PCOS (363), though it is still an unlicensed indication in PCOS.

1.4.2.1.1.1 The mechanism of action of Metformin

1.4.2.1.1.1.1 Hepatic glucose production

Metformin inhibits hepatic glucose production by activating the AMP-activated protein kinase (AMPK), a major glucose and lipid homeostasis cell regulator. The activation of AMPK is associated with glucose inhibition in the hepatocytes (364). Metformin is transported to the hepatocytes mainly via organic transporter 1 (OCT1) and the mitochondrial respiratory-chain-complex 1(NADPH), resulting in the reduction of the adenosine triphosphate (ATP) and the increase in adenosine monophosphate (AMP)/ATP and adenosine diphosphate (ADP)/ATP ratios which subsequently activate AMPK (365,366). The reduction of ATP and the accumulation of AMP reduces gluconeogenesis by reducing key gluconeogenic enzymes such as fructose 1,6-bisphosphatase. In addition, high AMP inhibits adenylate cyclase, thus reducing cyclic AMP (cAMP) and inhibiting glycerol conversion to glucose (367).

1.4.2.1.1.1.2 Regulation of lipid metabolism

Metformin has also been shown to improve lipid metabolism by reducing hepatic steatosis (368). It was also reported that metformin exerts beneficial effects by reducing the circulating plasma TGs by selectively increasing the VLDL-TGs uptake and FFA oxidation in the adipose tissues (369). Metformin-induced lipid storage reduction is mediated by both increases in FFA oxidation and the inhibition of lipid synthesis via its activation of AMPK (369).

1.4.2.1.1.2 Metformin intolerance

Treatment with metformin is associated with significant gastrointestinal (GI) side effects in around 20-30% of the patients (370). The common GI side effects associated with metformin are nausea, vomiting, diarrhoea and abdominal bloating (371); however, the prevalence of

these symptoms is variable, and the severity of the side effects can be reduced by titrating the dose guided by the severity of the symptoms, or by using modified-release preparations.

1.4.2.1.1.3 The rationale for metformin use in women with PCOS

Women with PCOS are at an increased risk of having prediabetes or T2DM. Despite this clear association, obesity sometimes confounds the link between PCOS and T2DM. Thus, prevention of T2DM in this cohort is crucial, and there is reliable evidence for the use of metformin to reduce the risk of T2DM in high-risk women with PCOS. A study comparing metformin and lifestyle intervention in women with PCOS showed a significant reduction in body mass index (BMI) observed in both groups; however, reduction in androgen levels were only seen in the metformin group (372). In an RCT of obese and morbidly obese women with PCOS assessing the effect of metformin on body weight, a significant decrease in BMI independent of lifestyle modification was reported (373). In a study of 3,234 non-diabetic participants with elevated fasting plasma glucose randomised to either metformin or lifestyle intervention with a mean follow up nearly three years, lifestyle changes reduced the new incidence of T2DM by almost 60%. In contrast, metformin reduced it by just over 30 % (374); however, this effect was lost entirely following the washout period. This was further confirmed in a similar study where the impact of metformin no longer existed after 12 months of withdrawal (375). Women with PCOS are also at an increased risk of CVD due to hyperinsulinemia, high androgen levels, obesity and dyslipidaemia (376). There is evidence that obesity and PCOS independently affect vascular endothelial function (377); however, the association between high insulin levels and CVD is independent of obesity (378, 379). In addition, women with PCOS have worse lipid profiles compared to the healthy population (380), and they typically have low high-density lipoprotein (HDL) and high triglyceride levels that are both strong predictors of CVD (381). Thus, the management of dyslipidaemia is

crucial in PCOS. Metformin improves dyslipidaemia by directly affecting the hepatic metabolism of free fatty acids or indirectly by reducing hyperinsulinemia (382). Many studies have reported that metformin significantly impacts dyslipidaemia (383, 384); however, there was no beneficial effect on total cholesterol levels (385).

In women with PCOS, metformin is usually prescribed at starting doses of 500-850 mg daily and can be titrated up to 2000 mg/day if tolerated (386). Higher metformin doses have been beneficial in reducing weight and improving lipid profiles, particularly in the obese PCOS population (373). However, there is also evidence of the development of vitamin B12 deficiency with long term metformin use (387-390). Therefore, due to metformin intolerance and its associated adverse events, it is essential to consider other therapeutic options for treating metformin-intolerant women with PCOS.

1.4.2.1.2 Thiazolidinediones

Pioglitazone and rosiglitazone are thiazolidinediones that are peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonists. Pioglitazone mainly acts by increasing peripheral glucose uptake and regulating adipogenesis and insulin action. Its effect in improving insulin resistance, hyperandrogenaemia and ovulatory dysfunction has been seen in women with PCOS (391). In a randomised control trial (RCT) investigating the effect of pioglitazone versus placebo in PCOS, pioglitazone resulted in significant reductions in fasting serum insulin and the free androgen index, whilst SHBG levels were increased (392). A meta-analysis comparing the effect of metformin and pioglitazone in treating PCOS reported a significant improvement in ovulation and menstrual cycle in the pioglitazone group. However, there was a marked increase in BMI score in the pioglitazone group compared to metformin (393). A randomised open-label study which assessed the effect of pioglitazone, metformin

and orlistat on mean insulin resistance (IR), and its biological variability in women with PCOS reported a significant overall reduction in IR and IR variability (394). Despite the desirable effect of pioglitazone on the metabolic parameters in PCOS, there is considerable concern about the potential risk of myocardial damage, congestive heart failure and pulmonary oedema due to fluid retention (395). However, whilst the absolute risk is low in young women with PCOS, weight gain is a concern with thiazolidinediones in women with PCOS who are obese, and its use is an unlicensed indication.

1.4.2.1.3 Acarbose

Acarbose is an α-glycosidase inhibitor widely used to reduce postprandial glucose excursion. Acarbose also inhibits the pancreatic α-amylase located in the intestinal lumen and prevents disaccharides' and oligosaccharides' cleavage into simple monosaccharides (396). Thus, it delays glucose absorption, which also affects insulin secretion. Acarbose has been used successfully in managing IGT and new patients with T2DM by significantly reducing HbA1c and improving glycaemic control (397). In a randomised trial of women with PCOS treated with clomiphene citrate (CC), metformin and acarbose 100 mg/day for three months, compared with metformin, treatment with acarbose improved ovulation and reduced BMI (398).

1.4.2.1.4 Anti-androgen therapies

This class of medication is taken to reduce and counteract the effect of excess androgen. This is mediated by its action by blocking androgen receptors, reducing the adrenal androgen synthesis, reducing the ovarian androgen synthesis, reducing the pituitary production of prolactin and inhibiting the action of the 5-α-reductase enzyme.

1.4.2.1.5 Combined oral contraceptives (COC)

Combined oral contraceptives (COC), including estrogen and progestin, affect androgen synthesis and metabolism and regulate menstrual irregularities in women with PCOS (399). The key mechanism of action of COC is to inhibit ovarian androgen production due to blocking the pituitary gonadotropins secretion (FSH/LH). Thus, improving hirsutism, acne and menstrual irregularities (400). Furthermore, the estrogenic component of the COC increases androgen binding capacity by increasing the hepatic production of SHBG and subsequently reduces the level of freely available androgen (401).

1.4.2.1.5.1 Evidence for COC use in PCOS

1.4.2.1.5.1.1 Glucose tolerance and insulin sensitivity

Both lean and obese women with PCOS are at increased risk of hyperinsulinemia due to insulin resistance, impaired insulin clearance and action (402). Two studies of obese women with PCOS reported a significant increase in the glucose level after OGTT with COC containing Desogestrel (DSG) and cyproterone acetate (CPA). However, no significant changes were observed in the non-obese PCOS women (403,404). In addition, a randomised controlled trial of 17 non-obese PCOS women compared metformin with CPA/ethinylestradiol (EE) did not find any significant change in the insulin sensitivity (405). A similar non-significant effect of COC on insulin sensitivity was reported in an observational study of non-obese PCOS and healthy control women treated with CPA (406).

1.4.2.1.5.1.2 COC and lipid abnormalities

Dyslipidaemia is a common consequence of PCOS, which manifests as increased LDL-C, TGs and reduced HDL-C. A European study of 20 women with hyperandrogenaemia and 13 healthy, regularly menstruating women treated with EE 35μg/CPA 2 mg was shown to

increase total cholesterol, LDL-C, and TGs with no changes in the HDL-C (407). On the contrary, administration of the progesterone-only pill (medroxyprogesterone acetate (MPA)) in women with PCOS did not show any significant effect on lipid metabolism (408, 409). Two studies that used drospirenone (DRSP) containing COC (DRSP 3mg/EE 30 µg) reported alteration of lipid profiles in women with PCOS, including increased LDL-C, TGs, VLDL-C and total cholesterol (55, 410). In non-obese adolescents with PCOS who were assigned for 1-year treatment with COC containing EE 35µg/CPA 2mg or DSG 150 µg/ EE 30µg experienced a significant increase in total cholesterol and TGs levels (411). However, few studies reported an increase in HDL-C when COC was used in women with PCOS. This could be due to the anti-androgenic effect of COC (412,413).

1.4.2.1.5.1.3 COC and hyperandrogenaemia

A randomised controlled trial (RCT) of 100 healthy women seeking family planning randomised to receive either DSG 150 µg/ EE 30 µg or levonorgestrel (LNG) 150 µg/EE 30 µg for six months showed a significant decrease in the acne, hirsutism, free testosterone and increase in the SHBG (414). Another study of 15 hirsute women with PCOS who received DRSP 3mg/EE 30 µg for 12 cycles showed that hirsutism score was entirely improved from the third cycle with a drop in the FAI, SHBG, LH, and total testosterone. Moreover, 17-OHP and DHEA also reduced significantly, and both LDL-C and TGs were increased (410). However, there are high discrepancies on the effect of the COC on PCOS. A study that evaluated EE with chlormadinone (CMA) in women with PCOS for six cycles reported a significant improvement of hirsutism and an increase in SHBG. The FAI and 17-OHP were reduced with a significant increase in the VLDL-C and LDL-C (415). However, a meta-analysis of three RCTs that compared the effect of EE/DRSP with EE/CMA in women with PCOS showed a favourable effect of EE/DRSP over EE/CMA in reducing androstenedione (A4), hirsutism and total

testosterone (416). A meta-analysis on the effects of COC on the clinical and biochemical parameters of hyperandrogenism in PCOS showed that 3-12 months treatment with COC was significantly associated with an increase in SHBG, decrease in the hirsutism score, total testosterone, FAI, A4 and DHEAS. However, the most noticeable effect of COC on the hirsutism was in COC containing CPA (417). This was also confirmed in a systematic review and meta-analysis comparing the effects of COC containing progestin with low androgenic activity on the H-P-G axis in women with PCOS. There was a significant decrease in FSH and LH with CPA and DRSP containing COC at 3, 6 and 12 months with no statistically significant effect with COC containing DSG (418).

1.4.2.1.5.2 Spironolactone

A non-selective mineralocorticoid receptors antagonist, potassium-sparing medication with anti-androgenic properties (419). However, spironolactone is relatively weak and not purely an anti-androgen medication. Spironolactone exerts its anti-androgenic effects by decreasing testosterone biosynthesis through inhibiting 17 alpha-monooxygenase (17α-hydroxylase) activity, leading to the destruction of cytochrome P-450 (CYP) in the ovarian and adrenal tissues. Spironolactone also competitively inhibit 5-α-reductase and, therefore, reduce DHT at the target tissues (420). It also has an effect in reducing the 17-OHP by inhibiting the 17α-hydroxylase. It also influences the ratio of LH/FSH by reducing the response of LH to GnRH. Spironolactone is licensed to use as a diuretic to treat various medical conditions. It is used off-label to treat hirsutism and acne (421).

Spironolactone demonstrated superiority over placebo in reducing the facial hair's growth and diameter in hirsute women. However, due to its potential teratogenicity, it is not recommended as initial therapy for hirsutism unless combined with rigorous methods of

contraception in sexually active women and those not seeking pregnancy (422). Another common side effect of spironolactone is hyperkalaemia, particularly in patients with severe renal impairment. Therefore, it is contraindicated in the aldosterone-deficient condition such as Addison disease or patients with hyperkalaemia (423).

1.4.2.1.5.3 Eplerenone

Eplerenone is a selective-aldosterone antagonist and potassium-sparing diuretic similar to spironolactone but has less affinity for androgen and progesterone receptors hence fewer adverse effects (424). Instead, it binds to its mineralocorticoid receptors competitively antagonising aldosterone. On the other hand, eplerenone is not like spironolactone which is associated with dose-dependent side effects (420).

1.4.2.1.5.4 Flutamide

Flutamide is a non-steroidal anti-androgen therapy that competes with androgens at their receptors in the target tissues, and it is primarily used to treat prostate cancer. Moreover, it has been used off-label as a treatment for hirsutism. There is also evidence that flutamide restores the sensitivity of the GnRH pulse generator to inhibition by estradiol and progesterone (335). It was also demonstrated successfully to treat hirsutism in women with PCOS (425). However, chronic therapy with flutamide is associated with hepatotoxicity manifested as elevated serum aminotransferase, but most of the flutamide side effect is transient (426).

1.4.2.1.5.5 Finasteride

Finasteride is anti-androgen therapy and selective inhibitor to 5α-reductase and prevents testosterone conversion to its active form DHT in the target tissue (427). Finasteride was primarily indicated in managing benign prostatic hyperplasia (BPH). Type II 5α-reductase is

mainly found in male genitalia and hair follicles, while type I 5α-reductase is abundant in skin, hair follicles and sebaceous glands (428). Finasteride reduces DHT serum levels and the local scalp DHT in the hair follicles (429). However, finasteride does not directly bind to the androgen receptors like the other traditional anti-androgen therapy but reduces DHT and secondary reduces the androgen receptors expression via feedback mechanism (430). However, finasteride is potentially teratogenic, and thus, caution should be taken when it is considered in child-bearing women with PCOS (431).

1.4.2.1.5.6 Cyproterone acetate

Cyproterone acetate (CPA) is a 17-OHP acetate derivative with strong progesterone properties, and it acts as anti-androgen by competing with DHT and testosterone for binding with the androgen receptor. In combination with ethinylestradiol (EE), CPA has been proven effective in treating PCOS-related skin problems such as acne and hirsutism (432). CPA is also stored in the adipose tissue, which causes a marked deposition, particularly when a high dose is used. Its effect in treating hirsutism is evident; however, its effect on the androgen hormone varies (433).

1.4.2.2 Fertility treatment

1.4.2.2.1 Clomiphene Citrate (CC)

A selective estrogen receptor modulator is indicated to treat anovulatory or oligoovulatory infertility in women with PCOS who desire to conceive (434). It selectively binds to the estrogen receptors in the ovary, hypothalamus and endometrium, producing estrogenic and anti-estrogenic effects. In the hypothalamus, the decrease in the estrogen negative feedback triggers the normal compensatory mechanism. It stimulates the hypothalamic GnRH secretion, leads to increased gonadotropins release and subsequently stimulates the ovarian

follicular activity (435). According to the recent recommendation from the international evidence-based guideline for the assessment and the management of PCOS-2018, CC should be used to induce ovulation in anovulatory PCOS women with no other fertility factors or could be added to metformin, particularly in those who are obese (436). Standard practice is to administer CC for five days for each cycle from the second or the third day of the menstrual cycle with starting dose of 50 mg/day and increasing up to 250 mg/day if tolerated. However, the CC dosage of 100-150 mg/day is effective with over 75% successful ovulation (437). An RCT where 626 infertile women with PCOS were randomised to receive either CC, metformin or the combination of both for up to 6 months showed a high birth rate with CC alone (22.5%), and in combination with metformin (26.8%) than with metformin alone (7.2%). There was also a higher rate of multiple pregnancies with CC (6%) and with the combination (3%) compared to metformin alone (0%)(437).

1.4.2.2.1.1 Clomiphene resistance

Clomiphene resistance is defined as failure to ovulate after receiving CC 150 mg daily for five days per cycle for at least three cycles. It is common and found in approximately 15-40% of women with PCOS (114,438). Obesity, hyperandrogenism and insulin resistance commonly observed in women with PCOS are the main drivers for clomiphene resistance (438). Therefore, other alternatives such as aromatase inhibitors and gonadotropins may be plausible options for clomiphene-resistance women with PCOS. However, in a prospective study of 200 clomiphene resistant women with PCOS, who received CC 150 mg/day for ten days for three consecutive cycles and were followed for three months, there was a significantly increased rate of ovulation and pregnancy rate. It emphasised that extending CC treatment is an excellent method of improving ovulation and pregnancy rate in women with PCOS and clomiphene resistance (439).

1.4.2.2.2 Letrozole

Letrozole is a potent aromatase inhibitor that reversibly binds to the aromatase enzyme and inhibits the estragon synthesis. Aromatase is a member of the CYP 450 enzymes family found in many tissues such as adrenals, ovaries and mammary glands. The aromatase enzyme catalyses the aromatisation of androgen into estrogen. It also influences the formation of C18, a rate-limiting step in estrogen production (440). Letrozole is used to treat estrogen-sensitive breast cancer and endometriosis and induce ovulation in women with PCOS (441). When letrozole inhibits estrogen production it decreases the negative feedback to the hypothalamus, thereby increasing the GnRH production, which subsequently stimulates FSH secretion and follicular development (441). Letrozole has gained popularity among many clinicians and is considered first-line therapy for ovulation induction. A large multi-centre RCT, comparing letrozole treatment with CC found a significant increase in the ovulation rate (13%) and live birth rate (8%) with letrozole (442). In another retrospective study of 320 women with PCOS underwent ovulation induction with CC and letrozole, the ovulation rate was higher with letrozole (93%) than CC (83.8%). There was also a higher clinical pregnancy rate with letrozole compared with CC (52% versus 41.2%, respectively) and a higher live birth rate (44% versus 33%, respectively) (443).

1.4.2.2.3 Gonadotropins

Gonadotropins are the second-line therapy for ovulation induction in PCOS, including recombinant FSH (r FSH), urinary FSH (u FSH) or the Human Menopausal Gonadotropin (HMG). Its mechanism of action is based on the concept that increasing the FSH above its threshold for a long duration will initiate and maintain follicular development and maturation (444). However, due to their high cost and extensive monitoring requirement, they are only recommended during timed intercourse or intrauterine insemination (IUI). A small starting

dose is recommended to achieve monofollicular development and reduce complications. It is also mandatory to monitor follicular development using ultrasound. To prevent complications, low-dose step-up or step-down is recommended. In the low-dose step-up protocol, a low starting dose of 37.5 IU to 75 IU is used, followed by a small increment and regular monitoring. In the step-down protocol, a higher starting dose is used and then reduced to mimic the endogenous surge of FSH (445,446). A systematic review and meta-analysis of 14 RCTs evaluating the effects of gonadotropin in ovulation induction in women with PCOS found no significant differences in the live birth rate between uFSH, uFSH and HMG (447). However, when metformin was used in combination with rFSH, significantly higher live birth and pregnancy rates were reported (448).

1.4.2.2.4 The complication of ovulation induction in PCOS

1.4.2.2.4.1 Ovarian hyperstimulation syndrome (OHSS)

Ovarian hyperstimulation syndrome (OHSS) is a complication due to excessive ovarian stimulation. OHSS is characterised by enlarged cystic ovaries, which manifests clinically as abdominal distension, nausea and poor appetite. Women with PCOS undergoing assisted reproductive technology (ART) are at high risk of developing OHSS. In a retrospective study of 2,699 women with PCOS who underwent ART, 24.8% had OHSS, while 75.2% had a normal response to controlled ovarian stimulation (449). However, the incidence of OHSS is significantly lower with CC (1-6%) (109). A systematic review and meta-analysis of 42 RCTs evaluating the effectiveness of letrozole in women with PCOS showed no difference in the rate of OHSS between CC and letrozole (450).

1.4.2.2.4.2 Multiple pregnancies

A retrospective study of 100 pregnancies was identified in women with PCOS who conceived after ovulation induction using CC, CC+metformin and gonadotropin. The women who received gonadotropins had higher multiple gestations (36%) compared with either CC or CC+ metformin (11% versus 0%, respectively)(451). On the other hand, letrozole is associated with the probability of monofollicular development and thus low risk of multiple pregnancies. In an extensive systematic review and meta-analysis of 42 RCTs, treatment with CC showed a significantly higher multiple pregnancy rate than letrozole (1.7% versus 1.3%, respectively) (450).

1.4.2.3 Bromocriptine

Bromocriptine is a dopaminergic agonist that binds to the dopamine receptor and inhibits the pituitary secretion of prolactin. Additionally, bromocriptine also induces cyclical and physiological estragon release and hence why used to induce ovulation. Even though they are different entities, hyperprolactinemia and PCOS share the common symptoms of anovulation in women (452). In a study of 330 women with PCOS evaluated for prolactin, 63.4% had normal prolactin, and 37% had higher prolactin levels. Of the 37% with hyperprolactinemia, 27% were later diagnosed with pituitary adenoma (453). In an RCT of 74 women with PCOS randomised to receive either bromocriptine added to CC or CC alone for ovulation induction, treatment with bromocriptine added to CC showed no significant difference in ovulation compared with CC (454). However, the limitation of the study was that all the participants had normal prolactin levels. Thus, the role of bromocriptine in the management of PCOS remains controversial.

1.4.2.4 Dexamethasone

Dexamethasone is a synthetic glucocorticoid with high immunosuppressive and anti-inflammatory properties, with a 20-30 fold higher affinity to bind with glucocorticoid receptors than endogenous cortisol. In an RCT of 38 women with PCOS who were randomised to receive either low-dose dexamethasone or placebo for 26 weeks, compared with placebo, dexamethasone was associated with a 27% reduction in testosterone levels, A4 by 21%, DHEAS by 46% and FAI by 50% in women with PCOS (455). In another study of 129 women with PCOS who underwent IVF/ICSI, 43 women received dexamethasone, and 74 were received a placebo. After six months, there was a high pregnancy rate among women who received dexamethasone compared with placebo (17.5% versus 4.3%, respectively) (456). Furthermore, treatment with dexamethasone as an add-on to CC in clomiphene resistant women improved the overall pregnancy rate compared with CC alone (457).

1.4.2.5 Statins

Dyslipidaemia reflected in elevated LDL-C, triglycerides and reduced HDL-C is prevalent in women with PCOS, a strong predictor of cardiovascular risk (458). Therefore, effective treatment of PCOS would encompass improvement in the lipid profile and subsequently reduced cardiovascular morbidity.

There is growing evidence that statins are beneficial in treating PCOS (459). Statins (atorvastatin, fluvastatin, pravastatin, rosuvastatin and simvastatin) are an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a rate-controlling enzyme essential in the cholesterol biosynthesis pathway. Blocking this enzyme will stop the conversion of HMG-CoA to mevalonate and subsequently block cholesterol synthesis (460). In a randomised placebo-controlled study, atorvastatin significantly reduced insulin

resistance, inflammatory markers and hyperandrogenaemia in women with PCOS compared to placebo (461). When both the atorvastatin and placebo group were followed up with metformin for another 12 weeks, demonstrated significant improvements in HOMA-IR, FAI, total testosterone and sex hormone-binding globulin (SHBG) in the atorvastatin pre-treated group compared to placebo pre-treated group, suggesting that atorvastatin augments the effect of metformin in PCOS patients (462). In this study, when the effect of atorvastatin on the markers of inflammation and adipose tissue dysfunction were examined, 12 weeks of treatment with atorvastatin significantly reduced acylation stimulating protein (ASP), interleukin-6 (IL-6) and monocyte-chemoattractant protein-1 (MCP-1). Subsequently, there was a substantial improvement in insulin resistance (HOMA-IR) and testosterone levels (463).

In the same study, the effect of atorvastatin on pancreatic β-cell function (HOMA-β) was examined, which showed a significant increase in HOMA-β. However, this result was maintained by metformin treatment for another 12 weeks, indicating a potential improvement of insulin resistance and, therefore, a reduction in β-cell requirement rather than an actual fall in β-cell function (464). Treatment with atorvastatin also significantly reduced serum malondialdehyde (MDA), a marker of oxidative stress among obese women with PCOS (465). Furthermore, atorvastatin significantly reduced A4 and DHEAS in this cohort of women with PCOS (466). Twelve weeks of atorvastatin also significantly raised the concentration of serum vitamin D (25OHD) level among women with PCOS compared to placebo (462). However, until further robust data are available to clarify its efficacy, it should not be used in young women of reproductive age due to its potential teratogenicity.

1.4.2.6 Weight loss medications

1.4.2.6.1 Orlistat

Orlistat is a gastric and pancreatic lipase inhibitor that reduces the absorption of prandial dietary fat by minimising triglyceride hydrolysis (467). Orlistat is recognised as an obesity treatment with proven though low efficacy. A study evaluated and compared the effect of treatment with orlistat versus metformin on the biochemical and hormonal factors in women with PCOS. Treatment with orlistat showed a significant reduction in weight and androgen level compared to metformin (468). A randomised open-labelled parallel study compared the change in insulin resistance (IR) and its biological variability after treatment with orlistat, metformin and pioglitazone in obese patients with PCOS. Orlistat significantly reduced both IR and its biological variability compared to metformin and pioglitazone (394). In another study, women with PCOS were treated with orlistat compared to metformin and lifestyle intervention, and there was an improvement in lipid profiles, weight, BMI and waist circumference (469). Also, orlistat reduced androgen levels, insulin resistance parameters and total cholesterol (470,471). Orlistat also modestly reduces blood pressure and plays a role in preventing T2DM in this high-risk population, possibly by its effect on weight reduction (472). However, orlistat at the recommended dose of 120 mg up to 3 times a day was taken with food has significant side effects that include fatty stool, diarrhoea, abdominal pain and flatulence (473). It may also cause fat-soluble vitamin deficiencies (474). While orlistat might have desirable effects in the management of obesity, its relevance in controlling the metabolic aspect of PCOS remains controversial.

1.4.2.6.2 Historical weight loss medications

1.4.2.6.2.1 Sibutramine

Sibutramine is an appetite suppressant used as an adjunct to lifestyle intervention in treating obesity. It is a monoamine reuptake inhibitor that reduces the uptake of neurotransmitters such as serotonin, noradrenaline and dopamine (475). Therefore, it increases their availability in the synaptic clefts, which helps reduce appetite, enhance satiety, and reduces food intake (476). An RCT reported significant weight reduction after six months of treatment with sibutramine at a daily dose of 15 mg (7.8 ± 5.1 kg) compared to placebo (2.8±6.2 kg) in women with PCOS (477). In addition, another RCT reported an even more significant weight reduction (−15.4±1.1 kg versus –11.1±1.9 kg) with a lower daily dose of 10 mg (478). However, sibutramine has a significant cardiovascular risk through increased cardiovascular mortality, stroke and myocardial infarction (479). It has been withdrawn from the markets and therefore, using sibutramine for weight loss in women with PCOS with high cardiometabolic risk is questionable.

1.4.2.6.2.2 Rimonabant

Rimonabant is an anorectic, selective cannabinoid receptor 1 (CB1) blocker used for obesity treatment. A study assessed the impact of rimonabant on the markers of hepatic injury in obese women with PCOS without non-alcoholic fatty liver disease (NAFLD), rimonabant significantly reduced alanine aminotransferase (ALT) and weight (480). A trial that compared the effect of treatment with rimonabant and metformin on incretin hormones in obese women with PCOS showed a significant increase in a glucose-dependent insulinotropic polypeptide (GIP). After three months of rimonabant treatment, no change was reported with metformin (481). Moreover, treatment with rimonabant augmented the weight loss effect

and enhanced the metabolic benefit of metformin treatment in obese women with PCOS (482). However, rimonabant demonstrated a superior impact in weight reduction, improved insulin resistance, and reduced androgen levels than metformin in women with PCOS (483). Nevertheless, data from clinical trials showed that rimonabant caused severe psychiatric problems, including a depressive disorder, mood changes and suicidal ideation (484). As a result, rimonabant has also been withdrawn from the market because of their side effect profile.

1.4.2.6.2.3 Naltrexone/ Bupropion

Naltrexone is an opioid receptors antagonist with great affinity to the μ opiate receptor, which is implicated in eating behaviours. In experimental studies, naltrexone has shown an ability to block dopamine release and subsequently reduces food intake, food eating and binge eating behaviour. However, in human clinical studies, naltrexone as monotherapy has not produced consistent results. It has recently been approved by the US Food and Drug Administration (FDA) to manage alcohol and drug addiction (485). Bupropion is an anti-depressant approved to manage depression and seasonal affective disorder and help with smoking cessation. It acts by blocking dopamine reuptake. In clinical studies, its main side effect was weight loss (486). Although these agents were not principally approved for the management of obesity, clinical trials suggest that the combination of these agents induces significant weight loss. Therefore, the combination of Naltrexone/Bupropion (N/B), marketed as CONTRAVE pills or COR for short, has recently been approved for obesity treatment both in the US and Europe.

In two double-blind placebo-controlled clinical trials CONTRAVE Obesity Research (COR-I and COR-II) in overweight and obese patients, the combination of N/B demonstrated significant

weight loss (-8.1% and -8.2%, respectively) and showed an improvement in cardiometabolic parameters (-1.8% and -1.4%) compared to placebo (487,488). Furthermore, in the COR-BMOD (COR-Behavioural Modification) trial, patients treated with the combination of N/B in addition to an intensive behavioural modification programme or placebo showed significant weight loss with N/B+BMOD compared to placebo+BMOD (-11.5% versus -7.3%; P<0.01 respectively) (489). Furthermore, in the COR-diabetes study where overweight and obese patients with T2DM were randomised to N/B or placebo, treatment with the N/B combination showed a significant weight loss effect regardless of concomitant diabetes medications (-5.9% versus -2.2%; P<0.01 respectively) compared to placebo (490).

The combination of N/B has not been used for PCOS treatment, so there is no evidence of its efficacy in this cohort. However, a few studies have demonstrated the positive effects of naltrexone monotherapy in PCOS treatment. For example, an RCT of 30 clomiphene-resistance obese women with PCOS treated with naltrexone (50 mg/day) for six months showed significant reductions in BMI, fasting serum insulin and the LH/FSH ratio (491). It also showed a significant effect combined with pulsatile GnRH by improving ovarian responsiveness to ovulation induction in obese women with PCOS than pulsatile GnRH alone (492). Thus, the combination of naltrexone and bupropion may have a significant clinical effect on weight management for the metabolic aspect of PCOS.

1.4.2.7 New therapeutic agents

1.4.2.7.1 Incretin-based therapeutic agents

A significant increase in the plasma insulin level has been observed after an oral glucose administration compared to intravenous glucose infusion; this phenomenon is known as the “incretin effect”; this makes up to 80 % of the total insulin secretion after oral glucose

ingestion (493,494). Incretins are gut-secreted hormones, including glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1), that are both secreted in response to meal ingestion, and they enhance the glucose-stimulated insulin secretion (495). Incretin hormones also maintain glucose homeostasis by reducing the hepatic glucagon release, slowing the gastric emptying and suppressing appetite, thus helping control body weight and improving glycaemic control (494). However, most studies have found impaired incretin secretion and activity in overweight/obese individuals, and relatively small studies have reported reduced, normal, or increased GLP-1 levels in patients with PCOS, though the data have been inconsistent (496,497). Increased GIP and lower GLP-1 concentrations have been reported after an oral glucose tolerance test (OGTT) in women with PCOS (498). A reduction of GLP-1 was also reported in individuals with impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), early markers for prediabetes and the progression to T2DM (499). Therefore, initial incretin-based therapy was suggested to reverse the risk of prediabetes by preserving β-cell function in patients with IFG and IGT (500,501).

Endogenous GLP-1 has a relatively short half-life of 1-2 minutes, and it is quickly degraded by the proteolytic enzyme dipeptidyl peptidase-4 (DPP-4) more rapidly than GIP, which has a half-life of 5 minutes (502). The DPP-4 inhibitors are a class of oral anti-diabetes medications that improve glycaemic control by increasing endogenous physiological levels of both GLP-1 and GIP (503). GLP-1 receptor agonists (GLP-1 RAs) mimic the action of native GLP-1, achieving pharmacological levels, and are resistant to DPP-4 degradation. They have been shown to improve glycaemic parameters, and some agents, such as semaglutide (504), show remarkable weight reduction in overweight and obese patients with or without diabetes (505). Studies in animal models and clinical settings have demonstrated that both DPP-4

inhibitors and GLP-1 RA are effective therapeutic agents in managing PCOS and preventing its metabolic consequences (506).

1.4.2.7.1.1 The expression of GLP-1 receptors in the hypothalamic-pituitary-gonadal system

GLP-1 mRNAs receptors are densely expressed on the cerebral cortex, hippocampus, thalamus and hypothalamus (507). GLP-1 has the potential to regulate the GnRH release from the hypothalamic neurons. It modulates nitric-oxide and the endocannabinoid pathways, regulating the GABAergic current in the postsynaptic GnRH neurons (508). There is also increased expression of the GLP-1 receptors in the area overlapping the hypothalamus' arcuate nucleus occupied by proopiomelanocortin (POMC) neurons. Increased activity in POMC neurons reduces appetite, and its inhibition causes obesity. The data showed that GLP-1 could increase the electrical activity in the hypothalamic POMC neurons by upregulating PKA and increasing L-type Ca^{2}+, which explains GLP-1 action in suppressing appetite (509). In an experimental study, activation of GLP-1 R in the lateral hypothalamus of male rats reduced food reinforcement, food intake and ingestive behaviour (510).

Additionally, GLP-1 RA, including liraglutide, stimulates brown adipose tissues thermogenesis by activating AMPK in the ventromedial nucleus of the hypothalamus, which leads to weight reduction independently to food intake (511). In humans, changes in energy expenditure do not seem to contribute significantly to the weight-lowering effect of these drugs. Lower GLP-1 mRNA is expressed in the pituitary gland than hypothalamus. However, in the pituitary gland, GLP-1 increases the release of LH via its effect on releasing the gonadotropin-releasing hormone (GnRH) (512). Acute intracerebral injection of GLP-1 promoted an immediate increase in the preovulatory luteinising hormone (LH), which provoked a significant rise in the

level of estrogen and progesterone and the number of mature follicles (513). GLP-1 R is also expressed in ovaries, and its effects were observed in both preclinical and clinical studies (514). In obese women with PCOS, treatment with liraglutide resulted in a significant reduction of androstenedione, free testosterone and increased sex hormone-binding globulin (SHBG) (515). GLP-1 also significantly suppressed progesterone levels with no effect on estrogen synthesis (514).

1.4.2.7.1.2 The potential mechanisms by which GLP-1 RAs and DPP-4 inhibitors improve the metabolic parameters in PCOS

In addition to its glycaemic effect, there is considerable evidence that GLP-1 improves insulin sensitivity in peripheral tissues. An increase in GLP-1 concentration achieved by administering GLP-1 RAs or DPP-4 inhibitors can enhance insulin sensitivity and glucose uptake in animal and human muscle and the fatty tissues (516). Figure 1-7. However, this was not a primordial role for GLP-1; the primary targets are weight reduction and the central anorectic effects. Furthermore, not all reported studies found an improvement in insulin sensitivity in obese women with PCOS. It has also been proposed that GLP-1 facilitates glucose disposal in an insulin-independent fashion; however, this could be attributed to the overall reduction of glucagon secretion and changing the insulin/ glucagon ratio (517). There is evidence suggesting that GLP-1 possesses anti-inflammatory properties. In obese individuals, as the inflammation of the adipose tissue is the main driver for insulin resistance, treatment with GLP-1 analogues suppresses the inflammatory response by reducing macrophage secretion of inflammatory cytokines including interleukin-1β (IL1-β), interleukin-6 (IL-6) and tumour necrosis factor-β (TNF-β) (518). Therefore, by reducing the inflammatory response, GLP-1 facilitates insulin sensitivity.

Moreover, GLP-1 reduces the stress in the endoplasmic reticulum (ER) and improves insulin resistance in adipose tissues by modulating the protein kinase R-like endoplasmic reticulum (PERK) pathway by targeting the activating transcription factor 4 (ATF4) and CHOP (C/EBP homologous protein) expression (519). Furthermore, it increases the inhibitory effect of insulin on glucose and very-low-density lipoprotein (VLDL) and triglyceride release and facilitates glucose disposal (520). GLP-1 has a significant impact on eating behaviour, intestinal motility, appetite and gastric emptying. Figure 1-7. It also directly affects the feeding centre in the hypothalamus; many GLP-1 receptors exist in the hypothalamic nuclei (521). GLP-1 decreases gastric emptying and intestinal motility by reducing gastric smooth muscle activity; therefore, delaying glucose absorption and inhibiting postprandial glucose excursions (522). Additionally, GLP-1 has a significant effect in suppressing appetite and inducing satiety; thus, it decreases food intake and facilitates weight loss in humans and animals (521).

The GLP-1 receptors are also expressed in β-cells of the pancreas, where GLP-1 exerts multiple actions. GLP-1 stimulates insulin release via various molecular pathways, including the production of cyclic adenosine monophosphate (cAMP), activation of voltage-dependent Ca^{2+} channels, and Ca^{2+} influx with increased intracellular $Ca^{2+,}$ which stimulates insulin-containing secreting granules and facilitates its release into the bloodstream (494, 523). In addition to its insulinotropic effects, GLP-1 expands pancreatic β-cell mass by promoting β-cell growth, differentiation, and proliferation by activating the epidermal growth factor receptors, promoting phosphatidylinositol-3 kinase (PI3-K) to synthesise DNA (494,524). Furthermore, GLP-1 utilises its β-cell proliferative effect by downregulating PI3-K, protein kinase B (PKB/Akt), extracellular signal-related kinase (ERK), p38, protein kinase and mitogen-

activated protein kinase (MAPK) (525,526). Besides, it has also been reported that GLP-1 enhances β-cell survival by reducing apoptosis caused by various cytotoxic stimuli (494).

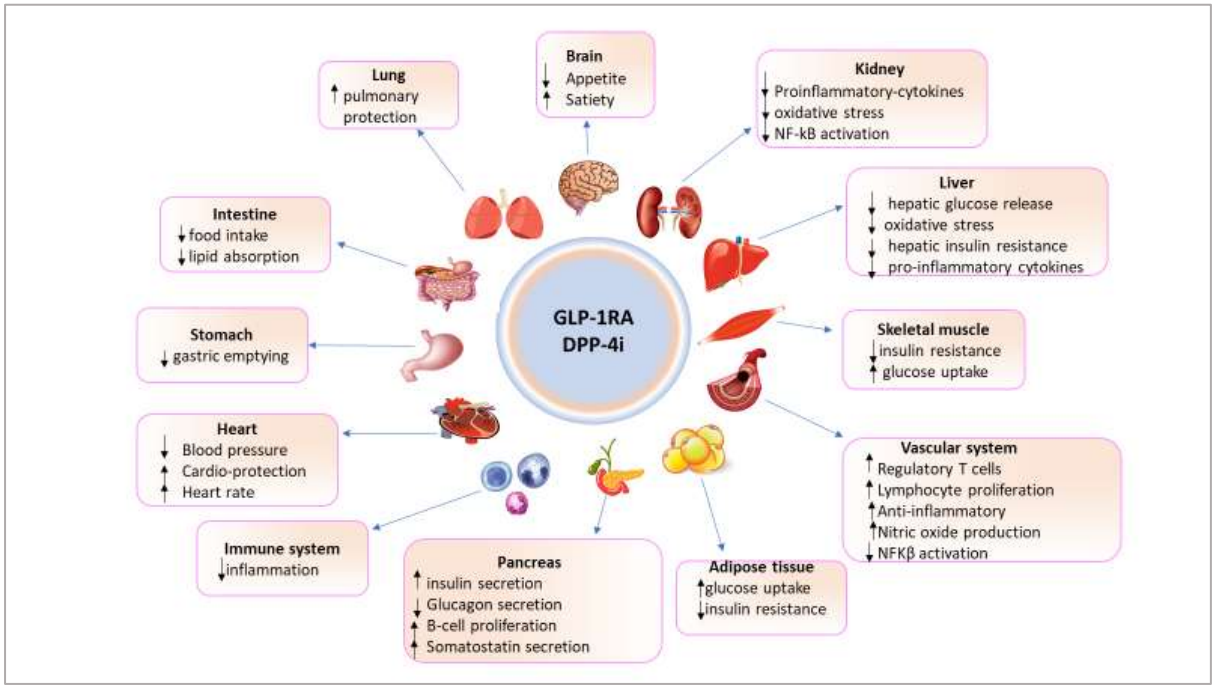


Figure 1-7: the potential mechanism of action of GLP-1 and DPP-4 inhibitors

1.4.2.7.1.3 Evidence for the therapeutic potentials of GLP-1RA in PCOS

1.4.2.7.1.3.1 Exenatide

1.4.2.7.1.3.1.1 Studies in animal models

Exenatide is one of the newest therapeutic agents for treating T2DM, and its use in PCOS has increased recently. In a rodent study, 50 female rats (25-day-old) were randomly allocated to a PCOS induced group (n= 37) or a control group (n= 13). The rats in the PCOS group were injected with dehydroepiandrosterone (DHEA) at a dose of 6 mg/100 g/ day and 0.2 ml of soybean oil to induce PCOS; meanwhile, 0.2 ml of soybean oil only was administered to the control group. The PCOS induced rats were then divided into three groups; PCOS-exenatide group were injected with 10 µg/ kg/day of exenatide, PCOS-metformin group that were given metformin 300 mg/kg/day, and a PCOS-normal saline group that was injected with 0.2 ml of

saline together with the control group for four weeks. In the exenatide treated group, the number and the size of endometrial glands were reduced due to an increase in the expression of AMP-activated protein kinase-α (AMPK-α) and the deacetylates enzyme (SIRT1)(527). Moreover, the high expression of AMPKα and SIRT1 improved the endocrine and reproductive profiles in PCOS induced rats treated with exenatide; for instance, there was a significant weight loss (from 222.64 ± 16.57 g to 218.63 ± 13.18 g) with exenatide group versus (238.30 g ± 12.26 g) in the metformin group (528). The homeostatic model assessment of insulin resistance (HOMA-IR) was also lower in the exenatide group (from 8.26±2.50 to 7.71±1.23) versus (12.66±1.44) in the metformin group. There was also a significant reduction in the level of serum testosterone (0.09 ± 0.03 ng/ml) versus (0.53 ± 0.41 ng/ml) in the exenatide and metformin group, respectively (528). The diminishing androgen effects of GLP-1 RA occur despite the ongoing daily injection of the DHEA.

In a randomised trial study of 45 female rats (3 weeks old) randomly allocated into DHEA induced group and a control group (529), the DHEA group was further divided into three groups: metformin treatment group (265 mg/kg), exenatide treatment group (10 µg/kg) and saline group (1ml) in addition to the control group for a total duration of 4 weeks. As a result, there was a comparable effect in weight reduction between metformin and exenatide. Furthermore, exenatide and metformin significantly reduced testosterone, LH and LH/FSH ratio and increased the level of SHBG (529).

1.4.2.7.1.3.2 Liraglutide

1.4.2.7.1.3.2.1 Studies in animal models

Liraglutide is a class of long-acting GLP-1 analogues with 97 % similarity to the human GLP-1. Compared to the endogenous GLP-1, liraglutide possesses an additional 16 carbon chain

which delays its absorption and slows its degradation by DPP-4, therefore, prolonging its half-life to over 13 hours (530, 531). The clinical effectiveness of liraglutide in the management of T2DM has led to its approval by the European Medicines Agency (EMA) in 2009 and by the Food and Drug Administration (FDA) in 2010 (532). Therefore, it increased the interest to consider liraglutide as a prospective therapeutic option for PCOS management (533). Besides its glycaemic reducing effect, liraglutide also has significant results in weight reduction, lowering blood pressure and lipid profiles. A study of 50-C57BL6 female mice aged 3-week old were randomly assigned into the DHEA group (n=40) and a vehicle group (n=10). The first group was injected with DHEA (6 mg/100g/ day) for 20 successive days, while the other group received sesame oil at a dose of 0.1 ml/100 g. At 32 days, the DHEA mice received a liraglutide injection at a dose of 0.2 mg/kg BID for 21 days, and the vehicle group received saline injections daily. After six weeks, liraglutide induced granulosa cell proliferation and promoted their viability in DHEA-induced PCOS mice by modifying the forkhead box protein O1 phosphorylation site (534). In another study of 20 Parkes strain mice, PCOS was induced using DHEA at a dose of 6mg/100g of body weight per day. The PCOS-induced mice received liraglutide either 100 or 200 μg/day twice a day for the 14 following days. Liraglutide enhanced adiponectin and IL-6 synthesis, reduced serum triglyceride levels, glucose, and testosterone. It also improved ovarian function and elevated the level of adiponectin by increasing the expression of Akt and PI3K (535).

1.4.2.7.1.3.3 Semaglutide

Semaglutide is another genetically engineered GLP-1 analogue with a longer half-life of 168-184 hours. It is used in managing T2DM either alone or combined with other anti-diabetes therapies. Recently, oral semaglutide has been approved for the treatment of T2DM (536). Most of the trials have been performed in patients with T2DM, where treatment with

semaglutide has shown significant improvements in glycaemic parameters, considerable weight reduction and lowering the cardiometabolic risk factors (536). Thus, semaglutide might potentially be the next therapeutic target in PCOS management; however, robust clinical trials are needed.

1.4.2.7.1.4 Evidence for the therapeutic potentials of DPP-4 inhibitors in PCOS

1.4.2.7.1.4.1 Sitagliptin

1.4.2.7.1.4.1.1 Studies in animal models

Sitagliptin was the first DPP-4 inhibitor used in clinical practice and the most studied in the class of DPP-4 inhibitors. It inhibits the action of DPP-4, the enzyme that inactivates incretin hormones, allowing endogenous GLP-1 to facilitate insulinotropic glucose-dependent postprandial insulin release. A study of spontaneously hypertensive obese strain (SHROB) rats with insulin resistance treated with the sulfonylurea glyburide (1 mg/kg per day) or sitagliptin (30 mg/kg per day) for 6-weeks was performed and compared with lean rats with hypertension. Sitagliptin enhances insulin secretion, normalises excess glucagon secretion, and lower plasma glucose (537). Sitagliptin was also administered in a dietary-induced obese mouse model using C57Bl/6J mice given a fat-rich- diet and treated for 12 weeks. Treatment with sitagliptin had significantly reduced body weight in fat-rich diet mice, inhibited the inflammation in adipose tissues and pancreatic islet cells, and lowered FBG and the serum insulin level (538).

Moreover, in streptozotocin-induced diabetic mice, long term sitagliptin for 2-3 months showed a significant rise in the number of insulin-positive β-cells in the pancreatic islets leading to the normalisation of the mass of the β-cell and an increased β-to-α-cell ratio (539). An experimental study of 6-weeks-old SD rats injected with insulin and HCG to establish

pathogenesis similar to PCOS. Then they were treated with a combination of dimethyl biguanide (DMBG, 300 mg/kg QD) and sitagliptin (10 mg/kg QD) for 12 days. As a result, co-treatment with TECOS and DMBG significantly decreased the levels of LH and estradiol and attenuated the IR via upregulating the expression of H19 (540). Another study of thirty rats (21-day-old) was randomised into the PCOS group, modelled by administering DHEA and a control group. The PCOS group was given sitagliptin (63mg/100 g) and 2 ml of distilled water for the control group. At 28 days, the treatment group showed a significant reduction in blood glucose and androgen levels and delayed the progression of ovarian fibrosis. This was suggested to reduce factors associated with the TGF-β1 and smad 2/3 signalling pathways (541). The TGF-β1 signalling has also been implicated in adipocyte pathology in women with PCOS. TGF-β signalling is crucial for adipocyte differentiation and implies the developmental origin of the visceral fat accumulation in PCOS (167,542).

1.4.2.7.1.5 Other DPP-4 inhibitors in PCOS

Alogliptin is a class of DPP-4 inhibitors approved for managing T2DM either as monotherapy or in combination with other anti-diabetes medications (543). In a 12-weeks randomised controlled study, 30 obese women with PCOS aged (34.4 ± 6.5 years) and BMI (39.0 ± 4.9 kg/m^2) were assigned to receive either alogliptin 25 mg QD or a combination of alogliptin 25 mg QD and pioglitazone 30 mg QD in addition to continuing metformin 1g/BID. Treatment with alogliptin-metformin alone or alogliptin-pioglitazone -metformin significantly reduced insulin resistance (HOMA-IR) and improved insulin sensitivity and the androgen index (544). In a 16-week randomised single-blinded study, 38 prediabetic women with PCOS were randomised to a combination of saxagliptin 5 mg/day and metformin 2000 mg/day or saxagliptin 5 mg or metformin 2000 mg as monotherapy. Treatment with saxagliptin +

metformin was superior to monotherapy, normalising glucose tolerance, the insulin sensitivity index, waist/height ratio and the free androgen index (545). Furthermore, saxagliptin + metformin was also effective in reducing weight, improving lipid profiles, and inhibiting the inflammatory response in women with PCOS newly diagnosed with T2DM (546).

A double-blind, randomised clinical trial of 105 women with PCOS was randomly randomised into three groups to receive pioglitazone 30 mg/QD (group 1), metformin 500 mg/ 3 times per day (group 2, control group), vildagliptin 50 mg/once a day (group 3) for six months was performed. In group 1, patients who received pioglitazone showed a significant reduction of BMI ($p<0.016$), Ferriman-Gallwey score (F-G scores) ($p<0.003$), DHEA ($p<0.001$) and improvement of menstrual irregularities ($p<0.035$). A similar result was found with metformin where BMI was reduced significantly ($p<0.010$), F-G score improved ($p<0.002$), free androgen level reduced ($p<0.034$) and menstrual irregularity improved ($p<0.004$). However, the vildagliptin group demonstrated significant reductions in BMI ($p<0.001$) and F-G score ($p< 0.046$) with no effect on the free androgen levels and menstrual irregularity (547).

1.4.2.7.2 Sodium-glucose co-transporter-2 (SGLT-2) inhibitors

Sodium-glucose co-transporter-2 (SGLT-2) inhibitors (e.g. empagliflozin and dapagliflozin) are a class of oral medications used to manage T2DM. Their mode of action is by inhibiting SGLT-2 in the kidney's proximal convoluted tubule (PCT), reducing glucose reabsorption and increasing urinary glucose excretion (548). As glucose is eliminated, its plasma levels drop and significantly improve glycaemic parameters (549). This mechanism of action is solely glucose-dependent, and unlike other agents, it is insulin-independent; therefore, the risk of hypoglycaemia is minimal (549). There is an emerging role of SGLT2 inhibitors for the treatment of obesity; their body weight effect is promising in addition to their protective

advantages for cardiovascular and renal events (550). In addition to their glucose-lowering effect, they can also improve insulin sensitivity via several molecular pathways, including reducing glucotoxicity and lipotoxicity, enhancing β-cells function, reducing oxidative damage and inflammatory processes, improve caloric deposition and weight loss (551).

Recently, treatment with SGLT2 inhibitors has shown promising results in trials involving patients with PCOS. In a 12-weeks randomised open-label study of empagliflozin versus metformin in obese women with PCOS, treatment with empagliflozin demonstrated significant anthropometric parameters and body composition improvement. However, no changes were observed in the metabolic parameters (561). This suggests that SGLT2 inhibitors could potentially help manage PCOS. Common adverse events reported for the SGLT2 inhibitors include genital infections, genitourinary tract infection, vulvovaginal candidiasis and vulvovaginitis (557).

1.4.2.7.3 Future treatment options

1.4.2.7.3.1 Dual GLP-1/GIP receptor agonist (Twincretins)

The term twincretins refers to a combination of a GLP-1 receptor agonist and a glucose-dependent insulinotropic polypeptide (GIP) receptor agonist, an example being Tirzepatide. Figure 1-8. It has shown a promising effect in reducing HbA1c and weight in T2DM patients. In a randomised, placebo-controlled, double-blind trial investigating the efficacy and tolerability of tirzepatide compared to a selective GLP-1 agonist (dulaglutide) in T2DM patients, tirzepatide reduced fasting blood glucose and had a greater significant weight reduction than dulaglutide, with tolerability that was comparable to the GLP-1 agonist (562). Furthermore, a recent randomised, placebo-controlled double-blind phase 2a trial compared the efficacy and the safety profile of a novel dual-action product (NNC0090-2746) in inadequately controlled patients with T2DM. Patients were randomised to 1.8 mg of NNC0090-2746 by subcutaneous injection daily or placebo as one arm. In addition, Liraglutide 1.8 mg subcutaneous daily injection with two weeks titration was given as an open-label arm. The results showed that NNC0090-2746 significantly improved glycaemic control (HbA1c) and reduced body weight compared to placebo (563). From the current data, it would seem that twincretins are promising new therapies to enhance the management of T2DM and weight control with potential utility for PCOS treatment.

1.4.2.7.3.2 Dual GLP-1/glucagon agonist

It is a recently developed GLP-1/glucagon co-agonist with enhanced metabolic efficacy as a therapeutic option for diabetes and obesity treatment. In animal models and non-human primates, GLP-1/glucagon agonist has shown a potency to induce glycaemic control and weight loss and reduce hepatic fat content (564). Furthermore, a novel GLP-1R/GCGR dual agonist used in DIO mice normalised glucose tolerance and improved adiposity and metabolic

parameters (565) suggesting that this combination could be potentially beneficial in patients with diabetes and possibly women with PCOS.

1.4.2.7.3.3 Triple GLP-1/GIP/glucagon agonist

The potential success of dual GLP-1/GIP and GLP-1/glucagon agonists has inspired the invention of a single combination of all three target receptors agonists. In an animal model, the tri-agonist had a significant weight lowering effect and higher than liraglutide (566). Moreover, it reduced plasma glucose and plasma cholesterol levels (567). HM15211 is a glucagon agonist with the ability to target all three receptors that showed a significantly higher weight loss effect than liraglutide, reduces hepatic fat mass and improves lipid profiles (568). Therefore, this could potentially be a therapeutic option in women with PCOS to improve metabolic risk if proven beneficial in clinical studies.

1.4.2.7.3.4 Glucagon receptor antagonist

Glucagon is a hormone produced by α-cells of the pancreas that potently regulates glucose homeostasis during fasting states by stimulating hepatic gluconeogenesis and glycogenolysis (569). High glucagon levels and increased glucagon to insulin ratio have been reported in patients with diabetes (570). Therefore, blocking glucagon receptors would reduce hepatic glucose production and improve glycaemic control. Glucagon has an opposing action to insulin; therefore, drugs targeting the inhibition of glucagon action are in development as potential therapies for T2DM, though their utility in PCOS is unclear.

1.4.2.7.3.5 Imeglimin

Imeglimin is a novel class of glucose-lowering agents developed to treat T2DM, though its mechanism of action remains elusive. However, experimental studies suggest that it acts by

blocking oxidative phosphorylation, which is a crucial step in hepatic gluconeogenesis (571). Additionally, it increases insulin secretion and improves muscle glucose uptake (572).

A recent study reported that imeglimin could improve insulin sensitivity through several molecular pathways, including insulin signalling transduction via activating Akt phosphorylation (573). In addition, imeglimin may also improve glucose homeostasis by improving β-cell function, suppressing gluconeogenesis, lowering insulin resistance, improving mitochondrial function and attenuating oxidative stress (574). This novel mechanism of action for imeglimin benefits patients with T2DM and potentially complements other oral antidiabetic therapies. However, clinical trials are needed to examine its efficacy and tolerability in women with PCOS.

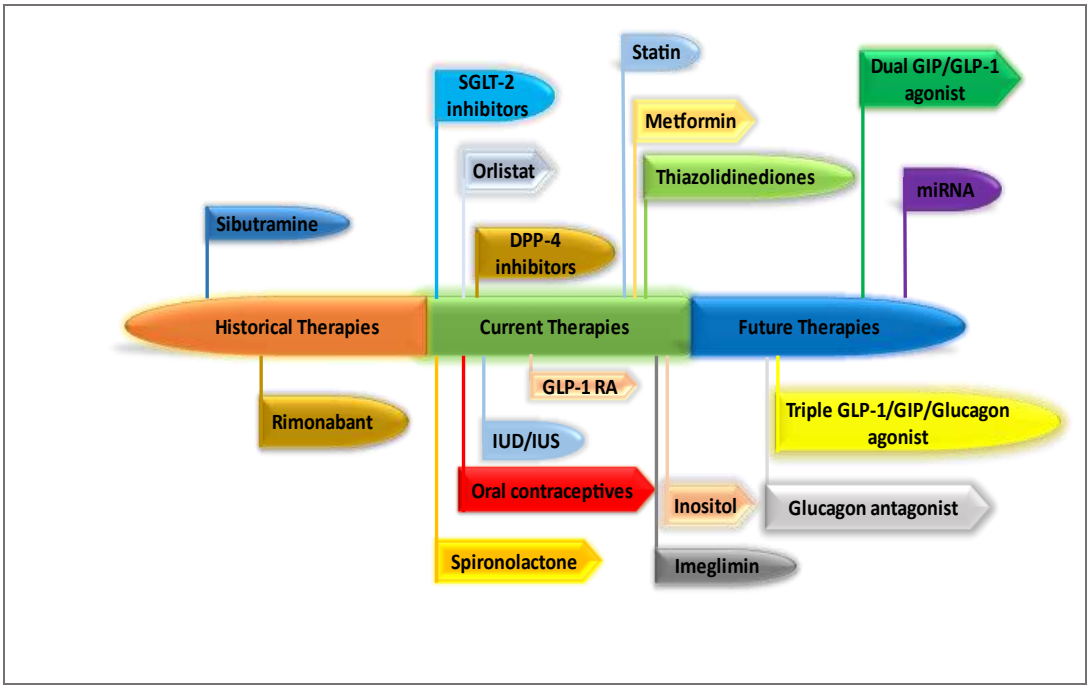


Figure 1-8: past, present and future therapeutic options for the management of PCOS

1.4.2.7.3.6 microRNA therapy

MicroRNAs (miRNAs) are a new class of endogenous, non-coding, single-stranded RNA molecules with 20-25 nucleotides that regulate post-transcriptional gene expression by

binding to the 3' untranslated location of the target messenger RNA (mRNA), thus, leading to the inhibition of mRNA expression and block post-transcriptional protein translation (575, 576). miRNAs are widely presented in the human body and can be isolated from urine, plasma, semen and saliva or encapsulated in microvesicles (577-580). They have also been expressed in different organs, including the liver, adipose tissue and muscle (581). A piece of accumulative evidence has shown that miRNAs regulate various critical regulatory biological functions, including cell growth and development, apoptosis, metabolism, stress response and hematopoietic differentiation (576, 582). A single miRNA has the potential to modulate the function and expression of various target genes, and amplification or inhibition of miRNA signal via the regulatory feedback mechanism may drive to a significant alteration of miRNA expression, which contributes to different diseases, including ovarian cancer, endometriosis, cardiovascular disease and inadequate ovarian response (583-585). There is also growing evidence demonstrating the influence of miRNAs in the pathogenesis of diabetes mellitus, and they could potentially be a novel biomarker for diabetes (586). There is also data showing differential expression of circulating miRNAs in women with and without PCOS (587).

1.5 Long-term monitoring in PCOS

Women with PCOS are at increased risk of developing IGT, T2DM and CVD compared with the general population. Accordingly, the Amsterdam ESHRE/ASRM-sponsored 3rd PCOS consensus workshop group (588) and the AE/PCOS society (89) recommended performing an OGTT as screening for IGT and T2DM in women with PCOS, particularly those who are obese (BMI> 30kg/m^2) or have increased visceral adiposity measured as waist circumference (WC), family history of GDM, acanthosis nigricans or hyperandrogenaemia (89,588). Moreover, due to the increased prevalence of abdominal obesity, it is recommended that BMI and WC be

performed at each visit and should be considered if the WC is > 80 cm (589). Regarding the CVD risk, women with PCOS who are obese, smokers, hypertensive, or with dyslipidaemia, IGT and family history of CVD are at risk of CVD. Moreover, those with T2DM, metabolic syndrome, and overt renal or vascular disease are at high risk of CVD. Thus, it is recommended that CVD risk assessment be performed at any age for blood pressure, dyslipidaemia (LDL-C, HDL-C, TG, and non-HDL-C), BMI, WC, glucose profiles and family history of CVD (589). Depression, anxiety and mood changes are expected with PCOS, and it is a recognisable risk factor for CVD. Therefore, it is suggested that women with PCOS be assessed for depression, anxiety and QoL (588).

1.6 Aims of the thesis

This thesis aimed to evaluate the impacts of the different pharmacological interventions and structured education on PCOS management. The following were the questions this thesis was determined to answer:-

1.6.1 Systematic review and meta-analysis

1) What is the impact of the various pharmacological interventions on insulin resistance in women with PCOS?
2) What is the impact of different pharmacological interventions on the lipid profile and the C-reactive protein in women with PCOS?
3) What is the impact of various pharmacological interventions on the anthropometric indices in women with PCOS?
4) What is the impact of various pharmacological interventions on the biochemical hyperandrogenaemia in women with PCOS?
5) What are the impacts of various pharmacological interventions on the fertility outcomes in women with PCOS?
6) What is the impact of metformin in the management of PCOS?
7) What is the impact of thiazolidinediones (TZDs) in PCOS management?

1.6.2 Living with PCOS- a structured education

1.6.2.1 The hypothesis of the study

The study hypothesised that developing and piloting an evidence-based structured education programme that can be run in groups will enable women with PCOS to make better lifestyle changes, which will help them improve their PCOS and reduce the risk of future PCOS-related complications.

1.6.2.2 Aims of the study

1.6.2.2.1 Aim 1

I) To develop an evidence-based structured education programme for women with PCOS. To do this, we have first surveyed women with PCOS to understand their perspectives and identify the need to develop an educational programme.

II) Based on the survey's outcome and the available literature, we developed an evidence-based educational programme written curriculum. Then the curriculum was peer-reviewed by healthcare professionals with experience in curriculum development and PCOS management and by women with PCOS attending our PCOS clinic or participating in our research activities. After the last draft of the curriculum was approved, written presentation material for the educational sessions and participants' handouts were developed.

III) We also develop educational material to train the educators to deliver the programme.

1.6.2.2.2 Aim 2

I) To pilot an evidence-based structured education programme for women with PCOS.

II) To evaluate the programme's impact on cognitive outcomes (i.e., health beliefs, awareness, and knowledge) related to PCOS.

III) To monitor and evaluate the delivery of the education sessions.

1.6.2.3 Endpoints of the study

I) To understand PCOS's aetiology, pathophysiology, prevalence, and diagnosis.

II) To understand the long-term consequences associated with PCOS.

III) To understand how lifestyle changes, including diet and physical activity, can help to improve PCOS symptoms and prevent long-term complications.

IV) To understand the concept of energy balance and how this can be used for weight management (i.e. diet and physical activity for weight loss and maintenance).

V) To know the pharmacological options for PCOS management (i.e. hormonal contraception, anti-androgens, and insulin sensitisers).

VI) Future planning (e.g. family planning, screening for long-term conditions).

2 Chapter 2: Overview of methods and materials

2.1 Study designs and protocols

2.1.1 Systematic review and meta-analysis

2.1.1.1 Register the protocol for systematic review and meta-analysis

The protocol of the systematic review and meta-analysis was developed and prospectively registered on the International Prospective Register of Systematic Reviews, PROSPERO (CRD42020178783), and was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement (590).

2.1.1.2 The eligibility criteria for the studies included in the systematic review and meta-analysis

Only randomised controlled trials (RCTs) defined based on PICO (population, intervention, comparator and outcome) were included in the review. Eligibility criteria are presented in Table 2. Briefly, only RCTs included women aged ≥ 18 years and diagnosed with PCOS based on a recognisable diagnostic criterion were eligible. RCTs that evaluated one pharmacological agent versus placebo or compared different pharmacological agents were eligible regardless of the design and methodology (open-labelled, double-blinded, parallel and crossover). RCTs reported anthropometric outcomes (body weight, BMI, WC and WHR), lipid profiles (LDL-C, HDL-C, total cholesterol, and TGs) and C-reactive protein were all included. Moreover, RCTs reported androgen hormones, including total testosterone, free testosterone, FAI, DHEAS, DHEA, A4, LH, FSH and prolactin. Studies reporting the effect of the different agents on the ovulation rate, pregnancy rate and live birth rates were included. The review also included RCTs that reported the effects of the various pharmacological agents on the FBG, FI, HOMA-IR and HOMA-β.

On the other hand, case-control studies, observational studies and animal studies were excluded. Also, studies that included the paediatric population (age ≤ 18 years), postmenopausal women and patients with other endocrine illnesses were excluded. Moreover, studies that evaluated non-pharmacological interventions, including diet and physical activity, and surgical treatment with other pharmacological interventions were also excluded. Finally, studies with a duration of fewer than two months were also excluded. Table 2.

Table 2: The inclusion criteria for the included studies in the systematic review and meta-analysis

Inclusion criteria
1. Study design: randomised controlled trials including (randomised open-label trials, double-blind controlled trials, crossover randomised trials, parallel randomised trials). 2. Patient population: adult females aged 18 and over with PCOS diagnosis based on a robust diagnostic criterion. 3. Comparators: reported pharmacological interventions compared to placebo or other pharmacological agents. 4. Outcomes: reported outcomes such as CRP, LDL-C, HDL-C, triglycerides, total cholesterol, body weight, BMI, WC, WHR, FBG, FI, HOMA-IR, HOMA-β, DHEAS, DHEA, FAI, TT, FT, A4, LH, FSH pregnancy rate, ovulation rate and live birth rate.
Exclusion criteria
1) Study design: case studies, observational studies and animal studies. 2) Patient population: adolescents females, postmenopausal women, and women without PCOS. 3) Comparators: non-pharmacological interventions, pharmacological interventions versus dietary interventions, pharmacological interventions versus physical activities or surgery. 4) Study duration < 2 months.

CRP: C-reactive protein, **PCOS**: polycystic ovary syndrome, **LDL-C**: low-density lipoprotein cholesterol, **HDL-C:** high-density lipoprotein cholesterol. **BMI:** body mass index, **WC**: waist circumference, **WHR:** waist to hip ratio, **FBG:** fasting blood glucose, **FI**: fasting insulin, **HOMA-IR:** homeostatic model assessment for insulin resistance**, HOMA-β:** homeostatic model assessment for β-cell function, **DHEAS:** dehydroepiandrosterone sulphate, **DHEA:** dehydroepiandrosterone. **A4:** androstenedione**, FAI:** free androgen index, **TT:** total testosterone, **FT:** free testosterone.

2.1.1.3 Literature search

A systematic literature search was conducted in six biomedical databases; PubMed, EMBASE, MEDLINE, Scopus, Cochrane Central Library and Web of Science in April 2020 and was updated in March 2021. Search terms were selected in close collaboration with a medical librarian specialising in systematic reviews. The search strategy was systematically developed in PubMed with the Medical Subject Headings (MeSH). All search terms were searched in a combination of title, abstract and MeSH to retrieve the best possible results. A filter for the English language was applied. All publication types and publication years were included in the search. The search strategy developed in PubMed was later repeated in all selected electronic databases and open access (Open Grey, ClinicalTrial.gov and Open thesis repository, EU clinical trial registry). The entire search strategy, including results, notes, and search technical specifications for all information sourced, is available in the appendix. All records found in the literature search were uploaded to Covidence (www.covidence.org) (591) for automatic de-duplication and blinded screening. Full-text review and data extraction was subsequently performed. Selected references were then uploaded to the software EndNote for reference management (592). The final reference list of the selected studies, and systematic reviews and meta-analyses located in the literature search, were also screened for additional undetected studies. Cabell's Predatory Report (593) was informed to verify the academic status of papers from open access journals included in the result.

2.1.1.4 Selection of the included studies

Titles and abstracts of the retrieved citations were screened and assessed for eligibility against the inclusion/exclusion criteria by two independent reviewers (Mohammed Abdalla & Najeeb Shah). The full-text assessment was undertaken and evaluated with the agreement of both reviewers. Any disagreements between reviewers about the inclusion were resolved

by consensus, discussion or consultation with a third reviewer (Thozhukat Sathyapalan). Non-pharmacological interventions and observational studies were excluded. Where duplicate publications for the same study on the same patients utilising the same intervention and measuring the same outcomes were identified, the most recent version of the study was selected.

2.1.1.5 Data extraction

From studies that were deemed eligible, two independent reviewers extracted relevant information. The information extracted covered the country of the trial, year of publications, design of the intervention, type of the RCT and comparators, number of participants, duration of the trials, baseline characteristics of the participants, and outcomes reported.

2.1.1.6 Risk of bias (RoB) assessment in the included studies

The Cochrane collaboration's tool for assessing the risk of bias (RoB) was used as recommended by Higgins et al.,(594). Six domains, including (selection bias, performance bias, detection bias, attrition bias, reporting bias and other biases) were assessed. Two independent reviewers assessed the RoB for each study, and a third reviewer mediated any conflict between reviewers. The recommendations from the Cochrane handbook (595) were followed, and any RoB was graded as either 'high RoB', 'low RoB', or 'unclear RoB'. The proportion of all studies regarded as either with 'high RoB', 'low RoB', or 'unclear RoB' for each specific RoB domain was also calculated and reported.

2.1.1.7 Grading the quality of evidence using GRADE

The robustness of evidence for each chosen outcome was examined following the recommendations from the Grading of Recommendations, Assessments, Development and Evaluation (GRADE) (596). In addition, the GRADEpro GDT software was consulted to value

the quality of the outcomes and generate a “Summary of findings table”. Initially, four points were given for each outcome. The points were then reduced in each outcome based on the presence of the following; the overall RoB for each RCT, inconsistency (significant heterogeneity), indirectness (significant differences in the population, comparisons, and outcomes), imprecision (the size of the cohort, width and significance of the confidence intervals (CIs)). Based on these factors, the overall GRADE scores were recorded for the outcome of each comparison as a high grade (at least 4 points), moderate grade (3 points), low grade (2 points) or very low-grade (1 point or less).

2.1.1.8 Assessment of heterogeneity

Heterogeneity for outcomes across the trials was assessed using the I-squared (I^2) test statistics. Heterogeneity was described as either not significant (I^2 0-40 %), moderate (I^2 30-60 %), substantial (I^2 50-90 %) or considerable (I^2 75-100 %) heterogeneity (595). For substantial heterogeneity, the source was investigated by removing the study that represented the largest weight from the analysis, and the I^2 was re-evaluated. If heterogeneity was still not resolved, subgroup analyses were performed.

2.1.1.9 Subgroup analysis

Subgroup analysis was performed at different levels according to the nature, dosages, frequencies of administration (one/day (QD), twice/ day (BID) and thrice/day (TDS)), and duration (weeks /months) of the pharmacological interventions.

2.1.1.10 Sensitivity analysis

The effect of each RCT on the heterogeneity and the strength of the result was reviewed by conducting a sensitivity analysis. Thus, small sample-sized RCTs and the one with an overall

high RoB were eliminated from the meta-analysis while inspecting their impacts on the collective results.

2.1.1.11 Assessment of publication bias

We have assessed for publication bias whenever more than 10 RCTs were included in any comparison. Furthermore, we used the funnel plot to examine any significant asymmetry that reflects the chance of publication bias.

2.1.1.12 Statistical analysis

The pooled effect estimate (mean difference (MD), standardised mean difference (SMD)), odds ratios (ORs) and its 95% confidence intervals (95% CIs) on the difference between the intervention and comparison group was quantified using the random-effects model and inverse variance *(595)*. The meta-analysis was performed if there were at least two effect estimates assuming that data for the reported continuous outcome variable are normally distributed. Extremely skewed data or data reported as range were excluded from the meta-analysis. Mean and standard deviation (SD) values for both post-intervention results and changes from baseline scores were combined for the meta-analysis. For data presented as standard error (SE), CIs, *p*-values and *t*-values, the RevMan calculator was used when necessary to convert them to means and standard deviations (SD). Mean difference (MD) was used when the same continuous data were presented using the same scales across the trials. Otherwise, SMD was used to pool estimates from trials using different scales to measure the outcomes. For trials with more than one intervention arm on the same outcome, data from all arms were combined using the method recommended in the *Cochrane Handbook's (595)*. Post-intervention scores and data from crossover trials were used from the last point the trials were reported. For missing data, the authors were contacted, asking them to provide the

missing information. The meta-analysis was performed using the Review Manager software (RevMan 5.4, The Cochrane collaboration).

2.1.2 Living with PCOS (LW-PCOS)-a structured education programme

2.1.2.1 Funding

The study was funded by the British Dietetic Association (BDA)-GTA grant.

2.1.2.2 Ethical approval

The study was sponsored by the research and development (R&D) department, Hull University Teaching Hospital NHS Trust. The LW-PCOS study was composed of two parts; a survey on the patient's perspective on developing an evidence-based educational programme for women with PCOS and developing and piloting the education programme. The pilot study was an evidence-based structured education programme conducted after receiving ethical approval. First, the study was approved by the London-Brent research ethics committee (REC reference: 20/PR/0840), then approved by the health research authority (HRA) and health and care research Wales (HCRW). Appendix. The study was also registered at ClinicalTrials.gov (ID: NCT04777461)- (IRAS project ID: 287175)).

2.1.2.3 Recruiting methods

Participants were identified from the electronic records and the PCOS clinic at the Centre for Academic Diabetes, Endocrinology and Metabolism, Hull University Teaching Hospitals NHS Trust. The participants were already consented to be contacted to participate in future research. After identifying the potential participants, they were directly invited through the post, where an invitation letter and patient information sheet were sent. Moreover, some participants were contacted via phone or face-to-face at the weekly PCOS clinic. We also publicly invited participants to participate in the study via social media platform (Verity social

media platform). After a detailed explanation of the study procedures, all participants consented. No participants who lacked mental capacity or were deemed vulnerable were recruited. Some of the participant's expenses, such as bus tickets and parking permits, were reimbursed.

2.1.2.4 Recruiting criteria

Women with a confirmed diagnosis of PCOS based on a robust diagnostic criterion (38, 597) were included if they were; aged 18-50 years, had BMI > 25 kg/m^2 and were willing and able to provide a signed informed consent before any study activity. Exclusion criteria were women < 18 years or > 50 years old, women who could not adequately understand verbal and written explanations given in English and those who lacked mental capacity.

2.1.2.5 Design of the study

The study was developed in three phases: I) A survey exploring the perspectives of women with PCOS on the development and testing of an education programme, II) curriculum development, and III) piloting the structured education programme (Figure 2-1).

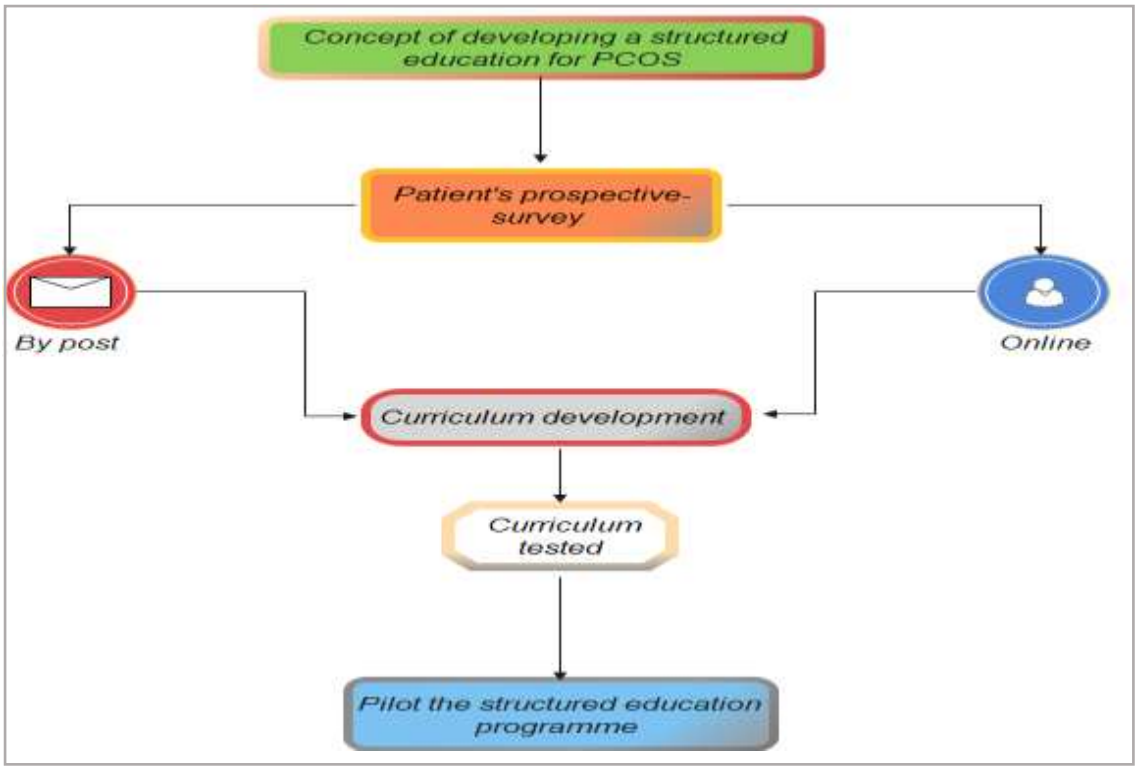


Figure 2-1: Conceptual diagram on the development of an educational programme for PCOS

2.1.2.5.1 Survey on patient's perspectives on the development of an educational programme for PCOS

2.1.2.5.1.1 Questionnaire development

A self-reported questionnaire was developed and used to capture qualitative data using close-ended and open-ended questions to establish a thematic overview of participants' experience living with PCOS. The closed-ended questions were designed to capture participants' responses in a 5-point Likert scale (1 and 2 = Not at all, 3= somewhat, and 4 and 5= very much). The questionnaire assessed the participants information and knowledge about PCOS, lifestyle modifications, PCOS symptoms, medications and long-term monitoring. The open-ended questions were used to establish common themes. Whereby the initial questions were about demographics. Appendix.

The questionnaire was then publicly uploaded online to create a link using an online survey tool (https://www.onlinesurveys.ac.uk). The survey link was then sent to participants via post or displayed at Verity's social media platform.

2.1.2.5.1.2 Curriculum development

Informed by the qualitative and quantitative outcomes of the survey and the gaps of knowledge identified from the participants, we reviewed the literature, and we developed a curriculum for an evidence-based educational programme. The programme covers a variety of sections, including PCOS definition, aetiology**,** pathophysiology, prevalence, and diagnosis. Moreover, the standard criteria used to diagnose PCOS, explain PCOS's health risks such as T2DM, cardiovascular problems, and infertility. The curriculum also included the current guidelines and recommendations for PCOS management, behavioural change strategies for effective weight loss and physical activity, healthy eating and physical activity and monitoring for PCOS-associated long-term complications. Healthcare professionals then reviewed the curriculum before developing the presenting materials.

2.1.2.5.1.3 Test curriculum

The initial draft of the presenting materials, developed as PowerPoint slides from the curriculum, was piloted in a cohort of participants (*n* =5). Those participants were employees at the centre for Academic Diabetes, Endocrinology and Metabolism, Hull University Teaching Hospital NHS Trust and not patients. They evaluated the time and the length of each section of the presentation. Their feedback was sought and implemented to produce the final draft of the presenting materials.

2.1.2.5.2 Piloting the structured education programme

The final programme consisted of one session of 3 hours of interactive PowerPoint presentation delivered face-to-face and/or online (self-directed study). Figure 2-2. At baseline, participants filled in self-administered questionnaires (pre-pilot evaluation form) after consent was obtained. In brief, the pre-pilot questionnaire captured participants' responses on a 5-point Likert scale (0 and 1= not at all, 3= much, and 4 and 5= very much). In addition, it measured their expectations from the session, how informative and engaging they expected the session would be, how much they know about PCOS, what is the causes of PCOS and how much they think it is running in families. Each education session was delivered by one trained educator, facilitator, and/or observer. We discussed PCOS symptoms, treatments, healthy eating, physical activity, weight-loss strategies, behavioural changes, long-term complications, and monitoring in the session. Each participant received a resource pack including abbreviations, a summary of each section and useful links. Participants were also asked to share their experiences and to ask questions at any point during the presentation.

At the end of the session, participants were asked to fill in another two questionnaires and reflect on the day. The first was a post-pilot questionnaire that captured participants' responses on a 5-point Likert scale (0 and 1 = Not at all, 3= much, and 4 and 5 = very much). It measured the feasibility of quickly accessing the education programme and their satisfaction with delivering the programme. They also had the opportunity to express themselves and ask questions. Moreover, they were also asked to reflect on the presented materials, the clarity of the information presented, information on the personal risk of developing long-term complications, knowledge about PCOS and its related symptoms, and

the recollection of the behavioural strategies of weight loss. The second questionnaire was the participant's knowledge, skills development, and illness perception evaluation form. In brief, the form had three parts and captured participants' responses on a 5-point Likert scale. It evaluated information on the knowledge gained about the anatomy and the physiology of the female reproductive system; symptoms and signs of PCOS; how PCOS could be diagnosed; PCOS management and PCOS-related complications. The skills development evaluated the improvement of goals setting skills, self-monitoring skills, self-efficacy skills, and overcoming barriers surrounding physical activity and dietary changes. Finally, the illness perception evaluated PCOS perception and measured the perceived knowledge on the cause of PCOS; timeline for the condition; personal control; management control and concerns.

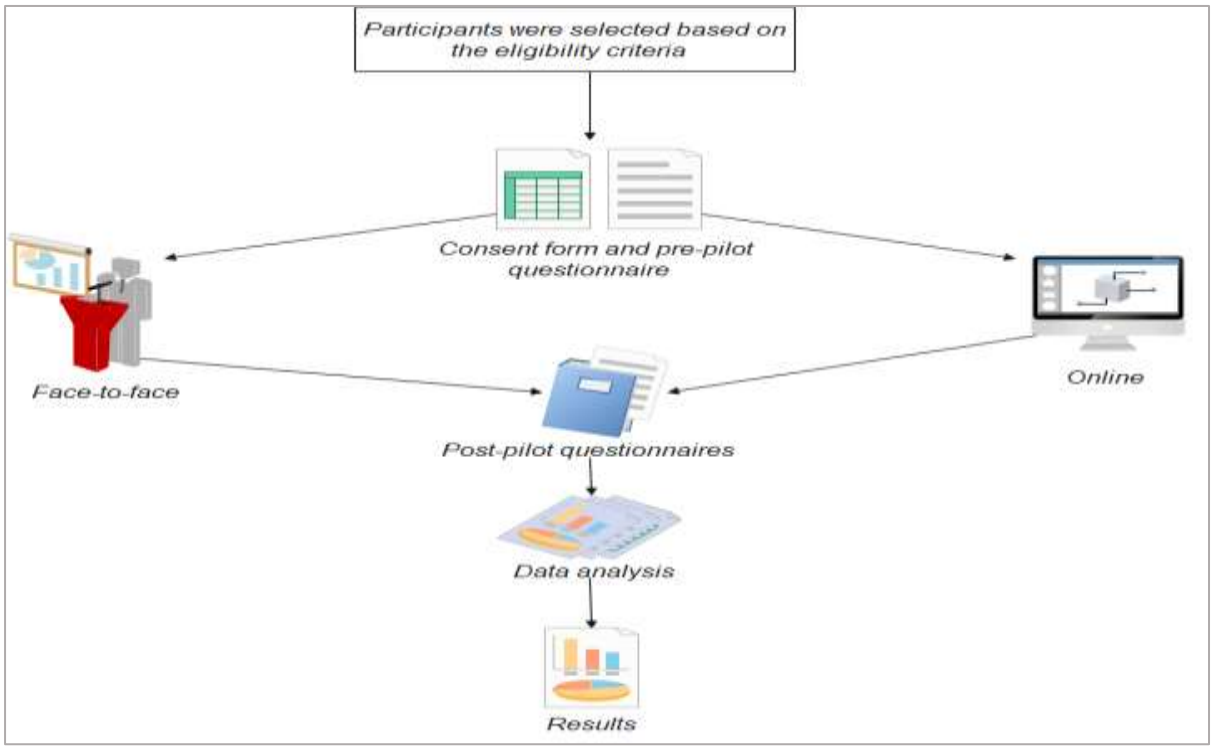


Figure 2-2: Schematic diagram for piloting the educational programme

2.1.2.6 Statistical analysis

2.1.2.6.1 Qualitative analysis

For qualitative data, thematic analysis employing the framework method was carried out using NVivo software (version 12)(598). Data were coded and mapped to establish significant patterns and common themes (599).

2.1.2.6.2 Quantitative analysis

Using Microsoft Excel, quantitative data were collected, analysed and presented as numbers and percentages *n* (%). The Fisher's Exact test of independence between two variables was used to calculate the difference between pre-and post-pilot results; p-value <0.05 was statistically significant.

3 Chapter 3: Effect of pharmacological interventions on lipid profiles and C-reactive protein in polycystic ovary syndrome: a systematic review and meta-analysis

3.1 Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous condition affecting up to 20% of women of reproductive age (55). PCOS is characterised by signs and symptoms of androgen excess and increased cardiovascular risk (506). The pathology behind this condition is unclear; however, it has been attributed to hormonal excess, environmental factors and increases in body weight (600). Lipid abnormalities including elevated triglycerides (TGs), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and decreased high-density lipoprotein cholesterol (HDL-C) are common in women with PCOS, with up to 70 % of women with PCOS having dyslipidaemia (307,380). Insulin resistance is also higher in obese women with PCOS, a feature of the metabolic syndrome associated with PCOS, and contributes to lipid disorders (601). Hyperandrogenism is a feature of PCOS that is also associated with an adverse metabolic risk by increasing intra-abdominal fat deposition, which promotes the metabolic dysfunction seen in the PCOS (602). Women with PCOS have significantly higher CRP which is an inflammatory marker and cardiovascular risk factor (313). Dyslipidaemia and high levels of CRP are associated with an increased risk of cardiovascular disease (CVD) (316,320). Moreover, anovulation has been associated with higher TC, TG, LDL-C and lower HDL-C in women with PCOS due to an increased release of the reactive oxygen species (ROS), which leads to ovarian damage and follicular atresia (603).

Lipid-lowering agents are occasionally used in PCOS for primary and secondary prevention of CVD. Besides lipid-lowering, these drugs can reduce oxidative stress and inflammation and

improve other metabolic parameters in PCOS (604). Statins can significantly reduce TC, TG, LDL-C and CRP in women with PCOS (605). Simvastatin or atorvastatin they have synergistic effects on the lipid profiles and can improve the menstrual cyclicity of women with PCOS (606). Therefore, this review aimed to evaluate and analyse the available evidence for the effectiveness of various therapeutic options for treating dyslipidaemia seen in PCOS.

3.2 Methods and materials

3.2.1 Protocol and registration

Explained in chapter 2, section 2.1.1.1.

3.2.2 Eligibility criteria for the included studies

The eligibility criteria, including inclusion/exclusion criteria, is explained in chapter 2, section 2.1.1.2.

3.2.3 Literature search

The search strategy, including the search in the medical databases, is explained in chapter 2, section 2.1.1.3.

3.2.4 Study selection

The study selection is explained in chapter 2, section 2.1.1.4.

3.2.5 Data extraction

The method used to extract information from the included studies is explained in chapter 2, section 2.1.1.5.

3.2.6 Risk of bias assessment in the included studies

The RoB assessment for the included studies is explained in chapter 2, section 2.1.1.6.

3.2.7 GRADE scoring

The robustness of evidence for each chosen outcome is assessed using the GRADE scoring system and is explained in chapter 2, section 2.1.1.7.

3.2.8 Statistical analysis

The statistical method used to estimate the pooled effects of the various interventions is explained in chapter 2, section 2.1.1.12.

3.2.9 Assessment of heterogeneity

Heterogeneity for the outcomes across each RCT was evaluated and explained in chapter 2, section 2.1.1.8.

3.2.10 Subgroup analysis

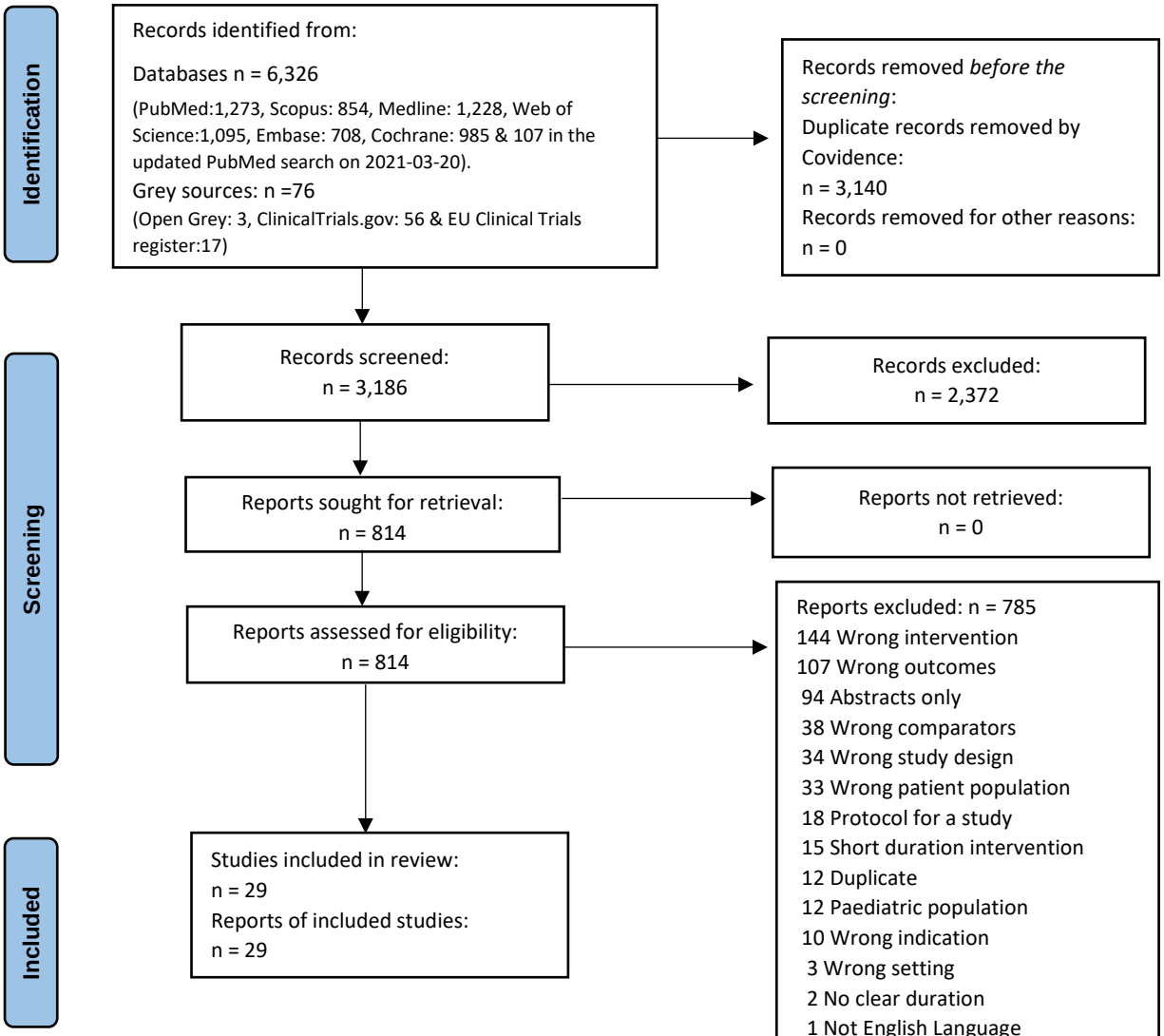
Subgroup analysis was conducted for the included RCTs and explained in chapter 2, section 2.1.1.9.

3.3 Results

3.3.1 Search results

Overall, 6,326 records were found in the electronic database, of which 3,186 records were initially scanned for eligibility criteria based on titles and abstracts after duplicates were removed. In total, 814 full-text articles were acquired to examine their eligibility, of which 29 RCTs met the eligibility criteria and were therefore included in the meta-analysis. Figure 3-1.

Figure 3-1: PRISMA flow diagram



3.3.2 Characteristics of the included studies

The 29 RCTs were published until 2020, of which fifteen RCTs (607-620) diagnosed PCOS based on the Rotterdam criteria-2003 (30), five RCTs (621-625) used the National Institute of Health 1990 (NIH, NICHD) criteria (626). In contrast, there were no diagnostic criteria for the remaining RCTs, table 3.

3.3.2.1 Interventions and comparisons details

Nine RCTs (31%) assessed the effect of metformin compared with placebo (607, 610, 616, 618,625,627-630). Five RCTs (17%) evaluated the effect of metformin compared with pioglitazone (614, 615, 620, 631, 632). Two RCTs (6.8%) examined the effect of pioglitazone compared with placebo (608,633). Two RCTs (6.8%) assessed the effect of rosiglitazone compared with metformin (612,622). Two RCTs (6.8%) evaluated the effect of liraglutide compared with liraglutide added to metformin (621, 623). Two RCTs (6.8%) examined the effect of exenatide compared with metformin (611,619). Two RCTs (6.8%) assessed saxagliptin compared with metformin (609,617). Two RCTs (6.8%) evaluated metformin compared with simvastatin (624,634). Three RCTs (10.3%) evaluated atorvastatin versus placebo (613, 635, 636).

3.3.2.2 Characteristics of the outcomes measured

All RCTs evaluated participants at baseline and post-intervention**.** Eleven RCTs (37.9%) reported changes in CRP (610-613, 616, 619, 622, 635, 636). Twenty-six RCTs (89.6%) reported changes in total cholesterol (607-615, 617-623, 625, 627-633, 635, 636). Twenty-seven RCTs (93.1%) reported changes in triglycerides (607-611, 613-615, 617-621, 623-625, 627-631, 633-636). Twenty-six RCTs (89.6%) reported changes in HDL (607, 609-615, 617-624, 627-630, 632,

634-636). Twenty-five RCTs (86.2%) reported changes in LDL (607, 609-615, 617, 619-623, 625, 627-632, 635, 636).

Table 3: Characteristics of the studies included in the systematic review and meta-analysis

Author	Study design	Country	POCS diagnostic Criteria	Participants characteristics (PCOS) mean ± SD	Interventions	Durations	Outcomes
Amiri et al (607)	RCT	Iran	Rotterdam	Age:25.6±4.02 BMI: 28.9±5	Metf, Flu, Metf+ Flu, Placebo	6 months	BMI, WHR, WC, FBG,LDL,HDL, TG
Akbari et al (635)	RCT	Iran	Rotterdam	Age: 27.7±3.4 BMI:26.6±3.6	Atorv, placeb	6 weeks	HDL, LDL, TG, TC
Brettenthaler et al (608)	RCT	Switzerland	Rotterdam	Age: 30.2± 1.4 BMI: 29.4± 1.7	Piog, placebo	3 months	TC, TG, BMI,WHR,FBG, FI, HOMA-IR
Elkind-Hirsch et al (609)	RCT	USA	NIH	Age: 29.9± 7 BMI: 39.9 ±1.5	Sax, Metf, Sax+Metf	16 weeks	FBG,FI, HDL,TG, LDL,HOMA-IR
Glintborg et al (637)	RCT	USA	N/A	Age: 32±0 BMI: N/A	Piog, placebo	16 weeks	TC, TG ,FI, HOMA-IR
Gambineri et al (627)	RCT	Italy	N/A	Age: 27·1 ± 3·6 BMI: 37·6 ± 4·1	Plac, Metfo, Flut, Metf + Flut	6 months	TC, TG,LDL, HDL
Puurunen et al (792)	RCT	Finland	N/A	Age: 40.5 ±5.9 BMI:> 19.9	Atorva, placebo	6 months	BMI, WHR,LDL, HDL
Heidari et al (610)	RCT	USA	Rotterdam	Age: 32.4±7.5 BMI: 37.1±9.1	Metf, placebo	3 months	CRP, TC, TG, LDL, HDL, FI
Jensterle et al (833)	RCT	Slovenia	NIH	Age: 27.6±7.2 BMI: 39.5±6.2	Metf, Rosi	6 months	BMI,WC, TC,TG,LDL,HDL ,FBG, FI
Jensterle Sever et al (779)	RCT	Slovenia	NIH	Age: 31.3±7.1 BMI: 37.1±4.6	Lira,Metf, Lira+Metf	12 weeks	FBG,BMI,WC,FI,TC,TG,HDL,LDL
Jensterle et al (622)	RCT	Slovenia	NIH	Age: 23.1±3.7 BMI: 39.5±6.2	Metf, Rosi	6 months	WC,BMI, FI,FBG,TC, TG,
Liu et al (767)	RCT	China	Rotterdam	Age: 27.69 ± 3.80 BMI: 28.29 ± 1.86	Metf, Exena	24 weeks	FI,FBG, HOMA-IR WC,BMI,TC, TG, WHR, LDL,HDL
Lord et al (784)	RCT	UK	N/A	Age: 27.76 ±4.89 BMI: 33.74± 6.74	Metf, placebo	3 months	WC,BMI, FI,FBG,TC, TG, WHR, LDL,HDL, HOMA-IR
Moghetti et al (625)	RCT	Italy	NICHD	Age: 23.9± 6 1.2 BMI: 27.1 ±6 1.5	Metformin, placebo	6 months	TC, TG, LDL
Mehrabian et al (835)	RCT	Iran	NIH	Age: 29.18±8.28 BMI: 29.83±4.1	Metf, Flut, Simva	6 months	WC,CRP,BMI,FBG,TG,HDL
Mohiyiddeen et al (768)	RCT	UK	Rotterdam	Age: 29.0 ±1.0 BMI: 29.7 ±1.0	Metf,Rosig	3 months	WC,BMI, FI,FBG,TC, TG, WHR, LDL,HDL
Naka et al (842)	RCT	Greece	N/A	Age: 23.3± 4.9 BMI: 28.7± 5.5	Metf,Piogl	6 months	WC,BMI, FI,FBG,TC, TG, WHR, LDL,HDL

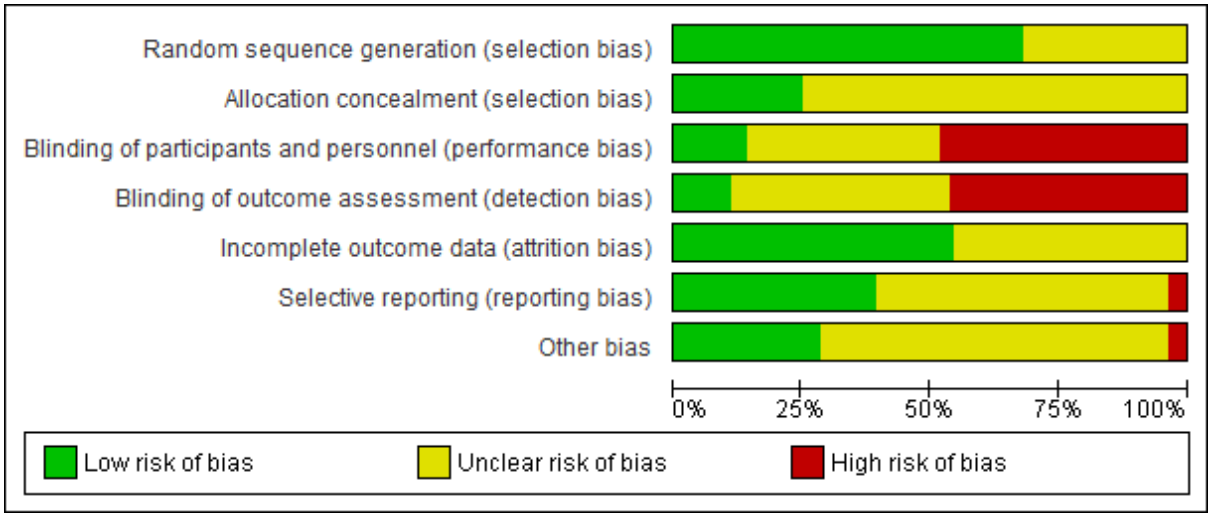
Navali et al (790)	RCT	Iran	N/A	Age:26.43±4.67 BMI:27.71±0.73	Metf, Simva	3 months	BMI, FI,FBG,TC, TG, WHR, LDL,HDL
Ng et al (785)	RCT	China	N/A	Age:30.5±0 BMI:N/A	Metf, placebo	3 months	BMI,FBG,FI,TC,TG
Ortega-González et al (788)	RCT	Mexico	N/A	Age: 28.8 ±0.9 BMI: 32.2 ±1.0	Metf, Piogl	6 months	TC, LDL, HDL, Wt, BMI,WHR ,FBG, FI
Sathyapalan et al (769)	RCT	UK	Rotterdam	Age: 27.7± 1.4 BMI: 33.20 ±1.4	Atorvas, placebo	12 weeks	HDL,LDL,TC, TG
Shahebrahimi et al (829)	RCT	Iran	Rotterdam	Age: 27.5 ± 3.68 BMI: 27.71±4.36	Metf, Piog	3 months	Wt, BMI,WC, FBG, LDL,HDL,TG
Sohrevardi et al (771)	RCT	Iran	Rotterdam	Age: N/A BMI: 27.5±3.6	Metf,Piog, Metf+Piog	3 months	TC, TG, LDL, HDL
Sova et al (772)	RCT	Finland	Rotterdam	Age: : 27.7 ±4.0 BMI: 27.5 ±6.2	Metf, placebo	3 months	CRP
Tao et al (773)	RCT	China	Rotterdam	Age: 30 ± 5 BMI: 27.2±0	Saxag, Metf	24 weeks	Wt, BMI,WC,WHR, LDL,HDL,TG, HOMA-IR
Trolle et al (786)	RCT	Denmark	N/A	Age: 31±0 BMI:32±0	Metf, placebo	6 months	Wt,WHR,FBG,FI,HOMA-IR, LDL,HDL
Underdal et al (774)	RCT	Denmark	Rotterdam	Age: 29.5 ±3.9 BMI: 28.7± 6.9	Metf, placebo	N/A	TC, TG, LDL, HDL
Zheng et al (775)	RCT	China	Rotterdam	Age: 27.70 ± 3.41 BMI: 28.27 ± 4.85	Exena, Metf	12 weeks	Wt, BMI ,WHR,FBG,FI,HDL,LDL, TG, TC
Ziaee et al (776)	RCT	Iran	Rotterdam	Age: 25.28±4.38 BMI: 26.13 ±3.03	Metf, Piog	12 weeks	BMI,HOMA-IR,HDL,LDL,TG

RCT: randomised clinical trial, **N/A**: not available, **HDL**: high-density lipoprotein**, LDL:** Low-density lipoprotein, **TG:** triglycerides, **TC**: total cholesterol, **NIH**: national institute for health, **NICHD**: national institute of child health and development. **Metf:** Metformin, **Saxa:** Saxagliptin, **Piog**: Pioglitazone, **Rosig**: Rosiglitazone,**Atrova:** Atorvastatin, **Simva**: Simvastatin, **WHO:** world health organisation, **CRP:** C-reactive protein,**Lira**: Liraglutide, **USA:** United states of America. **SD**: standard deviation.

3.3.3 Assessment of risk of bias in the included studies

The RoB item for each included RCT is presented in Figure 3-2. Briefly, fifteen RCTs (51.72%) were judged to have a high risk of performance bias due to lack of blinding the participants (607, 609-612, 615, 617, 619-621, 623, 631, 632). One RCT (3.4%) was judged to have a high risk of selective reporting bias (634). Low risk of bias was judged for the majority of domains among the included RCTs, and an unclear RoB was also judged due to insufficient reporting.

Figure 3-2: Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.



3.3.4 Effects of interventions on the lipid profiles outcomes and CRP

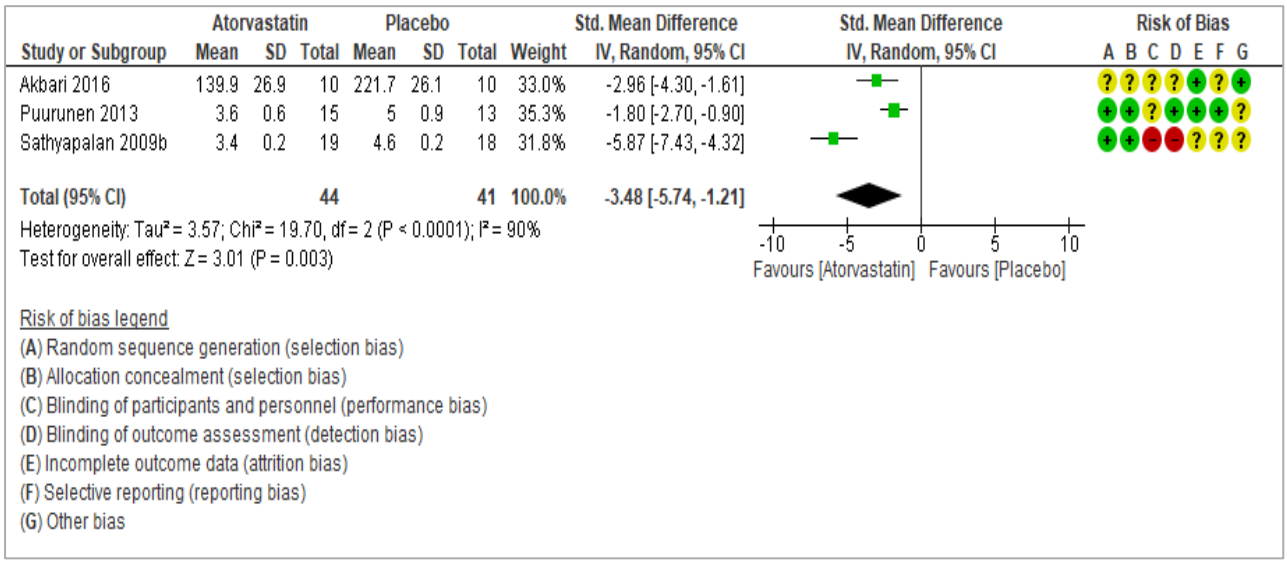
3.3.4.1 Lipid profiles

3.3.4.1.1 Total cholesterol (TC)

3.3.4.1.1.1 Atorvastatin versus placebo

In three RCTs, atorvastatin 20 mg QD significantly reduced the mean TC (SMD: -3.48; 95%CI: -5.74, -1.21, I^2 = 90%) (Figure 3-3) (very low-grade evidence, table 4).

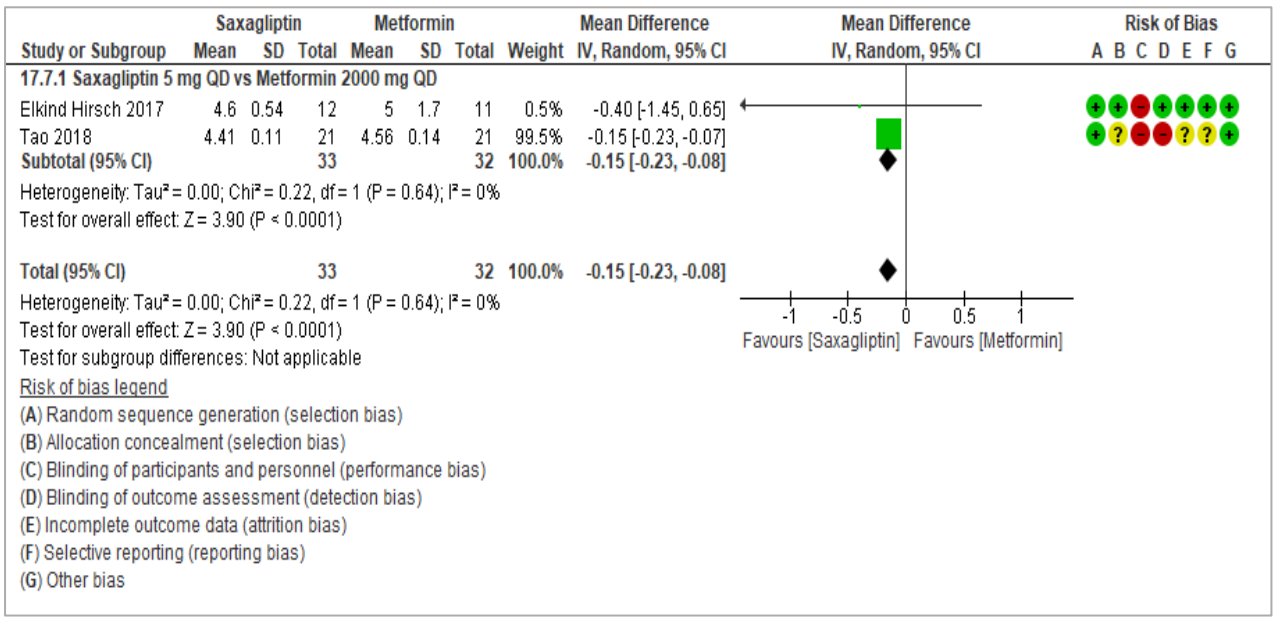
Figure 3-3: Forest plot of Atorvastatin versus placebo on TC



3.3.4.1.1.2 Saxagliptin versus Metformin

In two RCTs, compared with metformin 2000 mg QD, saxagliptin 5 mg QD significantly reduced the mean TC by 0.15 mmol/L (95% CI: -0.23, -0.08, I^2 = 0%) (Figure 3-4)(very low-grade evidence, table 4).

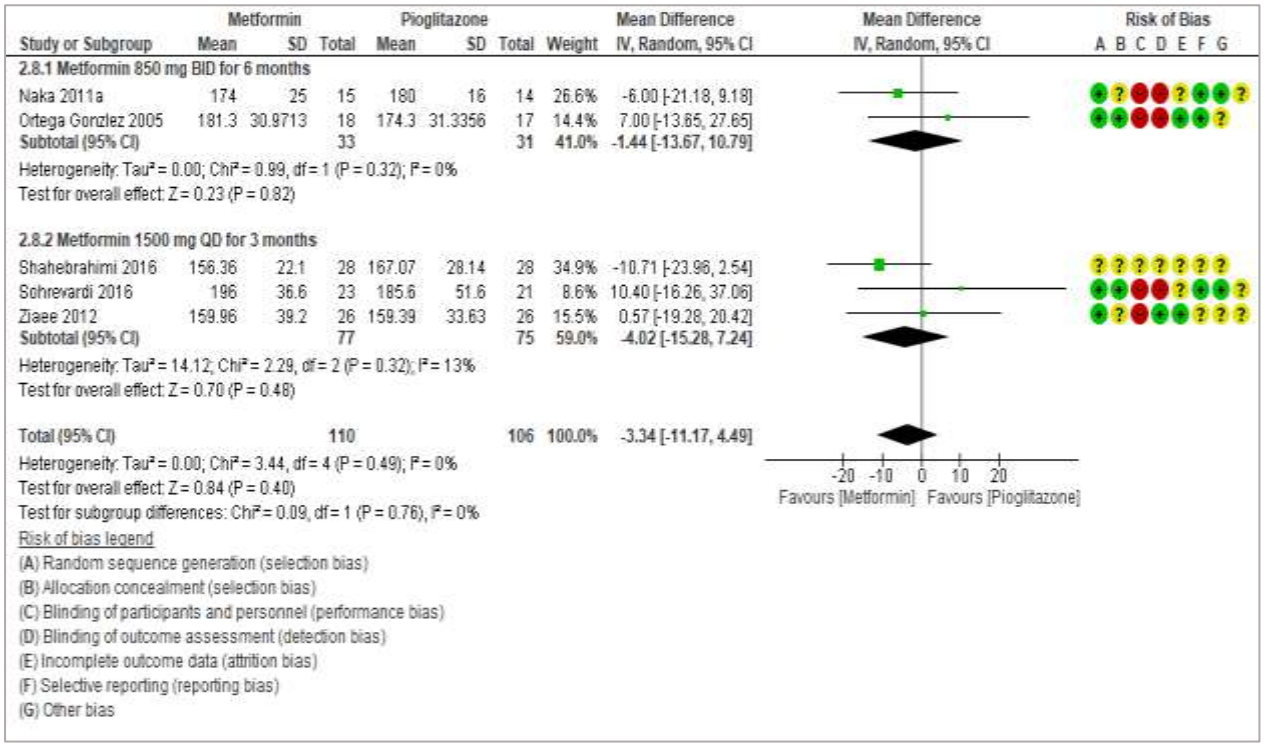
Figure 3-4: Forest plot of Saxagliptin versus Metformin on TC (mmol/L)



3.3.4.1.1.3 Metformin versus Pioglitazone

In two RCTs, metformin 850 mg BID compared with pioglitazone for six months has no effect on the mean TC (MD: -1.44 mmol/L; 95% CI: -13.67, 10.79). In three RCTs, metformin 1500 mg QD compared with pioglitazone for three months has no effect on the mean TC (MD: -4.02 mmol/L; 95%CI:-15.28, 7.24). Overall, metformin at various doses compared with pioglitazone has no effect on the mean TC (MD: -3.34 mmol/L; 95%: -11.17, 4.49, I^2= *0%)* (Figure 3-5) (Very low-grade evidence, table 4).

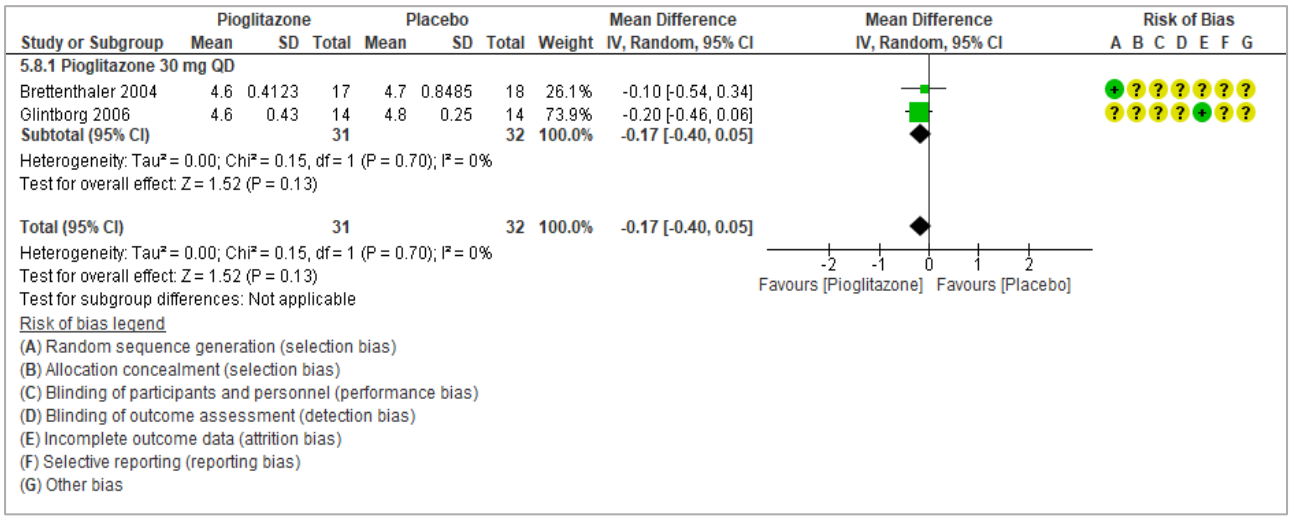
Figure 3-5: Forest plot of Metformin versus Pioglitazone on TC (mmol/L)



3.3.4.1.1.4 Pioglitazone versus placebo

In two RCTs, pioglitazone 30 mg QD compared with placebo has no effect on the mean TC (MD: -0.17 mmol/L: 95% CI: -0.40, 0.05, I^2= 0%) (Figure 3-6) (Very-low grade evidence, table 4).

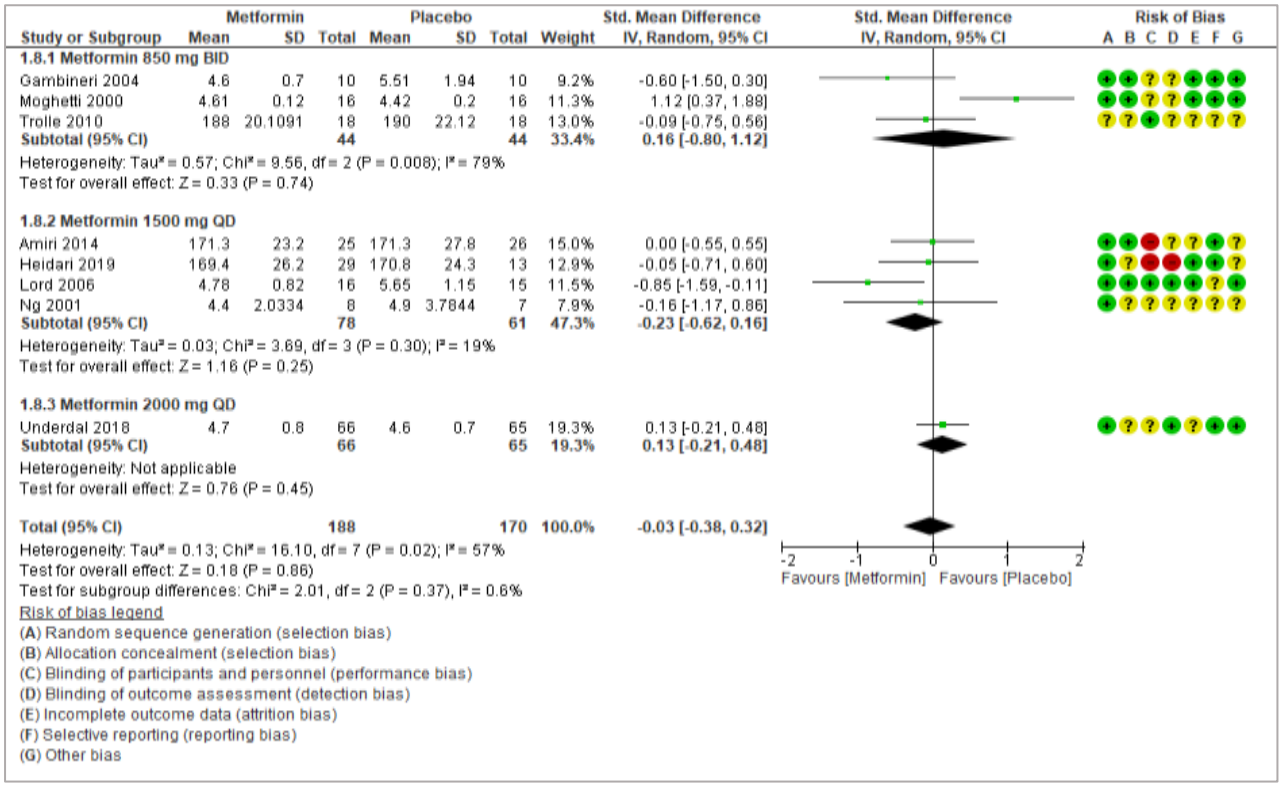
Figure 3-6: Forest plot of Pioglitazone versus placebo on TC (mmol/L)



3.3.4.1.1.5 Metformin versus placebo

In eight RCTs, metformin at various dosage has no effect on the mean TC when compared with placebo (SMD: -0.03; 95% CI: -0.38, 0.32, I^2= 0.6%) (Figure 3-7) (low-grade evidence, table 4).

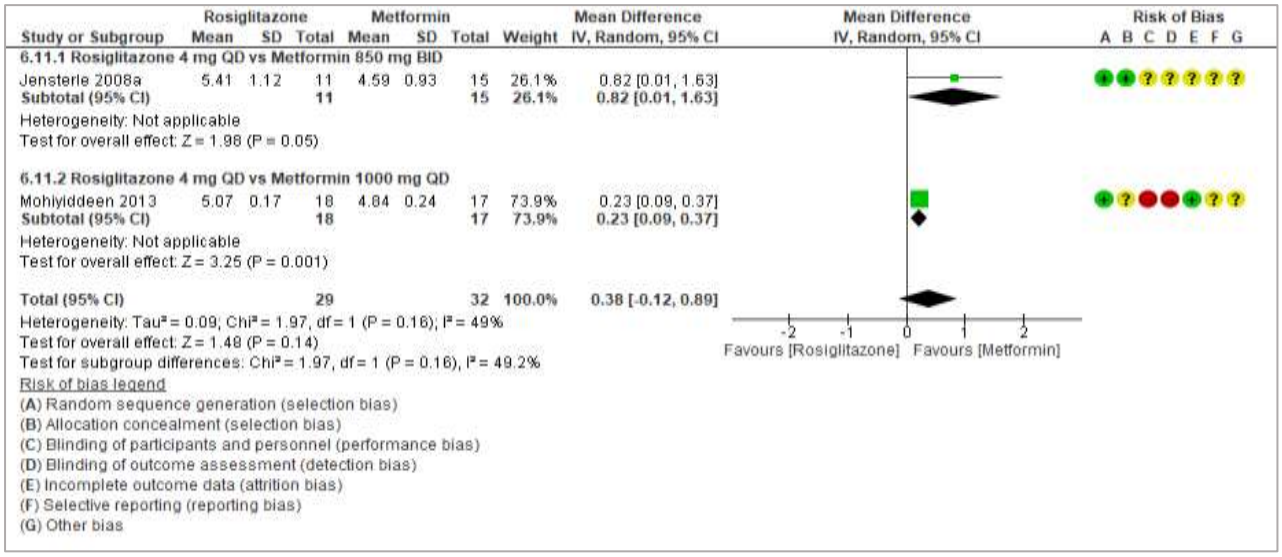
Figure 3-7: Forest plot of Metformin versus placebo on TC



3.3.4.1.1.6 Rosiglitazone versus Metformin

In two RCTs, rosiglitazone 4 mg QD compared with various dosages of metformin showed no effect on the mean TC (MD: 0.38 mmol/L; 95% CI: -0.12, 0.89, I^2= 49.2%) (Figure 3-8) (very low-grade evidence, table 4).

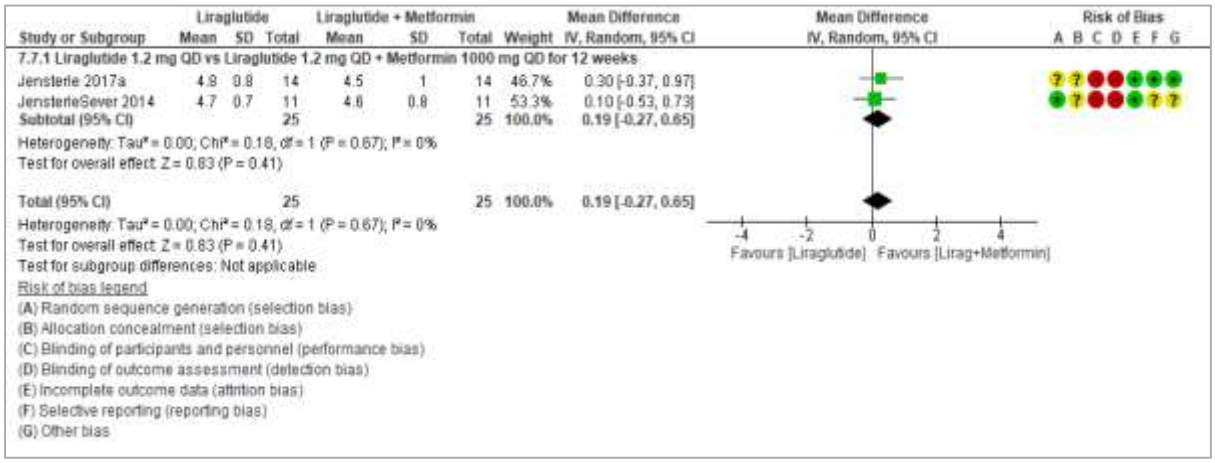
Figure 3-8: Forest plot of Rosiglitazone versus Metformin on TC (mmol/L)



3.3.4.1.1.7 Liraglutide versus Liraglutide+ Metformin

In two RCTs, Liraglutide 1.2 mg QD compared with Liraglutide 1.2 mg QD added to Metformin 1000 mg QD for 12 weeks has no effect on the mean TC (MD: 0.19 mmol/L; 95% CI: -0.27, 0.65, I^2= 0%) (Figure 3-9) (very low-grade evidence, table 4).

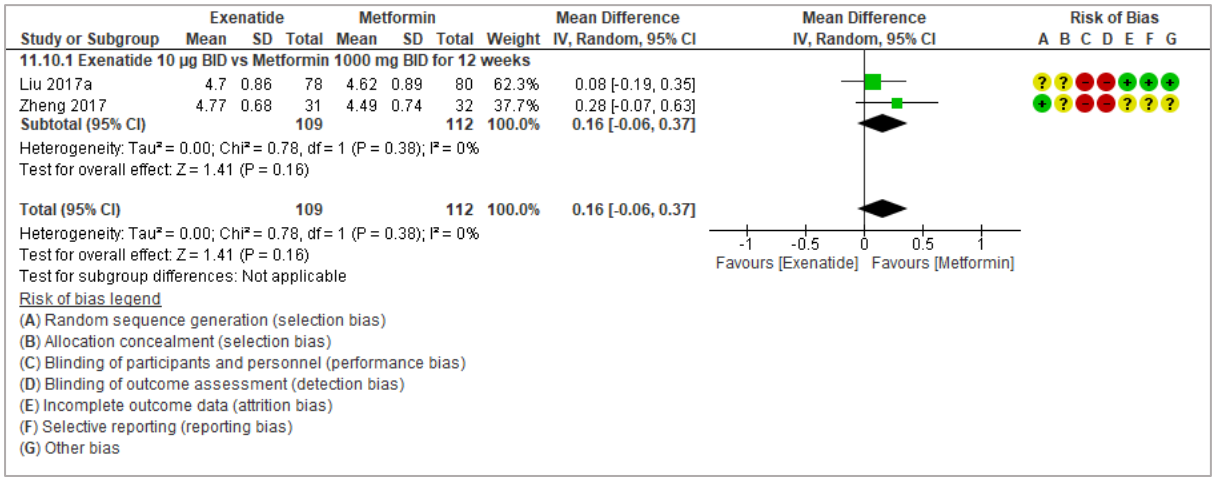
Figure 3-9: Forest plot of Liraglutide versus Liraglutide + Metformin on TC (mmol/L)



3.3.4.1.1.8 Exenatide versus Metformin

In two RCTs, exenatide 10 µg BID compared with metformin 1000 mg BID for 12 weeks has no effect on the mean TC (MD: 0.16 mmol/L; 95% CI: -0.06, 0.37, I^2= 0%) (Figure 3-10) (Very low-grade evidence, table 4).

Figure 3-10: Forest plot of Exenatide versus Metformin on TC (mmol/L)

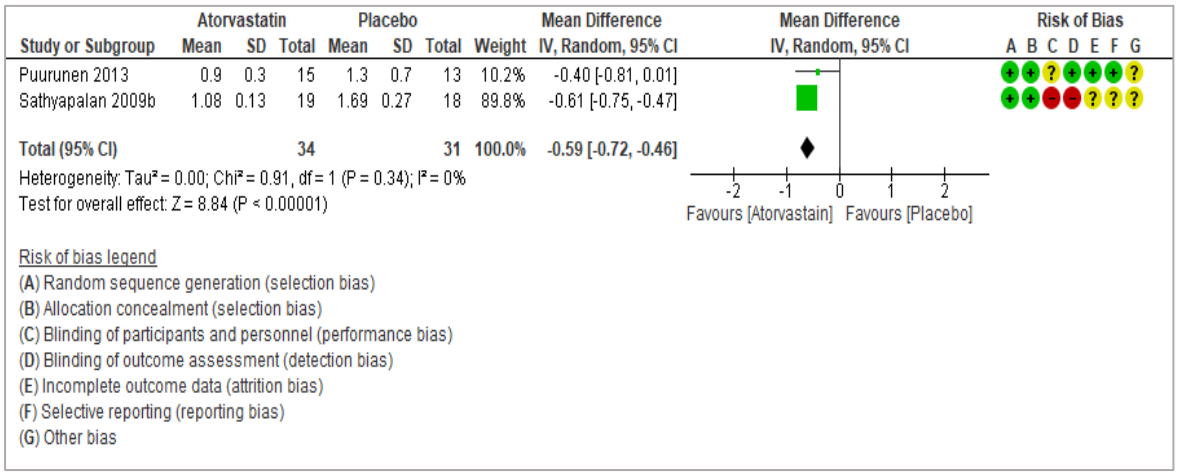


3.3.4.1.2 Triglycerides (TGs)

3.3.4.1.2.1 Atorvastatin versus placebo

In two RCTs, atorvastatin 20 mg QD significantly reduced the mean TGs by 0.59 mmol/L (95%CI: - 0.72,- 0.46, I^2= 0%*)* (Figure 3-11) (very low-grade evidence, table 4).

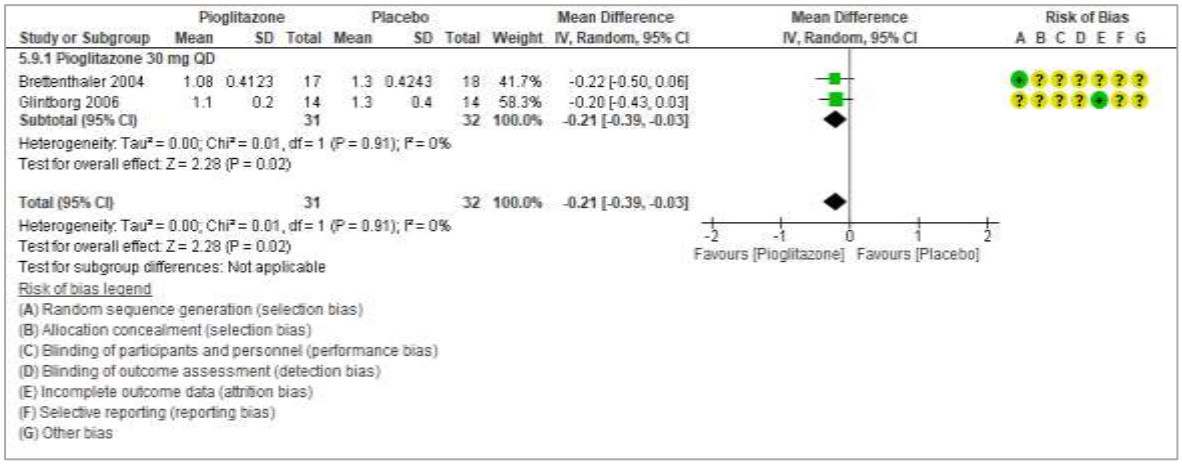
Figure 3-11: Forest plot of Atorvastatin versus placebo on TGs (mmol/L)



3.3.4.1.2.2 Pioglitazone versus placebo

In two RCTs, pioglitazone 30 mg QD significantly reduced the mean TGs by 0.21 mmol/L (95%CI: -0.39, -0.03, I^2 = 0%) when was compared with placebo (Figure 3-12) (very low-grade evidence, table 4).

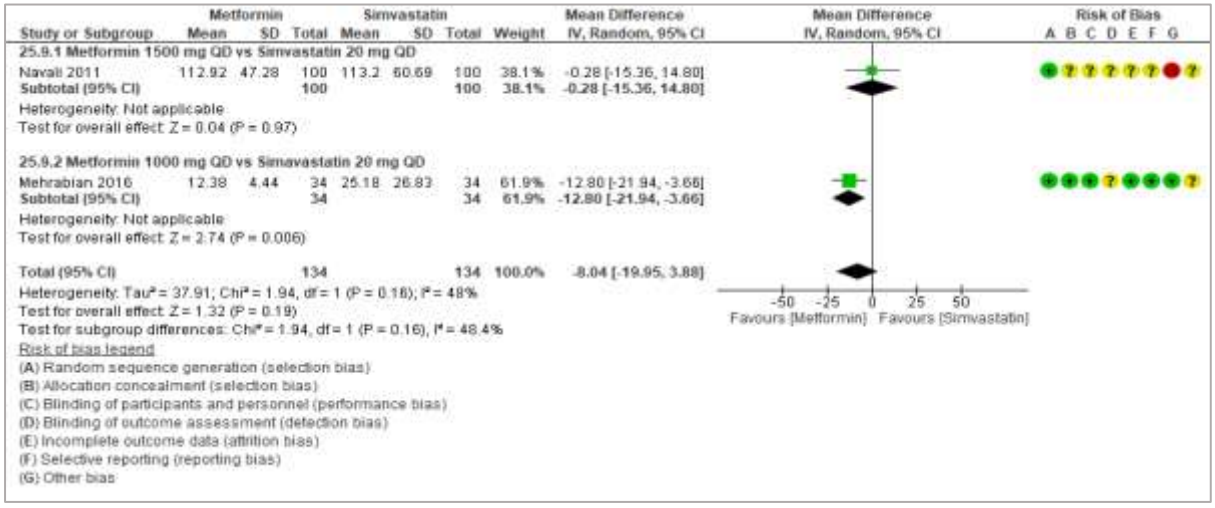
Figure 3-12: Forest plot of Pioglitazone versus placebo on TGs (mmol/L)



3.3.4.1.2.3 Metformin versus Simvastatin

In two RCTs, simvastatin 20 mg QD compared with metformin at various dosage has no effect on the mean TGs (MD: -8.04 mmol/L; 95% CI: -19.95, 3.88, I^2 = 48.4%) (Figure 3-13) (very low-grade evidence, table 4).

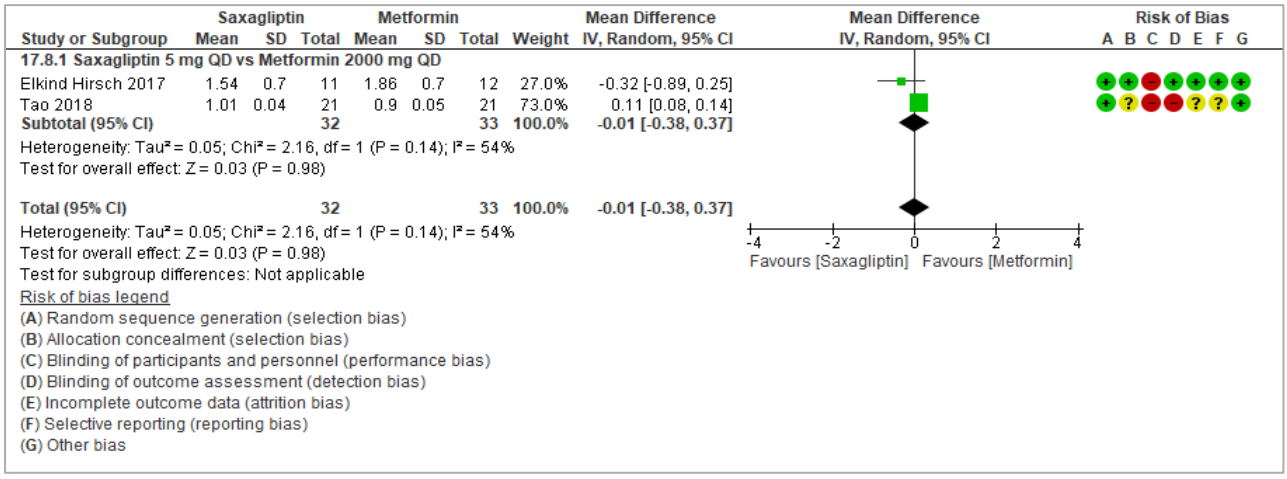
Figure 3-13: Forest plot of Metformin versus Simvastatin on TGs (mmol/L)



3.3.4.1.2.4 Saxagliptin versus Metformin

In two RCTs, saxagliptin 5 mg QD compared with metformin 2000 mg QD has no effect on the mean TGs (MD: -0.01 mmol/L; 95% CI: -0.38, 0.37, I^2 = 54%) (Figure 3-14) (very low-grade evidence, table 4).

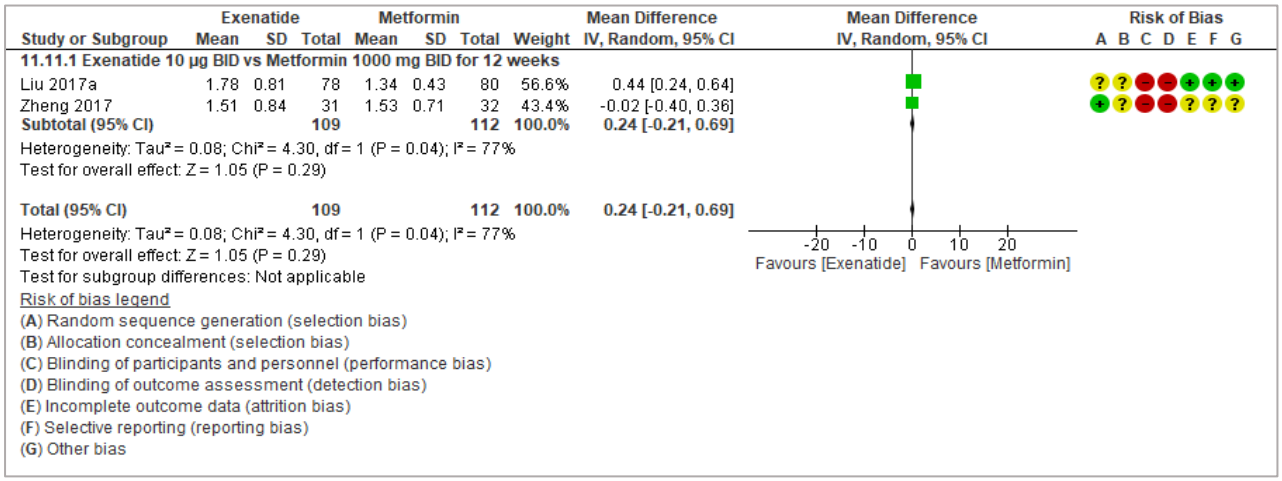
Figure 3-14: Forest plot of Saxagliptin versus Metformin on TGs (mmol/L)



3.3.4.1.2.5 Exenatide versus Metformin

In two RCTs, exenatide 10µg BID compared with metformin 1000 mg BID for 12 weeks has no effect on the mean TGs (MD: 0.24 mmol/L; 95% CI: -0.21, 0.69, I^2 = 77%) (Figure 3-15) (very low-grade evidence, table 4).

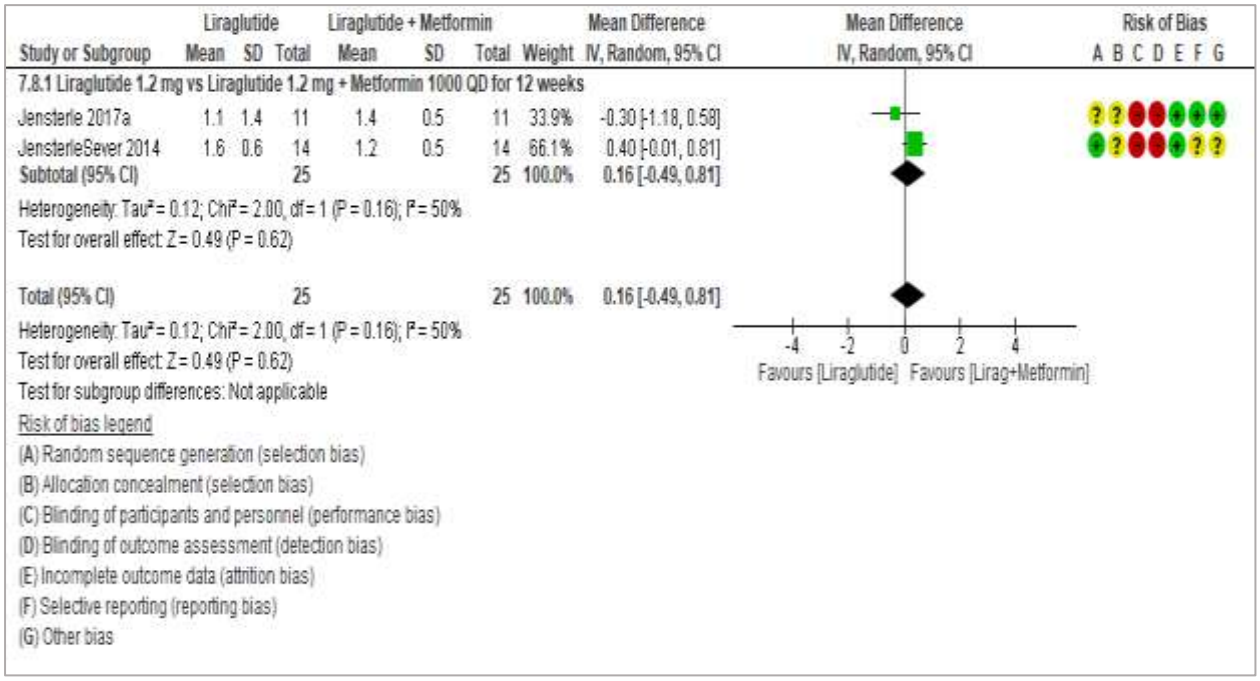
Figure 3-15: Forest plot of Exenatide versus Metformin on TGs (mmol/L)



3.3.4.1.2.6 Liraglutide versus Liraglutide + Metformin

In two RCTs, liraglutide 1.2 mg compared with liraglutide 1.2 mg added to metformin 1000 mg QD for 12 weeks has no effect on the mean TGs (MD: 0.16 mmol/L; 95% CI: -0.49, 0.81, I^2 = 50%) (Figure 3-16) (very low-grade evidence, table 4).

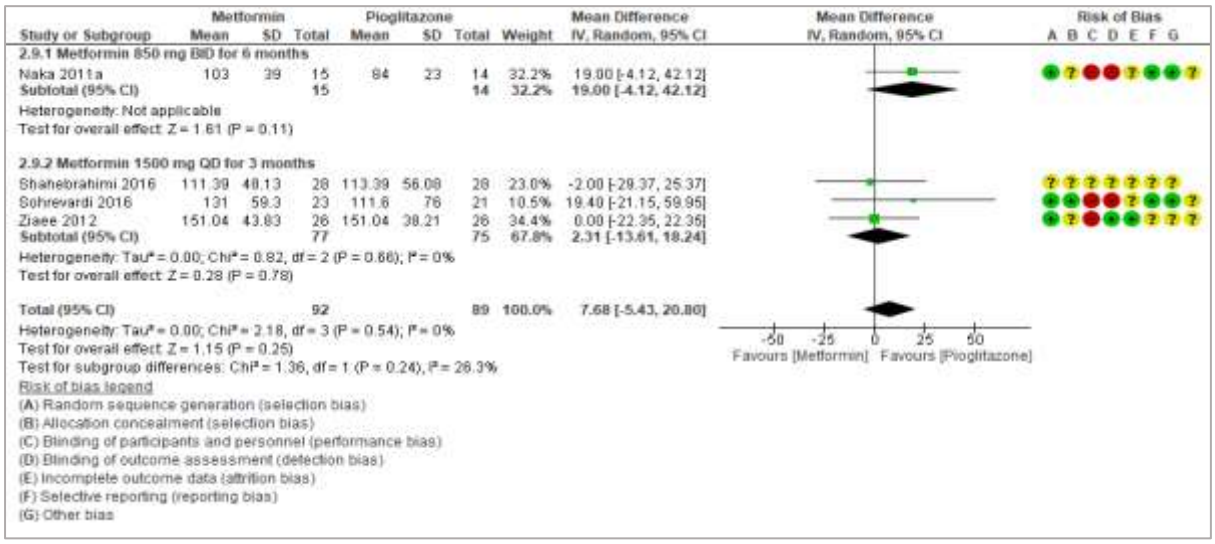
Figure 3-16: Forest plot of Liraglutide versus Liraglutide + Metformin on TGs (mmol/L)



3.3.4.1.2.7 Metformin versus Pioglitazone

In one RCT, metformin 850 mg BID compared with pioglitazone for six months has no effect on the mean TGs. In three RCTs, metformin 1500 mg QD compared with pioglitazone for three months has no effect on the mean TGs. Overall, regardless of the dosage and the duration, metformin does not affect the mean TGs when compared with pioglitazone (MD:7.68 mmol/L; 95% CI: -5.43, 20.80, I^2 = 26.3%) (Figure 3-17) (very low-grade evidence, table 4).

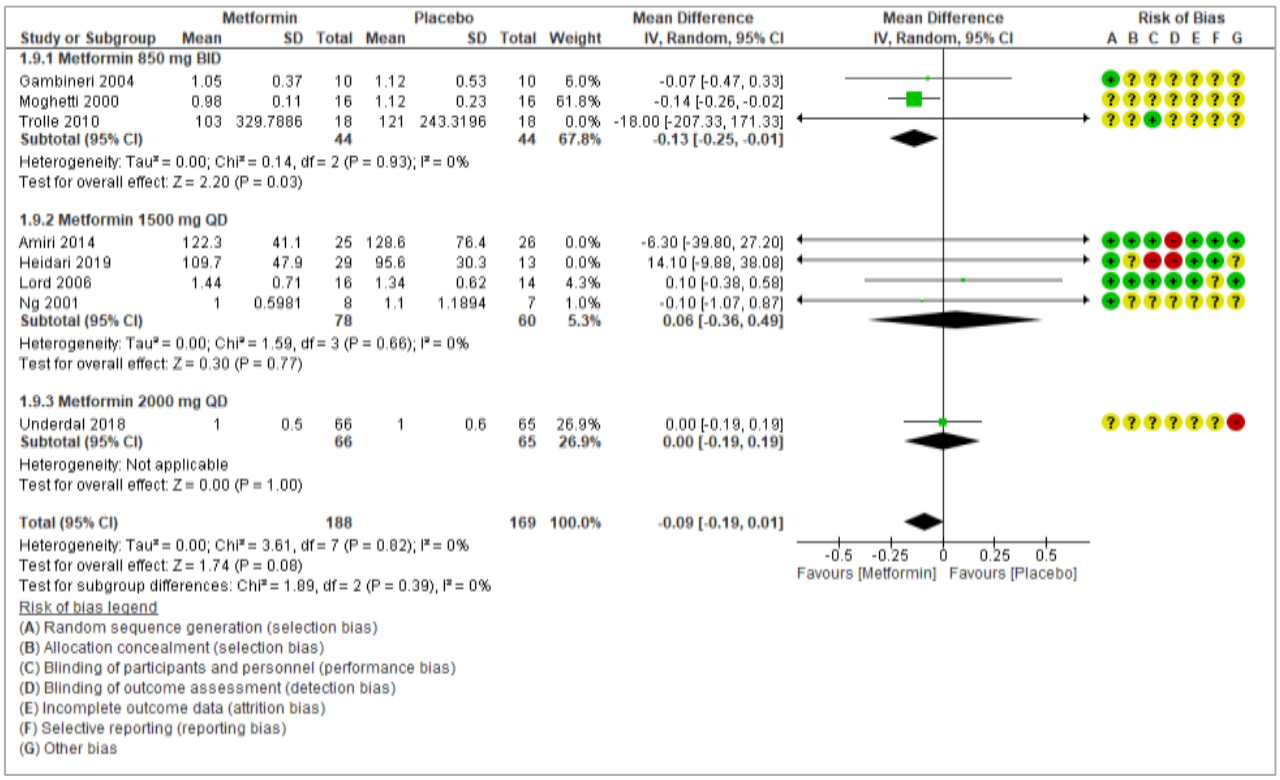
Figure 3-17: Forest plot of Metformin versus Pioglitazone on TGs (mmol/L)



3.3.4.1.2.8 Metformin versus placebo

In eight RCTs, regardless to the administered dosage metformin has significantly reduced the mean TGs when compared with placebo (MD: -0.09 mmol/L; 95% CI: -0.19, 0.01, I^2 = 0%) (figure 3-18) (moderate grade evidence, table 4).

Figure 3-18: Forest plot of Metformin versus placebo on TGs (mmol/L)

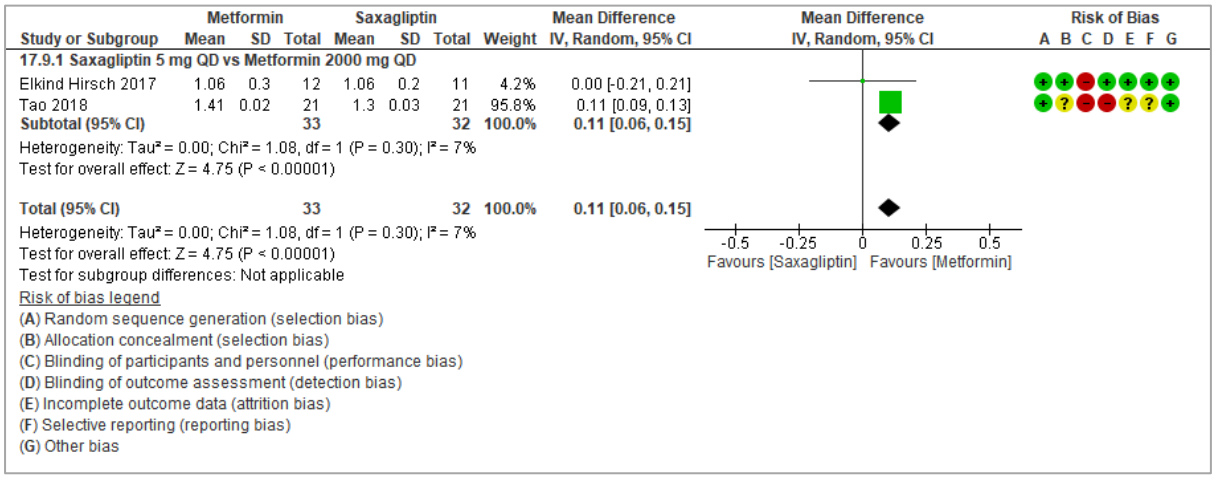


3.3.4.1.3 High-density lipoprotein cholesterol (HDL-C)

3.3.4.1.3.1 Saxagliptin versus Metformin

In two RCTs, compared with saxagliptin 5 mg QD, metformin 2000 mg QD significantly increased the mean HDL-C by 0.11 mmol/L (95%CI: 0.06, 0.15, I^2 = 7%) (figure 3-19) (very low-grade evidence, table 4).

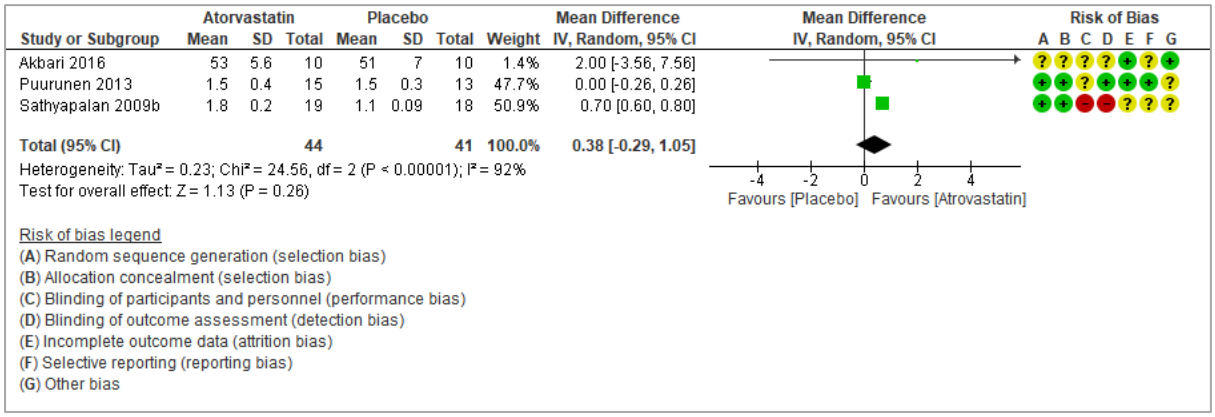
Figure 3-19: Forest plot of Saxagliptin versus Metformin on HDL-C (mmol/L)



3.3.4.1.3.2 Atorvastatin versus placebo

In three RCTs, atorvastatin has no effect on the mean HDL-C compared with placebo (MD: 0.38 mmol/L; 95% CI: -0.29, 1.05, I^2 = 92%) (figure 3-20) (very low-grade evidence, table 4).

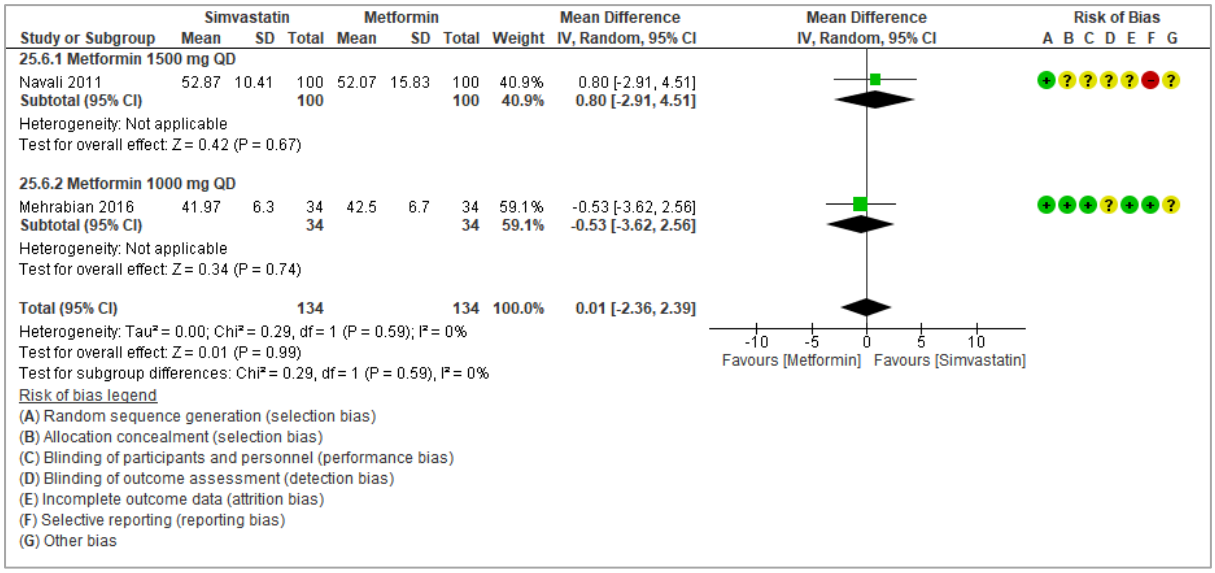
Figure 3-20: Forest plot of Atorvastatin placebo on HDL-C (mmol/L)



3.3.4.1.3.3 Metformin versus Simvastatin

In two RCTs, regardless of the administered dosage metformin has no effect on the mean HDL-C compared with simvastatin (MD: 0.01 mmol/L; 95% CI: -2.39, 2.36, I^2 = 0%) (figure 3-21) (very low-grade evidence, table 4).

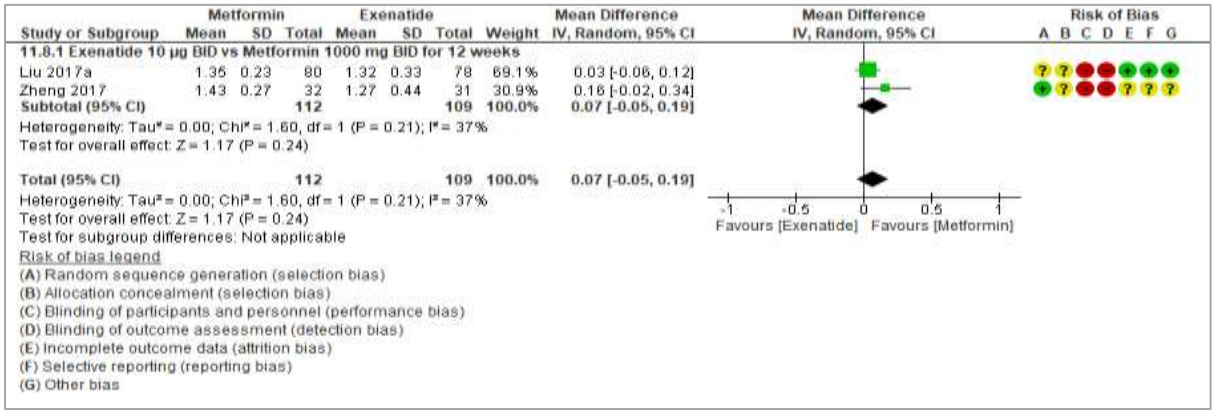
Figure 3-21: Forest plot of Metformin versus Simvastatin on HDL-C (mmol/L)



3.3.4.1.3.4 Exenatide versus Metformin

In two RCTs, exenatide 10 µg BID compared with metformin 1000 mg BID for 12 weeks has no effect on the mean HDL-C (MD: 0.07 mmol/L; 95% CI: -0.05, 0.19, I^2 = 37%) (figure 3-22) (very low-grade evidence, figure 4).

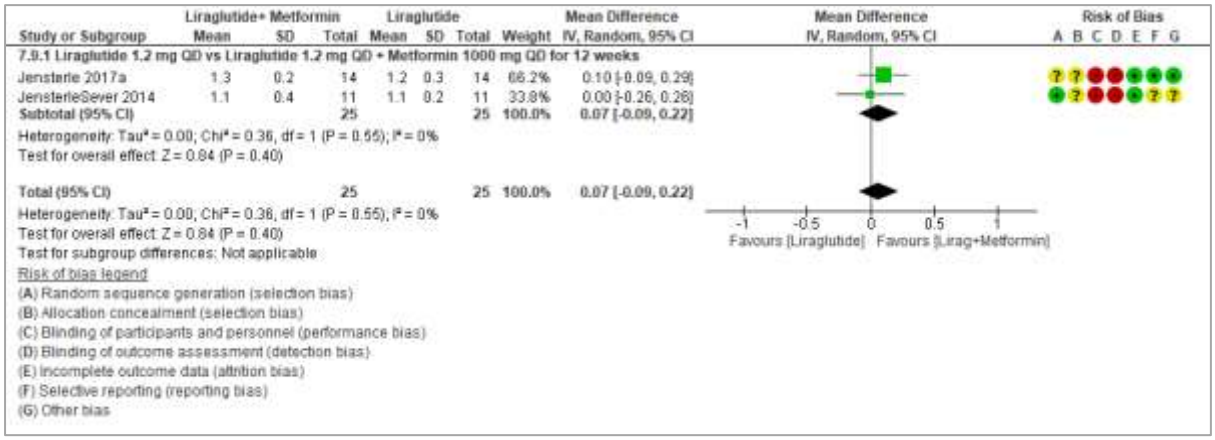
Figure 3-22: Forest plot of Exenatide versus Metformin on HDL-C (mmol/L)



3.3.4.1.3.5 Liraglutide versus Liraglutide+ Metformin

In two RCTs, liraglutide 1.2 mg QD compared with liraglutide 1.2 mg QD added to metformin 1000 mg QD for 12 weeks has no effect on the mean HDL-C (MD: 0.07 mmol/L; 95% CI: -0.09, 0.22, I^2 = 0%) (figure 3-23) (very low-grade evidence, table 4).

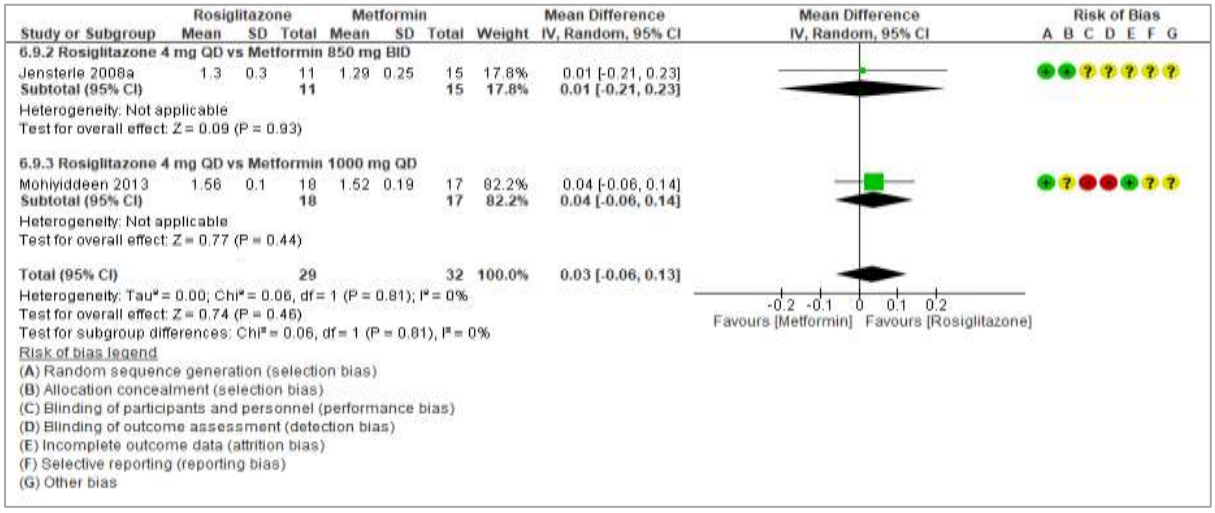
Figure 3-23: Forest plot of Liraglutide versus Liraglutide + Metformin on HDL-C (mmol/L)



3.3.4.1.3.6 Rosiglitazone versus Metformin

In two RCTs, when rosiglitazone 4 mg QD was compared with either metformin 850 mg BID or metformin 1000 mg QD has no effect on the mean HDL-C (MD: 0.03 mmol/L; 95% CI: -0.06, 0.13, I^2 = 0%) (figure 3-24) (very low-grade evidence, table 4).

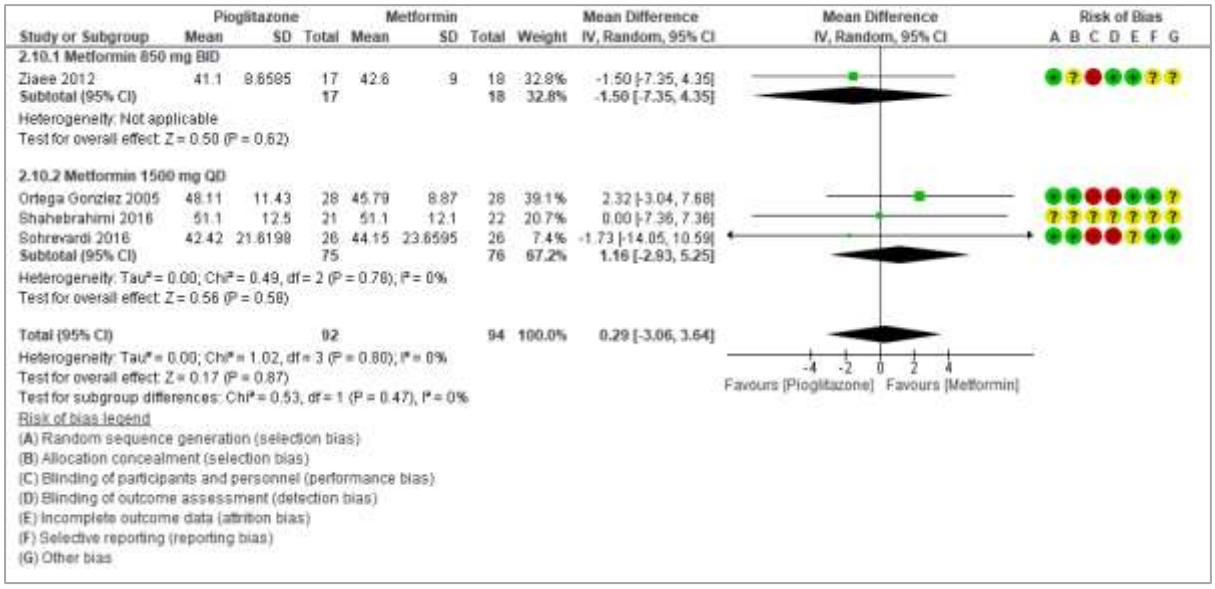
Figure 3-24: Forest plot of Rosiglitazone versus Metformin on HDL-C (mmol/L)



3.3.4.1.3.7 Metformin versus Pioglitazone

In four RCTs, when metformin 850 mg BID for six months and metformin 1500 mg QD for three months was compared with pioglitazone has no effect on the mean HDL-C (MD: 0.29 mmol/L; 95% CI: -3.06, 3.64, I^2 = 0%) (figure 3-25) (very low-grade evidence table 4).

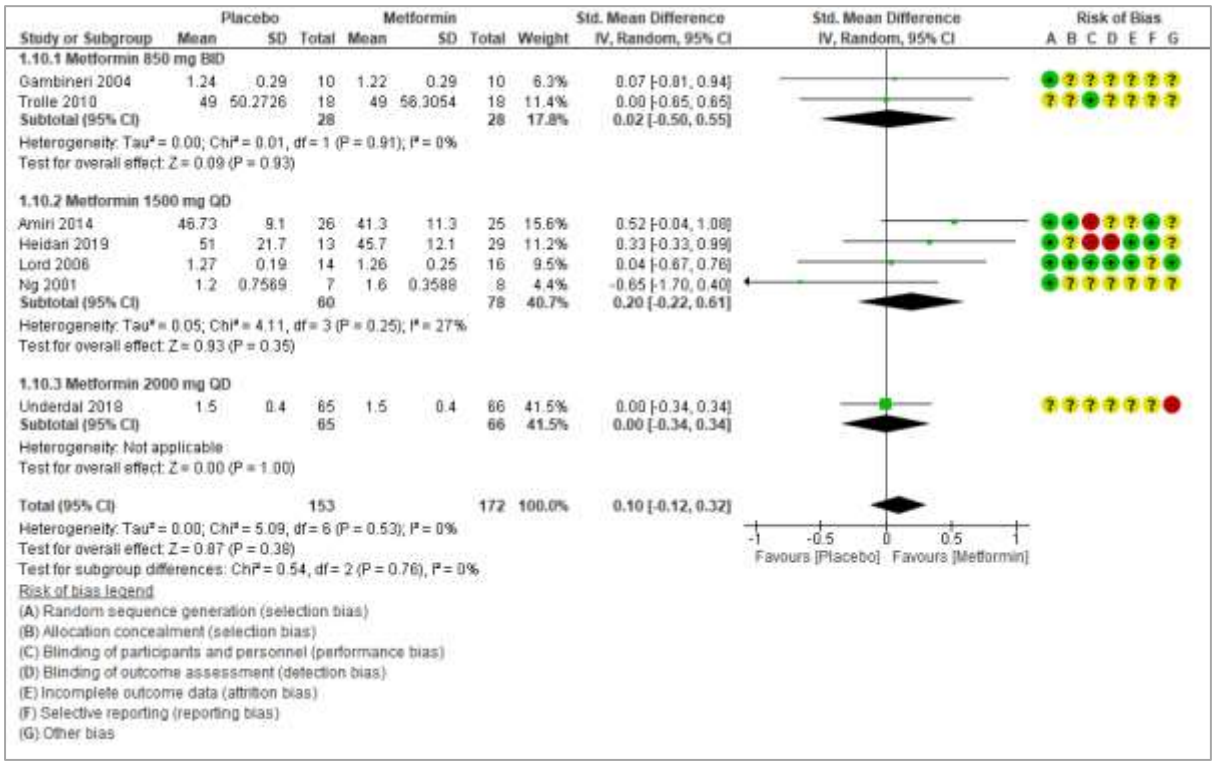
Figure 3-25: Forest plot of Metformin versus Pioglitazone on HDL-C (mmol/L)



3.3.4.1.3.8 Metformin versus placebo

In two RCTs, metformin 850 mg BID compared with placebo has no effect on the mean HDL-C. In four RCTs, metformin 1500 mg QD compared with placebo has no effect on the mean HDL-C. In one RCT, metformin 2000 mg QD has no effect on the mean HDL-C compared with placebo. Overall, regardless of the dosage metformin has no effect on the mean HDL-C compared with placebo (SMD: 0.10 mmol/L; 95% CI: -0.12, 0.32, I^2 = 0%) (figure 3-26) (low-grade evidence, table 4).

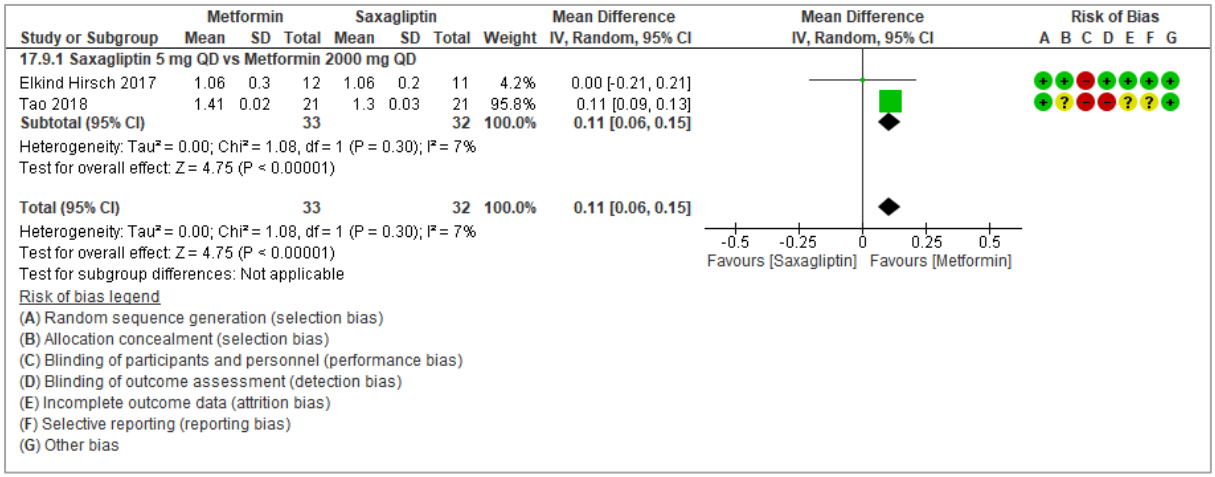
Figure 3-26: Forest plot of Metformin versus placebo on HDL-C (mmol/L)



3.3.4.1.3.9 Saxagliptin versus Metformin

In two RCTs, compared with saxagliptin 5 mg QD, metformin 2000 mg QD significantly increased the mean HDL-C by 0.11 mmol/L (95% CI: 0.06, 0.15, I^2 = 7%) (figure 3-27) (very low-grade evidence, table 4).

Figure 3-27: Forest plot of Saxagliptin versus Metformin on HDL-C (mmol/L)

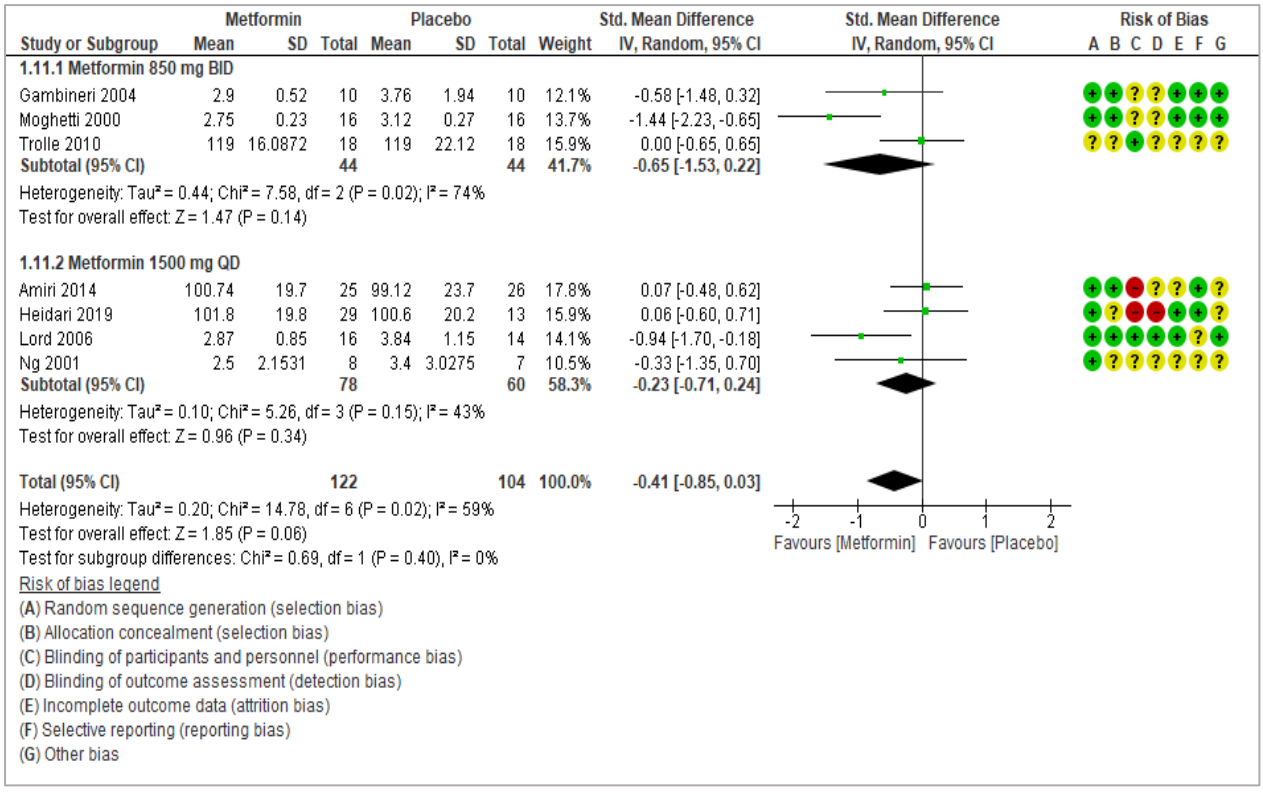


3.3.4.1.4 Low-density lipoprotein cholesterol (LDL-C)

3.3.4.1.4.1 Metformin versus placebo

In three RCTs, metformin 850 mg BID had no effect on the mean LDL-C (SMD: -0.65; 95%CI: -1.53, 0.22), and in four RCTs, metformin 1500 mg QD was also associated with no effect in the mean LDL-C (SMD: -0.23; 95%CI: -0.71, 0.24). Overall, regardless of the administered doses, metformin was associated with a significant reduction in the mean LDL-C when compared with placebo (SMD: -0.41; 95%CI: -0.85, 0.03, I^2 = 59%) (Figure 3-28) (low-grade evidence, table 4).

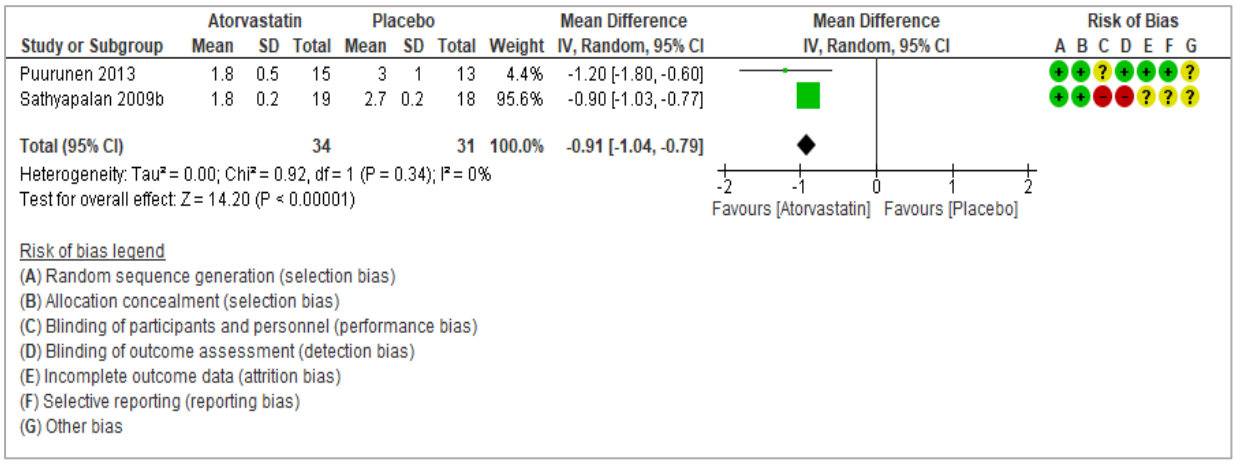
Figure 3-28: Forest plot of Metformin versus placebo on LDL-C



3.3.4.1.4.2 Atorvastatin versus placebo

In two RCTs, atorvastatin 20 mg QD significantly reduced the mean LDL-C by 0.91 mmol/L (95%CI: -1.04, 0.79, I^2 = 0%) when compared with placebo (Figure 3-29) (very low-grade evidence, table 4).

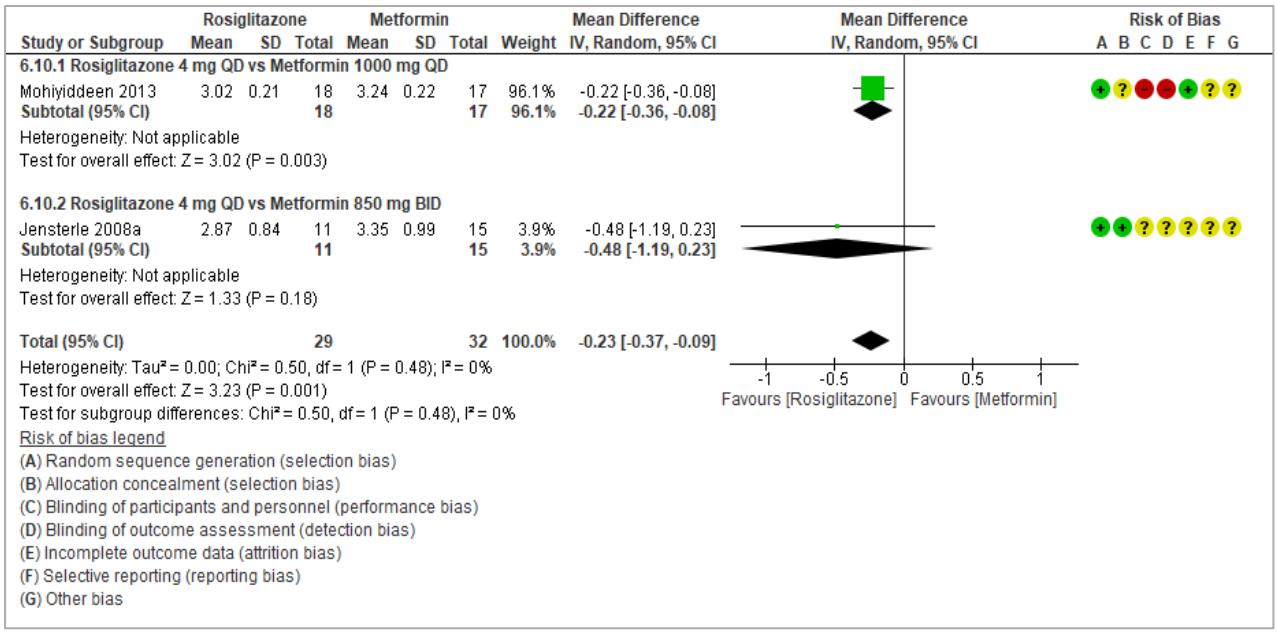
Figure 3-29: Forest plot of Atorvastatin versus placebo on LDL-C (mmol/L)



3.3.4.1.4.3 Rosiglitazone versus Metformin

In one RCT, rosiglitazone 4 mg QD significantly reduced the mean LDL-C by 0.22 mmol/L (95%CI: -0.36, -0.08) compared with metformin 1000 mg QD. In one RCT, rosiglitazone 4 mg QD also significantly reduced the mean LDL-C by 0.48 mmol/L (95%CI: -1.19, 0.23) compared with metformin 850 mg BID. Overall, rosiglitazone 4 mg QD significantly reduced the mean LDL-C by 0.23 mmol/L (95%CI: -0.37,-0.09, I^2 = 0%*)* when compared with various doses of metformin (Figure 3-30) (very low-grade evidence, table 4).

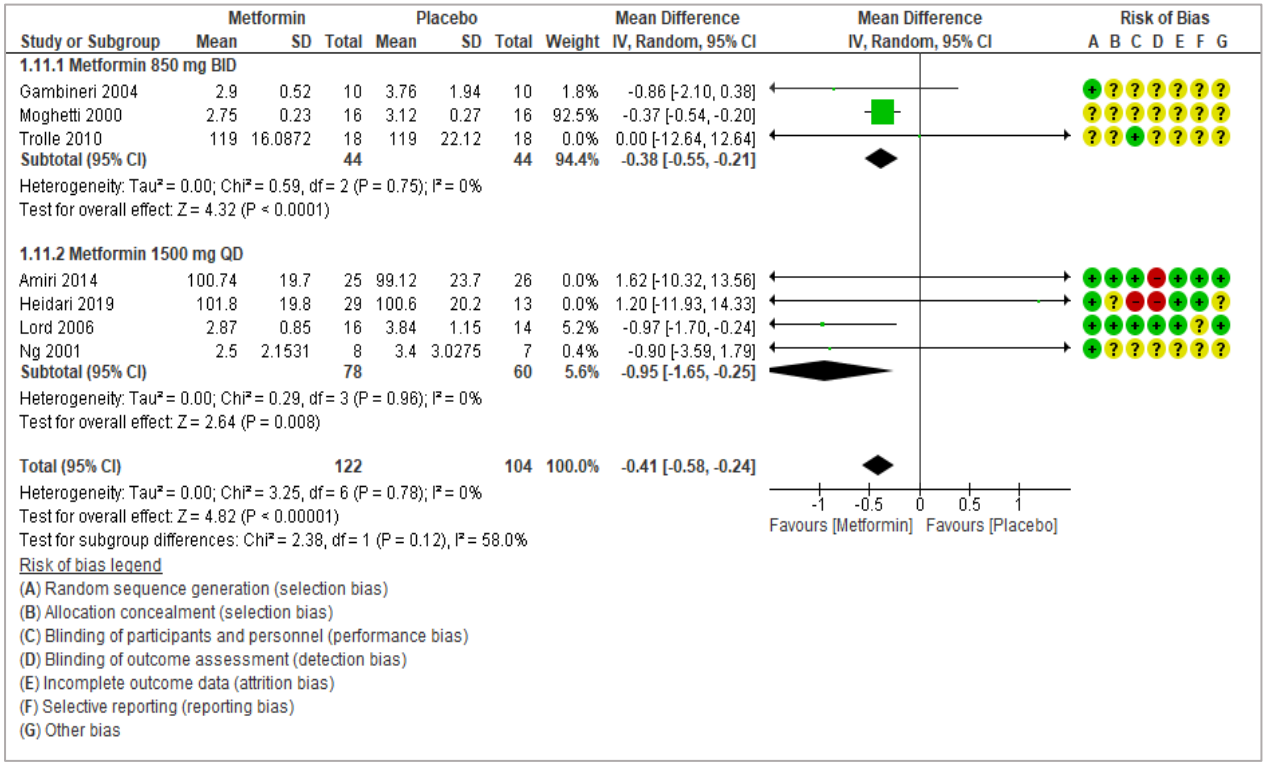
Figure 3-30: Forest plot of Rosiglitazone versus Metformin on LDL-C (mmol/L)



3.3.4.1.4.4 Metformin versus placebo

In three RCTs, metformin 850 mg BID significantly reduced the mean LDL-C compared with placebo (MD: -0.38 mmol/L; 95% CI: -0.55, -0.21). In four RCTs, metformin 1500 mg QD significantly reduced the mean LDL-C compared with placebo (MD: -0.95 mmol/L; 95% CI: -1.65, -0.25). Overall, regardless of the administered dosage metformin significantly reduced the mean LDL-C compared with placebo (MD: -0.41 mmol/L; 95% CI: -0.58, -0.24, I^2 = 58%) (Figure 3-31) (low-grade evidence, table 4).

Figure 3-31: Forest plot of Metformin versus placebo on LDL-C (mmol/L)

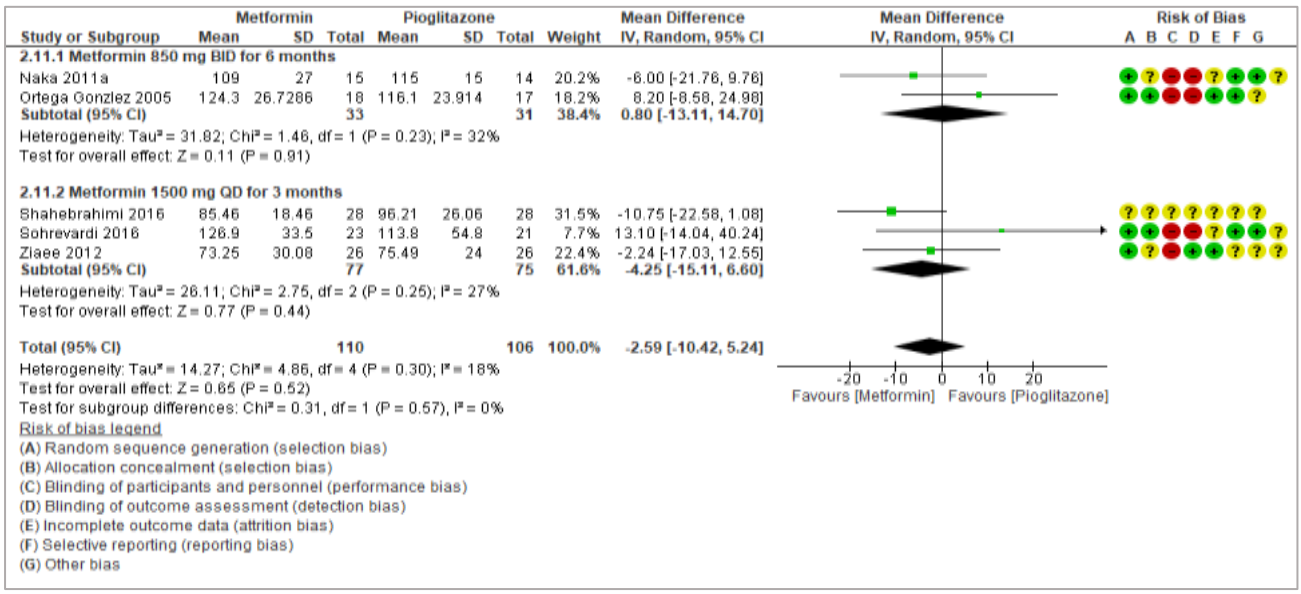


3.3.4.1.4.5 Metformin versus Pioglitazone

In two RCTs, metformin 850 mg BID for six months has no effect on the mean LDL-C compared with pioglitazone (MD: 0.80 mmol/L; 95% CI: -13.11, 14.70). In three RCTs, metformin 1500 mg QD for three months compared with pioglitazone has no effect on the mean LDL-C (MD: -4.25 mmol/L; 95% CI: -15.11, 6.60). Overall, regardless to the dosage metformin has no effect

on the mean LDL-C compared with pioglitazone (MD: -2.59 mmol/L; 95% CI: -10.42, 5.24, I^2 = 0%) (Figure 3-32) (very low-grade evidence, table 4).

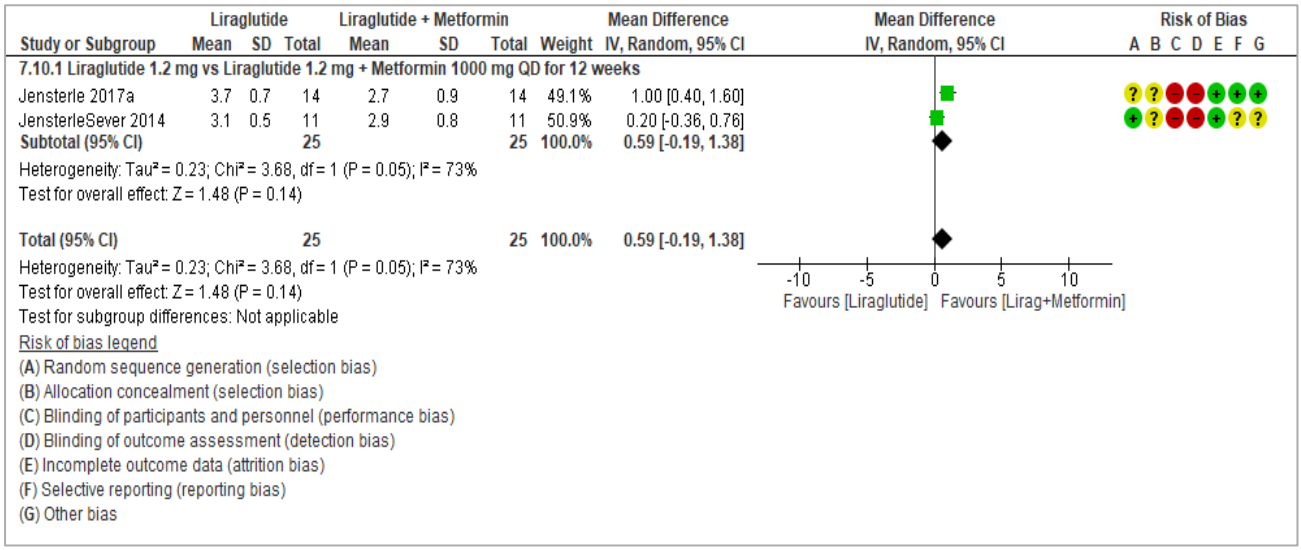
Figure 3-32: Forest plot of Metformin versus Pioglitazone on LDL-C (mmol/L)



3.3.4.1.4.6 Liraglutide versus Liraglutide + Metformin

In two RCTs compared liraglutide 1.2 mg QD with liraglutide 1.2 mg QD added to metformin 1000 mg QD for 12 weeks had no effect on the mean LDL-C (MD: 0.59 mmol/L; 955 CI: -0.19, 1.38, I^2 = 73%) (Figure 3-33) (very low-grade evidence, table 4).

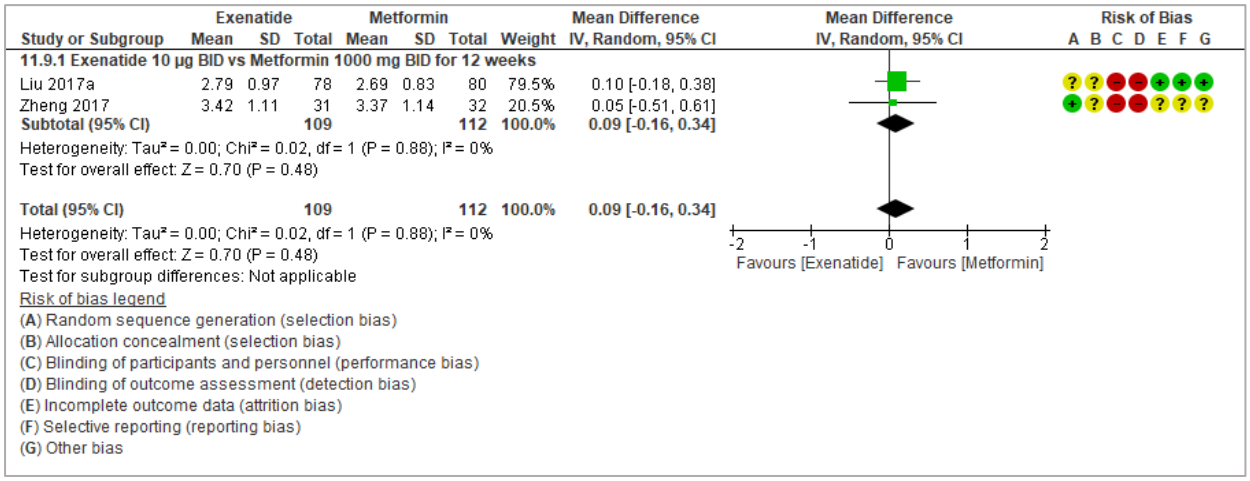
Figure 3-33: Forest plot of Liraglutide versus Liraglutide + Metformin on LDL-C (mmol/L)



3.3.4.1.4.7 Exenatide versus Metformin

In two RCTs exenatide 10 µg BID compared with metformin 1000 mg BID for 12 weeks has no effect on the mean LDL-C (MD: 0.09 mmol/L; 95% CI: -0.16, 0.34, I^2 = 0%) (Figure 3-34) (very low-grade evidence, table 4).

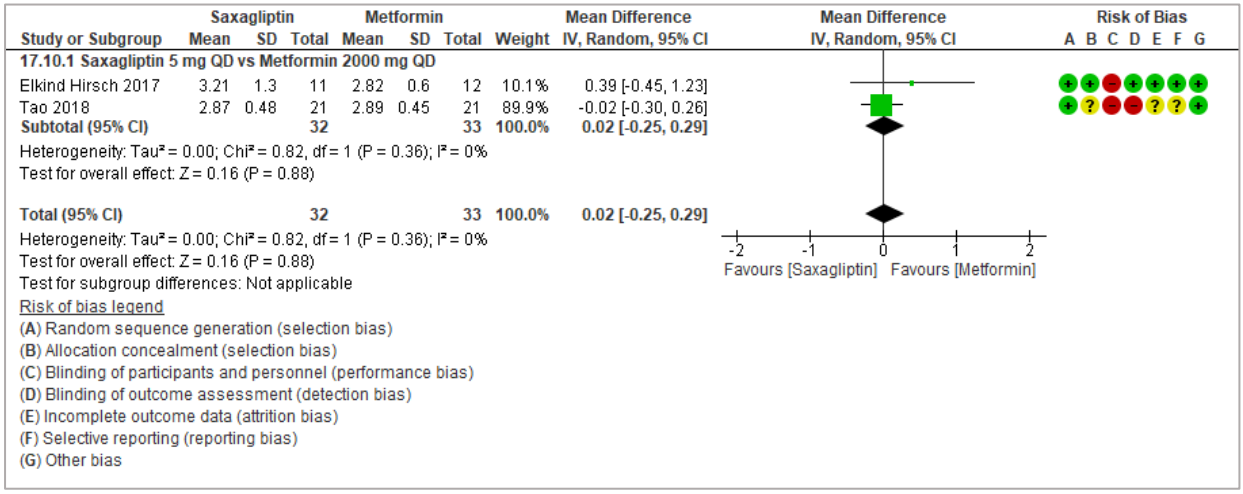
Figure 3-34: Forest plot of Exenatide versus Metformin on LDL-C (mmol/L)



3.3.4.1.4.8 Saxagliptin versus Metformin

In two RCTs compared saxagliptin 5 mg QD with metformin 2000 mg QD has no effect on the mean LDL-C (MD: 0.02 mmol/L; 95% CI: -0.25, 0.29, I^2 = 0%) (Figure 3-35) (very low-grade evidence, table 4).

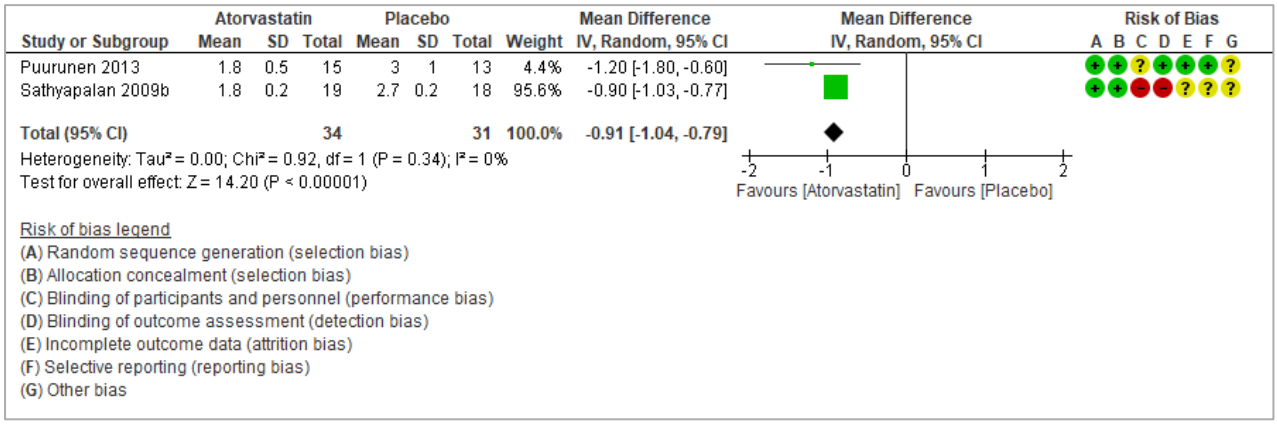
Figure 3-35: Forest plot of Saxagliptin versus Metformin on LDL-C (mmol/L)



3.3.4.1.4.9 Atorvastatin versus placebo

In two RCTs atorvastatin showed a significant reduction on the mean LDL-C compared with placebo (MD: -0.91 mmol/L; 95% CI: -1.04, -0.79, I^2 = 0%) (Figure 3-36) (very low-grade evidence, table 4).

Figure 3-36: Forest plot of Atorvastatin versus placebo on LDL-C (mmol/L)

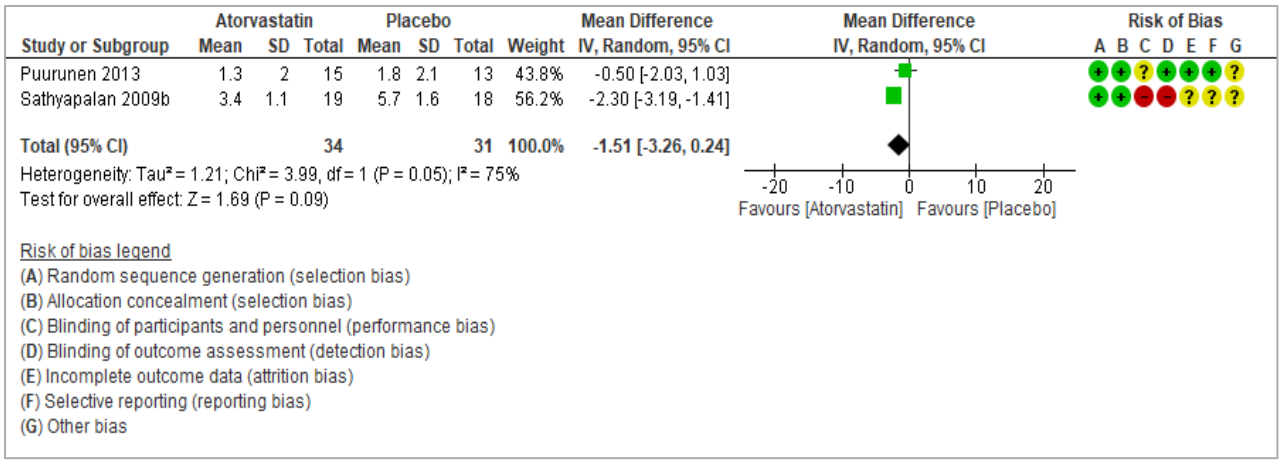


3.3.4.1.5 C-reactive protein (CRP)

3.3.4.1.5.1 Atorvastatin versus placebo

In two RCTs, atorvastatin 20 mg QD was associated with a significant reduction in the mean CRP by 1.51 mg/L (95%CI: -3.26, 0.24, I^2 = 75%, p = 0.09) (Figure 3-37) (very low-grade evidence, table 4).

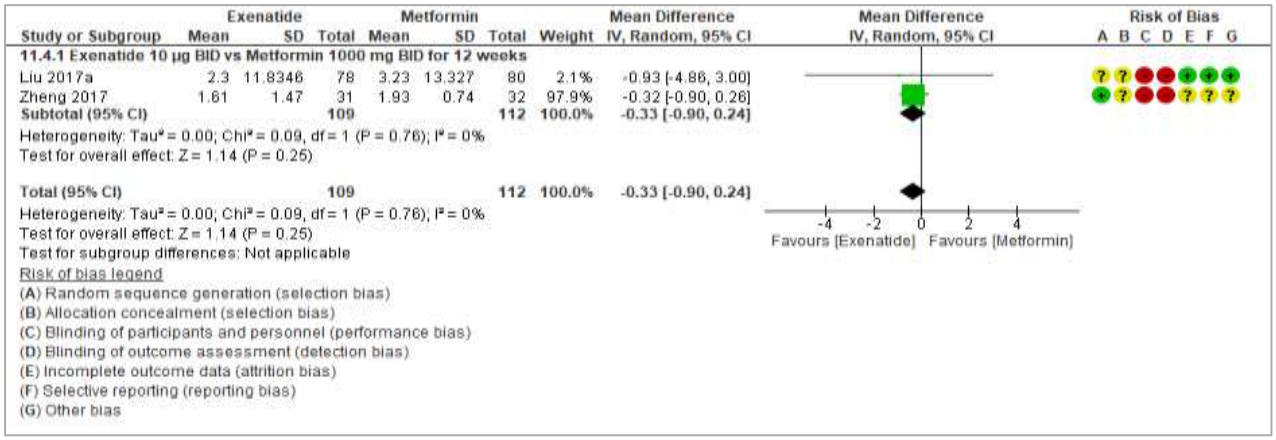
Figure 3-37: Forest plot of Atorvastatin versus placebo on CRP (mg/L)



3.3.4.1.5.2 Exenatide versus Metformin

In two RCTs exenatide 10 µg BID compared with metformin 1000 mg BID for 12 weeks has no effect on the mean CRP (MD: -0.33 mg/L; 95% CI: -0.90, 0.24, I^2 = 0%) (Figure 3-38) (very low-grade evidence, table 4).

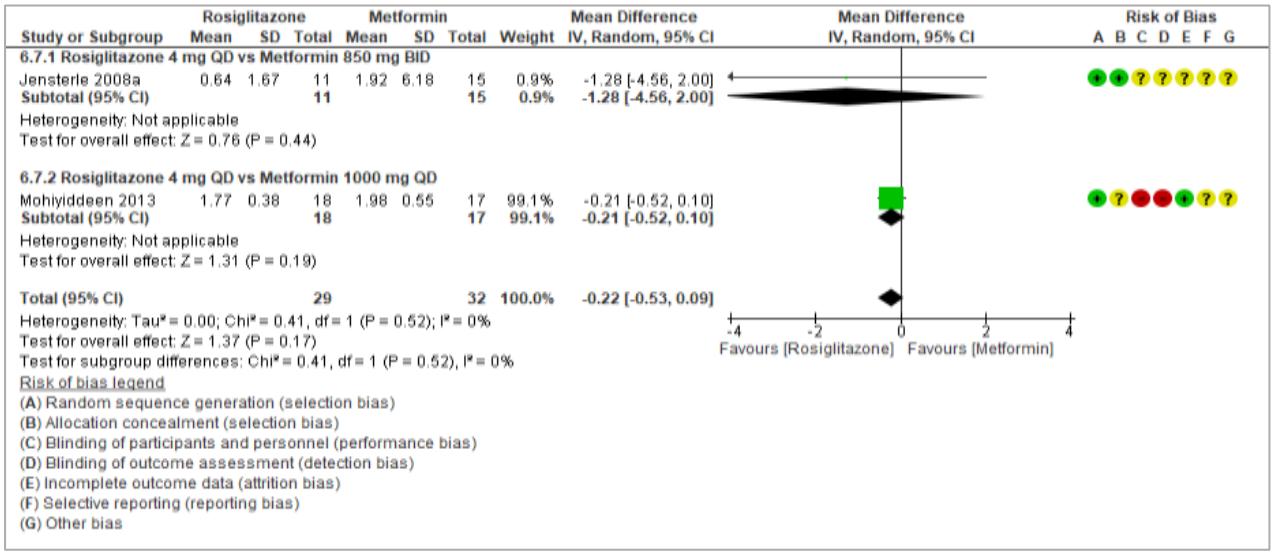
Figure 3-38: Forest plot of Exenatide versus Metformin on CRP (mg/L)



3.3.4.1.5.3 Rosiglitazone versus Metformin

In two RCTs compared rosiglitazone 4 mg QD with metformin 850 mg BID and metformin 1000 mg QD showed no effect on the mean CRP (MD: -0.22 mg/L; 95% CI: -0.53, 0.09, I^2 = 0%) (Figure 3-39) (very low-grade evidence, table 4).

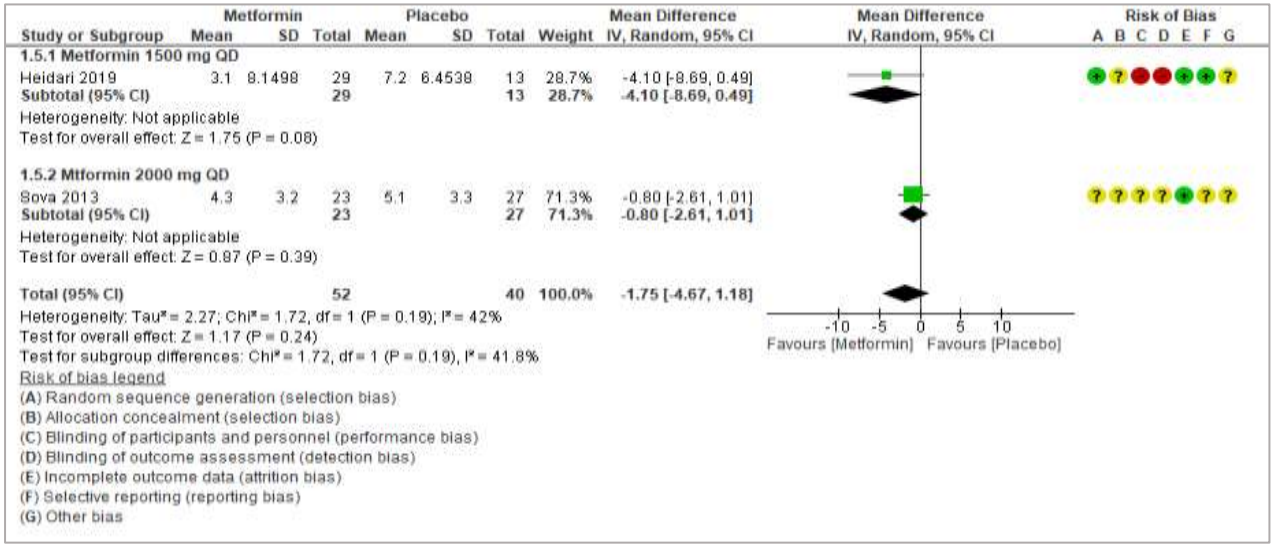
Figure 3-39: Forest plot of Rosiglitazone versus Metformin on CRP (mg/L)



3.3.4.1.5.4 Metformin versus placebo

In one RCT compared metformin 1500 mg QD with placebo showed significant reduction on the mean CRP by 4.10 mg/L (95% CI: -8.69, 0.49). In one RCT metformin 2000 mg QD had no effect on the mean CRP compared with placebo (MD: -0.80 mg/L (95% CI: -2.61, 1.01). Overall, metformin at various dosage insignificantly reduced the mean CRP by 1.75 mg/L (95% CI: -4.67, 1.18, I^2 = 41.8%) (Figure 3-40) (very low-grade evidence, table 4).

Figure 3-40: Forest plot of Metformin versus placebo on CRP (mg/L)



3.3.5 Sensitivity analysis

The effect of each RCT on heterogeneity and the strength of the result was reviewed by conducting a sensitivity analysis. Thus, small sample-sized RCTs and the one with an overall high RoB were eliminated from the meta-analysis while inspecting their impacts on the collective results. As a result, no substantial effect was found, and thus, no RCT was removed from the meta-analysis.

3.3.6 Publication bias

No assessment for publication bias was performed as there were fewer than 10 RCTs in each comparison.

Table 4: Summary of findings for the outcomes of the lipid profiles and CRP

Patient or population: PCOS **Setting:** **Intervention: First treatment (T1)** **Comparison: Second treatment (T2)**					
Outcome	**№ of participants (studies)**	**Certainty of the evidence (GRADE)**	**Relative effect (95% CI)**	**Anticipated absolute effects**	
				Assumed risk	
				Risk difference with intervention	**Risk difference with comparison**
Meformin versus placebo					**(T1 minus T2)**
CRP	92 (2 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean CRP was 5.1-7.2	MD 1.75 lower (4.67 lower to 1.18 higher)
Total cholesterol	358 (8 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean total Cholesterol was 4.42-190	MD 0.02 lower (0.3 lower to 0.26 higher)
Triglycerides	357 (8 RCTs)	⊕⊕⊕◯ MODERATE a	-	The mean triglycerides was 1-128.1	MD 0.09 lower (0.19 lower to 0.01 higher)
HDL	401 (8 RCTs)	⊕⊕◯◯ LOW a,c	-	The mean HDL was 1.2-51	MD 0 (0.09 lower to 0.1 higher)
LDL	226 (7 RCTs)	⊕⊕◯◯ LOW a,c	-	The mean LDL was 3.4-100.6	MD 0.41 lower (0.58 lower to 0.24 lower)
Metformin versus Pioglitazone					**(T1 minus T2)**
Total cholesterol	216 (5 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean total Cholesterol was 159.39-185.6	MD 3.34 lower (11.17 lower to 4.49 higher)
Triglycerides	181(4 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean triglycerides was 84-151.04	MD 7.68 higher (5.43 lower to 20.8 higher)
HDL	186 (4 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean HDL was 41.1-51.1	MD 0.29 lower (3.64 lower to 3.06 higher)
LDL	216 (5 RCTs)	⊕◯◯◯VERY LOW a,b,c	-	The mean LDL was 75.49- 116.1	MD 2.59 lower (10.42 lower to 5.24 higher)
Pioglitazone versus placebo					**(T1 minus T2)**
Total cholesterol	63 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean total Cholesterol was 4.7-4.8	MD 0.17 lower (0.4 lower to 0.05 higher)
Triglycerides	63 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean triglycerides was 1.3-1.3	MD 0.21 lower (0.39 lower to 0.03 lower)
Rosiglitazone versus Metformin					**(T1 minus T2)**
CRP	61 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean CRP was 1.92-1.98	MD 0.22 lower (0.53 lower to 0.09 higher)
HDL	61 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean HDL was 1.29-1.52	MD 0.03 higher (0.06 lower to 0.13 higher)
LDL	61 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean LDL was 3.24-3.35	MD 0.23 lower (0.37 lower to 0.09 lower)
Total cholesterol	61 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean total Cholesterol was 4.59-4.84	MD 0.38 higher (0.12 lower to 0.89 higher)
Liraglutide versus Liraglutide +Metformin					**(T1 minus T2)**
Total cholesterol	50 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean total Cholesterol was 4.5-4.6	MD 0.19 higher (0.27 lower to 0.65 higher)
Triglycerides	50 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean triglycerides was 1.2-1.4	MD 0.16 higher (0.49 lower to 0.81 higher)
HDL	50 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean HDL was 1.1-1.3	MD 0.07 lower (0.22 lower to 0.09 higher)
LDL	50 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean LDL was 2.7-2.9	MD 0.59 higher (0.19 lower to 1.38 higher)

<u>Exenatide versus Metformin</u>					**(T1 minus T2)**
CRP	221 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean CRP was 1.93-3.23	MD 0.33 lower (0.9 lower to 0.24 higher)
HDL	221 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean HDL was 1.35-1.43	MD 0.07 lower (0.19 lower to 0.05 higher)
LDL	221 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean LDL was 2.69-3.37	MD 0.09 higher (0.16 lower to 0.34 higher)
Total cholesterol	221 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean total Cholesterol was 4.49-4.62	MD 0.16 higher (0.06 lower to 0.37 higher)
Triglycerides	221 (2 RCTs)	⊕◯◯◯VERY LOW a,b,c	-	The mean Triglycerides was 1.34-1.53	MD 0.24 higher (0.21 lower to 0.69 higher)
<u>Saxagliptin versus Metformin</u>					**(T1 minus T2)**
Total cholesterol	65 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean total Cholesterol was 4.5-5	MD 0.15 lower (0.23 lower to 0.08 lower)
Triglycerides	65 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean triglycerides was 0.9-1.86	MD 0.01 lower (0.38 lower to 0.37 higher)
HDL	65 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean HDL was 1.06-1.41	MD 0.11 lower (0.15 lower to 0.06 lower)
LDL	65 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean LDL was 2.82-2.89	MD 0.02 higher (0.25 lower to 0.29 higher)
<u>Metformin versus Simvastatin</u>					**(T1 minus T2)**
HDL	268 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean HDL was 41.97- 52.87	MD 0.01 lower (2.39 lower to 2.36 higher)
Triglycerides	268 (2 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean triglycerides was 25.18-113.2	MD 8.04 lower (19.95 lower to 3.88 higher)
<u>Atorvastatin versus placebo</u>					**(T1 minus T2)**
CRP	65 (2RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean CRP was 5.7-1.3	MD 1.51 lower (3.26 lower to 0.24 higher)
LDL	65 (2RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean LDL was 3.0-1.8	MD 0.91 lower (1.04 lower to 0.79 lower)
HDL	85 (3 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean HDL was 153-0.21	MD 0.38 higher (0.29 lower to 1.05 lower)
Total cholesterol	85 (3 RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean Total cholesterol was221.7-3.4	MD 1.88 lower (3.86 lower to 0.11 higher)
Triglycerides	65 (2RCTs)	⊕◯◯◯ VERY LOW a,b	-	The mean triglycerides was 10.3-1.69	MD 0.59 lower (0.72 lower to 0.46 lower)

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI). **CI:** Confidence interval; **MD:** Mean difference. **T1:** first tretament, **T2:** second treatment.

GRADE Working Group grades of evidence

High certainty: We are very confident that the true effect lies close to that of the estimate of the effect

Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different

Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect

Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

Explanations

a. There is a high risk of performance bias across the studies. Thus, we downgraded one level.

b. Very high level of heterogeneity; we downgraded one level.

c. Very small sample size, significantly wider CI, we downgraded one level.

3.4 Discussion

This systematic review provides an overview of the current evidence on the effect of pharmacological interventions on the lipids profile and CRP of women with PCOS. In the current review, we found that when metformin and atorvastatin were administered at various doses, compared with placebo, there were significant reductions in the mean CRP, TC, TG, LDL-C and increase in HDL-C. In addition, saxagliptin, pioglitazone and rosiglitazone also showed significant reductions in the mean TC, TG and LDL-C compared with metformin or placebo.

Metformin significantly reduced the mean TC, TGs, LDL-C and incresed HDL-C. In a systematic review and meta-analysis of 12 RCTs, metformin significantly affected LDL-C reduction. However, no effect was seen for the other parameters of the lipid profiles (638). However, an RCT that compared metformin with placebo reported a significant increase in the mean HDL-C and a decrease in the mean TC (639,640). The lipid-lowering mechanism of action of metformin is that it activates the AMP-activated protein kinase (AMPK), which regulates the sterol regulatory element-binding protein-1 (SREBP-1) and inhibits the hepatic lipogenesis (641). In addition, statins reduce cholesterol production by competitively inhibiting the 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis (642).

Metformin at various therapeutic doses showed no effect on CRP compared with other agents. The subgroup analysis did not indicate any significant effect of metformin at various doses and durations on CRP compared with placebo. This is the converse to a meta-analysis of 20 RCTs (643) that assessed the effect of Metformin on CRP and reported a significant

reduction in CRP. However, in the above study, there was a significantly high level of heterogeneity among the studies due to the small smaple size ; therefore, care must be taken when interpreting the study results. Dawson et al. reported in an open clinical trial of exenatide (5 mcg BID administered for four weeks then titrated to 10 mcg for 12 weeks), there was significant reduction in CRP from baseline (8.5 ± 1.4 to 5.6 ± 0.8 mmol/L, p = 0.001)(644).

Conversely, in this study, we did not observe any effect for exenatide on CRP compared with metformin. Furthermore, no effect on CRP was seen in this study when rosiglitazone was also compared with metformin which differs from a study of rosiglitazone 4 mg QD as monotherapy administered for 12 months that showed a significant reduction in CRP (645). The review was conducted based on a systematic search for the related databases and grey sources. It also included RCTs and crossover trials, excluded observational and non-randomised studies. To date, this is the most inclusive systematic review and meta-analysis of the effect of pharmacological interventions on lipid profiles in women with PCOS. However, one of the limitations of this systematic review is that a language filter was applied, and only RCTs reported in the English language were included. This could have significantly affected the inclusion of several studies published in foreign languages. In addition, retrieving such studies require translation to English which could be challenging and may also influence the methodology of this review.

Moreover, we only included fully published studies, and there may be unpublished trials that could not be retrieved. The majority of the RCTs reported in this review had a small sample size and lacked statistical rigour used to identify sample size. Additionally, most of the RCTs had a short duration; thus, the long-term effect of the various pharmacological interventions on the lipid profiles in women with PCOS is not apparent. This systematic review recognises

the poor quality of the included RCTs, which is also shown in the summary of evidence of the GRADE score. Because of the design of some clinical trials (e.g. open-label), there was a substantially high level of performance bias. In some studies, reporting and the selection bias were inadequately evaluated, leading to the adjudication of an unclear RoB in 69% of the included RCTs. In addition, only 49% of the RCTs reported information of the method used to blind the participants and the outcome assessor and 45% were judged to have an unclear risk of attrition bias. For the lipid profile outcomes, the grade of evidence was rated as very low, low, or moderate due to the unclear or high risk of performance bias. There was a lack of blinding for the participants and the outcome assessors, lack of allocation, unclear risk of attrition bias, unclear risk of selective reporting and considerable heterogeneity.

This study highlights a lack of robust RCTs evaluating various pharmacological agents used in PCOS treatment. Moreover, currently available RCTs assessing the effectiveness of these pharmacological interventions are of low or very low quality. Therefore, the present results do not allow a definitive conclusion and recommendation for clinical practice. Furthermore, these RCTs are of a small sample size that may not have had the power to exclude false-negative outcomes. Thus, this review acknowledges the need for RCTs with rigorous design to facilitate a better-informed clinical decision to draw recommendations and to help develop guidelines.

3.5 Conclusion

Dyslipidaemia and a high level of CRP are associated with a significantly increased risk of cardiovascular disease (CVD). Data pooled in this review showed that metformin, atorvastatin, saxagliptin, rosiglitazone and pioglitazone have significant effects by reducing

the mean CRP, TC, TG, and LDL-C while increasing HDL-C. Therefore, these agents could potentially reduce the cardiovascular risk associated with PCOS.

4 Chapter 4: Impact of pharmacological interventions on insulin resistance in women with polycystic ovary syndrome: a systematic review and meta-analysis of randomised controlled trials

4.1 Introduction

Polycystic ovary syndrome (PCOS) is a complex disease that affects women of reproductive age with a prevalence of up to 13 % (646, 647). It has been estimated that 50-70% of women with PCOS exhibit metabolic abnormalities, including insulin resistance, abnormal glucose tolerance and an increased risk of type 2 diabetes mellitus (T2DM)(506). Insulin resistance results from an insulin action defect, including insulin-mediated glucose transport and signalling pathways (648). However, further evidence suggests a bidirectional link between hyperinsulinemia and androgen production, with high insulin stimulating ovarian androgen production (649). Acanthosis nigricans is a velvety or brownish-black skin lesion commonly seen around the neck, and it is a common sign of insulin resistance. The majority of obese and lean women with PCOS have been shown to have clinical evidence of acanthosis nigricans (72).

Moreover, hyperinsulinemia increases the risk of T2DM, and over 11% of overweight/obese women with PCOS develop diabetes (650). The liver has a significant role in regulating glucose and lipid metabolism, and the hepatic insulin effect is thought to be the primary driver of insulin resistance. In the postprandial state, the reduction in glucagon and the increase of insulin levels enhance hepatic glucose consumption, reduce hepatic glucose production and

store excess glucose as lipids and glycogen (651). Therefore, in disease states such as PCOS, obesity and diabetes, the insulin effect regulating hepatic metabolism will be affected, leading to excess glucose and lipid production, commonly referred to as hepatic insulin resistance (652). Insulin regulates lipid metabolism by regulating triacylglycerol secretion via the very-low-density lipoprotein cholesterol (VLDL-C)(653). Women with PCOS have an abnormal lipoprotein profile characterised by a high level of triglycerides, elevated low-density lipoprotein cholesterol (LDL-C) and reduced high-density lipoprotein cholesterol (HDL-C), which is a risk for cardiovascular disease (CVD)(282). The hyperinsulinemic glucose clamp is the standard method to determine insulin sensitivity in which concomitant glucose and insulin are infused, followed by measuring the insulin and glucose levels. Otherwise, the homeostasis model assessment (HOMA) is used as an alternative to determine insulin resistance (IR) and pancreatic β-cell function that shows a close correlation with the hyperinsulinemic glucose clamp (654).

Therapeutic approaches for PCOS are varied in their targets and effects and include pharmacological and non-pharmacological interventions. Metformin, a widely used insulin sensitising agent, has a beneficial effect on glucose metabolism and metabolic syndrome (393). At a molecular level, metformin activates AMP-activated protein kinase (AMPK), restoring the compromised energy balance by switching on the catabolic pathway, enhancing insulin sensitivity and reducing hepatic glucose production (655). A similar effect was also evident with thiazolidinediones (TZDs) such as pioglitazone and rosiglitazone (656). They alter gene transcription influencing glucose and lipid metabolism, and improve insulin resistance by activating the proliferator-activated receptor gamma (PPAR-gamma)(657). Statins may have a place in PCOS management because of their mode of action to reduce total cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C)(459). Recently, N-

acetylcysteine (NAC), a mucolytic agent with insulin-sensitising properties, has been used as a supporting agent in managing PCOS, particularly in clomiphene resistance (658). However, the relative effectiveness of these therapeutic options remains elusive, with a significant gap in the available evidence; therefore, this review aimed to evaluate and analyse the available evidence for the effectiveness of various pharmacological options for the treatment of insulin resistance in PCOS.

4.2 Methods and materials

4.2.1 Protocol and registration

The protocol and the registration of this systematic review and meta-analysis are explained in chapter 2, section 2.1.1.1.

4.2.2 Eligibility criteria for the included studies

The eligibility criteria, including inclusion/exclusion criteria, is explained in chapter 2, section 2.1.1.2.

4.2.3 Literature search

The search strategy, including the search in the medical databases, is explained in chapter 2, section 2.1.1.3.

4.2.4 Study selection

The study selection, including screening titles and abstracts of the retrieved studies, is explained in chapter 2, section 2.1.1.4.

4.2.5 Data extraction

The method used to extract information from the included studies is explained in chapter 2, section 2.1.1.5.

4.2.6 Risk of bias assessment in the included studies

The RoB assessment for the included studies is explained in chapter 2, section 2.1.1.6.

4.2.7 GRADE scoring

The robustness of evidence for each chosen outcome is assessed using the GRADE scoring system and is explained in chapter 2, section 2.1.1.7.

4.2.8 Statistical analysis

The statistical method used to estimate the pooled effects of the various interventions is explained in chapter 2, section 2.1.1.12.

4.2.9 Assessment of heterogeneity

Heterogeneity for the outcomes across each RCT was evaluated and explained in chapter 2, section 2.1.1.8.

4.2.10 Subgroup analysis

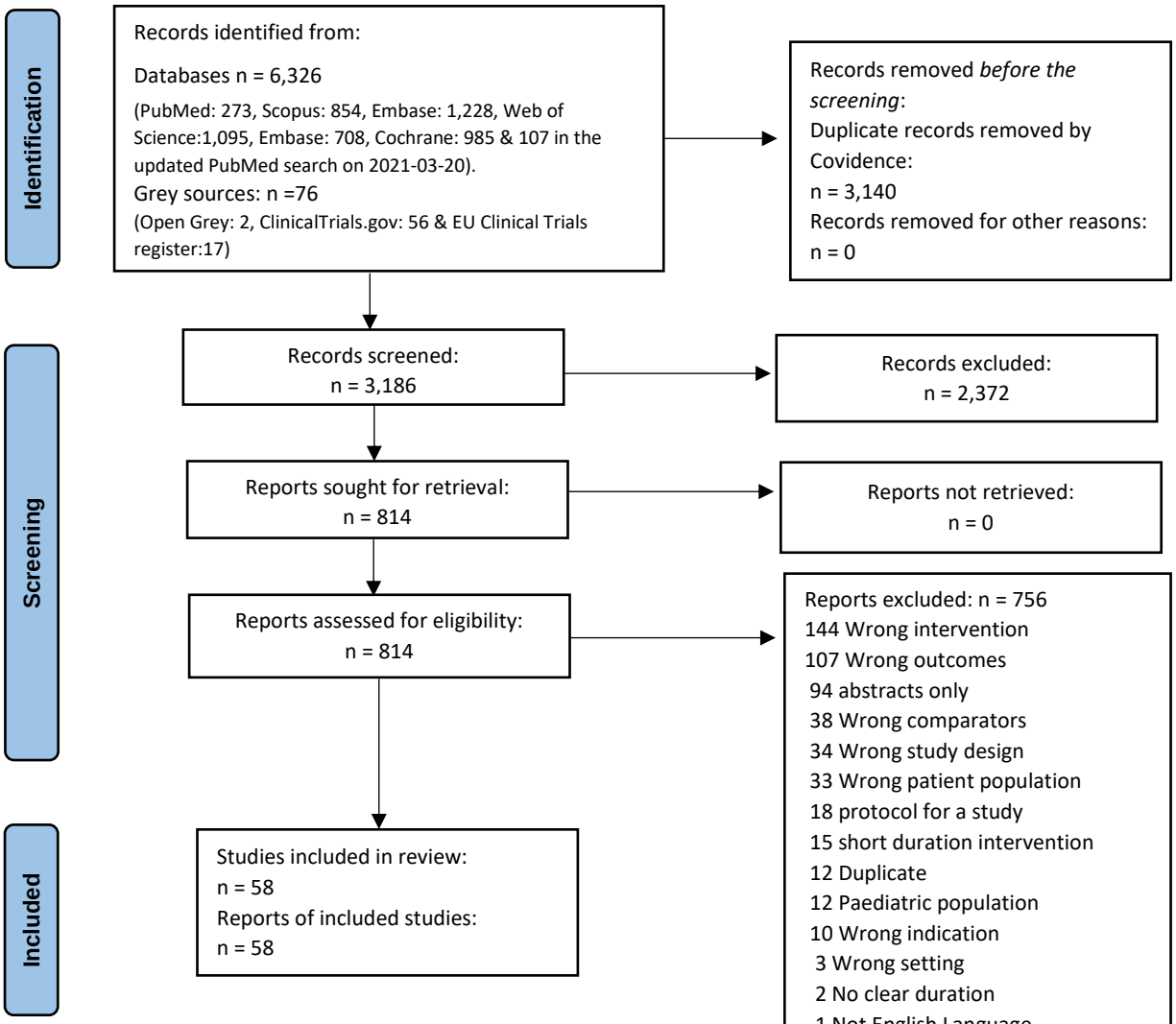
Subgroup analysis was conducted for the included RCTs and explained in chapter 2, section 2.1.1.9.

4.3 Results

4.3.1 Search results

In total, 6,326 articles were identified from the database search, of which 3,186 were screened for eligibility based on titles and abstracts after removing duplicates. In addition, 814 full-text articles were retrieved for detailed assessment for eligibility, of which 58 RCTs were found eligible and included in the study (Figure 4-1).

Figure 4-1: PRISMA flow diagram



4.3.2 Characteristics of the included studies

The 58 RCTs were published until 2020, of which 35 RCTs (60.3%) (607,608,610-613,615-620,622,659-678) diagnosed PCOS based on the Rotterdam criteria 2003 (30); nine RCTs (15.5%)(623,625,679-685) used the National Institute of Health 1990 (NIH, NICHD) criteria (626) while no diagnostic criteria were given for the rest of the RCTs (Table 5)**.**

4.3.3 Interventions and comparisons details

Sixteen (27.5%) RCTs assessed the effect of metformin compared with placebo (607, 610, 616, 618, 625, 627-630, 663, 670, 673, 682, 686-689). Six (10%) RCTs evaluated metformin compared with pioglitazone (615, 620, 632, 677, 686, 690). Four (6.8%) RCTs assessed pioglitazone compared with placebo (608, 633, 637, 679). Eight (13.8%) RCTs examined rosiglitazone compared with metformin (612, 622, 659, 672, 678, 681, 685, 691). Three (5.2%) RCTs assessed liraglutide compared with liraglutide added to metformin (623, 668, 669). Two (3.4%) RCTs examined sitagliptin compared with placebo (661, 666). Two (3.4%) RCTs assessed exenatide compared with metformin (611, 619). Two (3.4%) RCTs compared orlistat with placebo (662, 674). Three (5.2%) RCTs examined acarbose with metformin (676, 680, 684). Two (3.4%) RCTs compared saxagliptin with metformin (617, 664). Two (3.4%) RCTs compared simvastatin with metformin (634, 683). Two (3.4%) RCTs assessed metformin with N-Acetylcysteine (NAC) (667, 675). Two (3.4%) RCTs examined atorvastatin compared with placebo (613, 636). Two (3.4%) RCTs assessed sitagliptin added to metformin compared with metformin alone (665, 692). Two (3.4%) RCTs examined acarbose compared with placebo (693, 694).

4.3.4 Characteristics of the outcomes measured

All RCTs were assessed outcomes at baseline and post-intervention. Forty-six (79.3%) RCTs reported changes in FBG (607, 608, 610, 611, 613, 615-619, 627-629, 632, 634, 636, 637, 661-664, 666-670, 672-677, 679, 683-685, 689-691). Forty-eight (82.8%) RCTs reported FI (607, 608, 610-613, 615, 616, 618, 619, 622, 623, 625, 628-630, 632, 633, 636, 637, 659, 661-663, 666-669,671-675,677-682,684-688,690).Thirty-seven (63.8%) RCTs reported the homeostatic model assessment of insulin resistance (HOMA-IR) (608, 610, 611, 615, 617-620, 622, 623, 627, 628, 630, 632, 637, 660-666, 668, 669, 673,674, 681, 685, 686,691-696). Two (3.4%) RCTs reported the homeostatic model assessment of β-cells (HOMA-B) (628, 660).Table 4 presents more descriptive information on the included 58 RCTs.

Table 5: Characteristics of the studies included in the systematic review and meta-analysis

Author	Study design	Country	POCS diagnostic Criteria	Participants characteristics (PCOS) mean±SD	Interventions	Durations	Outcomes
Amiri et al (607)	RCT	Iran	Rotterdam	Age:25.6±4.02 BMI: 28.9±5	Metf, Flu, Metf+ Flu, Placebo	6 months	FBG
Aroda et al (679)	RCT	USA	NIH	Age: 27.87 ±0.87 BMI: 36.29 ±1.34	Piog, Placebo	6 months	FBG,FI
Brettenthaler et al (608)	RCT	Switzerland	Rotterdam	Age: 30.2± 1.4 BMI: 29.4± 1.7	Piog, placebo	3 months	FBG, FI, HOMA-IR
Cetinkalp et al (659)	RCT	Turkey	Rotterdam	Age: N/A BMA:25.82±6.12	Met, Rosigl , ECA	4 months	FBG,FI, HOMA-IR
Cheng et al (660)	RCT	Australia	Rotterdam	Age: 26 ± 4 BMI:24.2±5.3	Metf, placebo	6 months	HOMA-IR, HOMA-B
Cho et al (686)	RCT	UK	Rotterdam	Age: 26·4 ± 1·5 BMI: 36·0 ± 1·2	Metf, Orlistat, Piog	12 weeks	HOMA-IR
Ciotta et al (693)	RCT	Italy	N/A	Age:20.5±0.6 BMI:22.7±0.34	Acarbose, Placebo	3 months	HOMA-IR
Devin et al (661)	RCT-crossover	USA	Rotterdam	Age: N/A BMI:N/A	Sitag, placebo	4 weeks	FBG
Diamanti-Kandarakis et al (662)	RCT	Greece	Rotterdam	Age: 27·52 ± 5·77 BMI: 35·43 ± 5·3	Orli, placebo	6 months	HOMA-IR
Eisenhardt et al(663)	RCT	Germany	Rotterdam	Age: 27.0±0 BMI: 28.9±0	Metf,placebo	12 weeks	FBG.FI,HOMA-IR
Elkind-Hirsch et al(664)	RCT	USA	Rotterdam	Age: 28.2 ± 1.1 BMI: 39.9 ±1.5	Exen, Metf,Exen+Metf	24 weeks	FBG
Ferjan et al(666)	RCT	Slovenia	Rotterdam	Age: 34.3 ± 6.8 BMI: 36.3 ±5.2	Metf, Metf+Sitag	12 weeks	HOMA-IR
Ferjan et al(665)	RCT	Slovenia	Rotterdam	Age: 35.0 ± 7.2 BMI: 36.9 ± 5.5	Sitag, placebo	12 weeks	HOMA-IR, HOMA-B, FBG
Gambineri et al(627)	RCT	Italy	N/A	Age: 27·1 ± 3·6 BMI: 37·6 ± 4·1	Plac, Metfo, Flut, Metf + Flut	6 months	FBG,FI,HOMA-IR
Glintborg et al(637)	RCT	USA	N/A	Age: 32±0 BMI: N/A	Piog, placebo	16 weeks	FI, HOMA-IR
Glintborg et al(633)	RCT	USA	N/A	Age: 32±0 BMI: 32.2±0	Piog,plcebo	16 weeks	FI
Hanjalic-Beck et al(680)	RCT	Germany	NIH	Age: N/A BMI:N/A	Metf, Acarbose	12 weeks	FBG,FI

Heidari et al(610)	RCT	USA	Rotterdam	Age: 32.4±7.5 BMI: 37.1±9.1	Metf, placebo	3 months	FBG, FI
Javanmanesh et al(667)	RCT	Iran	Rotterdam	Age: 29.75 ± 4.90 BMI: 29.05 ± 2.80	Metf, NAC	24 weeks	FBG,FI, HOMA-IR
Jayagopal et al(695)	RCT	UK	N/A	Age: 27 ±0.9 BMI: 36.7 ±3.3	Orlistat, Metf	3 months	FBG, FI
Jensterle et al(833)	RCT	Slovenia	NIH	Age: 27.6±7.2 BMI: 39.5±6.2	Metf, Rosi	6 months	FBG, FI
Jensterle et al(778)	RCT	Slovenia	Rotterdam	Age: 30.7 ± 7.9 BMI: 38.6 ± 6.0	Metfo, Rosi	6 months	FI, FBG, HOMA-IR
Jensterle et al(821)	RCT	Slovenia	Rotterdam	Age: 33.1 ± 6.1 BMI: 37.2±4.5	Met+Lira, Lira	12 weeks	FBG,FI, HOMA-IR
Jensterle et al(587)	RCT	Slovenia	Rotterdam	Age: 34.4 ± 6.5 BMI: 39.0 ± 4.9	Met+Lira,Lira	12 weeks	FI,FBG
Jensterle Sever et al(779)	RCT	Slovenia	NIH	Age: 31.3±7.1 BMI: 37.1±4.6	Lira,Metf, Lira+Metf	12 weeks	FBG,FI
Kazerooni et al(822)	RCT	Iran	Rotterdam	Age: 25.6± 4.32 BMI: 28.52± 1.61	Metf, Simva,placebo	12 weeks	FI,FBG
Kocak et al(823)	RCT	Turkey	Rotterdam	Age: 26.2 ±3.7 BMI: 31.91± 5.38	Metf, placebo	2 months	FI,FBG
Ladson(834)	RCT	USA	NIH	Age: 29±4.5 BMI: 38±7.8	Metfo, placebo	6 months	FBG, FI
Li et al(824)	RCT	China	Rotterdam	Age: 25.95± 4.36 BMI: 27.54 ±2.21	Rosi, Metformin	6 months	FI,FBG
Lingaiah et al(825)	RCT	Finland	Rotterdam	Age: 27.6 ±4.0 BMI: 26.5 ±6.0	Metf, placebo	3 months	FI,FBG
Liu et al(767)	RCT	China	Rotterdam	Age: 27.69 ± 3.80 BMI: 28.29 ± 1.86	Metf, Exena	24 weeks	FI,FBG, HOMA-IR
Lord et al(784)	RCT	UK	N/A	Age: 27.76 ±4.89 BMI: 33.74± 6.74	Metf, placebo	3 months	FI,FBG, HOMA-IR
Mehrabian et al(835)	RCT	Iran	NIH	Age: 29.18±8.28 BMI: 29.83±4.1	Metf, Flut, Simva	6 months	FBG
Moghetti et al(781)	RCT	Italy	NICHD	Age: 23.9 ± 1.2 BMI: 27.1 ±6 1.5	Metformin , placebo	6 months	FBG, FI
Mohiyiddeen et al(768)	RCT	UK	Rotterdam	Age: 29.0 ±1.0 BMI: 29.7 ±1.0	Metf,Rosig	3 months	FI,FBG
Moini et al(826)	RCT	Iran	Rotterdam	Age: 27.42 ± 3.31 BMI: 29.01 ± 2.09	Orlistat, placebo	3 months	FI,FBG
Naka et al(842)	RCT	Greece	N/A	Age: 23.3± 4.9 BMI: 28.7± 5.5	Metf,Piogl	6 months	FI,FBG

Navali et al(790)	RCT	Iran	N/A	Age:26.43±4.67 BMI:27.71±0.73	Metf, Simva	3 months	FI,FBG
Nemati et al(827)	RCT	Iran	Rotterdam	Age: N/A BMI: 36.3± 8.4	Metf, NAC	12 weeks	FBG,FI
Ng et al(785)	RCT	China	N/A	Age:30.5±0 BMI:N/A	Metf, placebo	3 months	FBG,FI
Ortega-González et al(788)	RCT	Mexico	N/A	Age: 28.8 ±0.9 BMI: 32.2 ±1.0	Metf, Piogl	6 months	FBG, FI
Paredes Palma et al(845)	RCT	Mexico	N/A	Age: N/A BMI: N/A	Metf, Sitag	N/A	HOMA-IR
Penna et al(847)	RCT	Brazil	N/A	Age: 26.69 ±1.46 BMI: 35.8± 2.60	Acarbose, placebo	6 months	FI
Puurunen et al(792)	RCT	Finland	N/A	Age: 40.5 ±5.9 BMI: 30.4 ±8.6	Atorva, placebo	6 months	FI, HOMA-IR
Rezai et al(828)	RCT	Iran	Rotterdam	Age: 26.3±4 BMI: 26.9 ± 1.8	Metf, Acarbose	3 months	FBG
Sathyapalan et al(769)	RCT	UK	Rotterdam	Age: 27.7± 1.4 BMI: 33.20 ±1.4	Atorvas, placebo	12 weeks	HOMA-IR, FBG,FI
Shahebrahimi et al(829)	RCT	Iran	Rotterdam	Age: 27.5 ± 3.68 BMI: 27.71±4.36	Metf, Piog	3 months	FBG
Sohrevardi et al(771)	RCT	Iran	Rotterdam	Age: N/A BMI: 27.5±3.6	Metf,Piog, Metf+Piog	3 months	HOMA-IR, FBG, FI
Sönmez et al(836)	RCT	Turkey	NIH	Age: 26.13 ±5.08 BMI: 27 ±2.2	Metf, Acarbose	3 months	FBG,FI
Sova et al(772)	RCT	Finland	Rotterdam	Age: : 27.7 ±4.0 BMI: 27.5 ±6.2	Metf, placebo	3 months	FBG,FI
Steiner et al(837)	RCT	Germany	NIH	Age: 22.9±4.5 BMI: 27.4±6.0	Metf, Rosig	6 months	HOMA-IR, FBG,FI
Tao et al(773)	RCT	China	Rotterdam	Age: 30 ± 5 BMI: 27.2±0	Saxag, Metf	24 weeks	HOMA-IR
Trolle et al(786)	RCT	Denmark	N/A	Age: 31±0 BMI:32±0	Metf, placebo	6 months	FBG,FI,HOMA-IR
Underdal et al(774)	RCT	Denmark	Rotterdam	Age: 29.5 ±3.9 BMI: 28.7± 6.9	Metf, placebo	N/A	FBG, FI
Vandermolen et al(840)	RCT	USA	N/A	Age: 29 6 ±1.2 BMI: 37.6 ± 4.3	Metf, placebo	7 weeks	FBG,FI
Yarali et al(839)	RCT	Turkey	N/A	Age:29.7±5.6 BMI:28.6±4	Metf, placebo	6 weeks	FBG,FI
Yilmaz et al(830)	RCT	Turkey	Rotterdam	Age: 24.67+4.60 BMI: 27.12+6.18	Metf, Rosig	24 weeks	FBG,FI

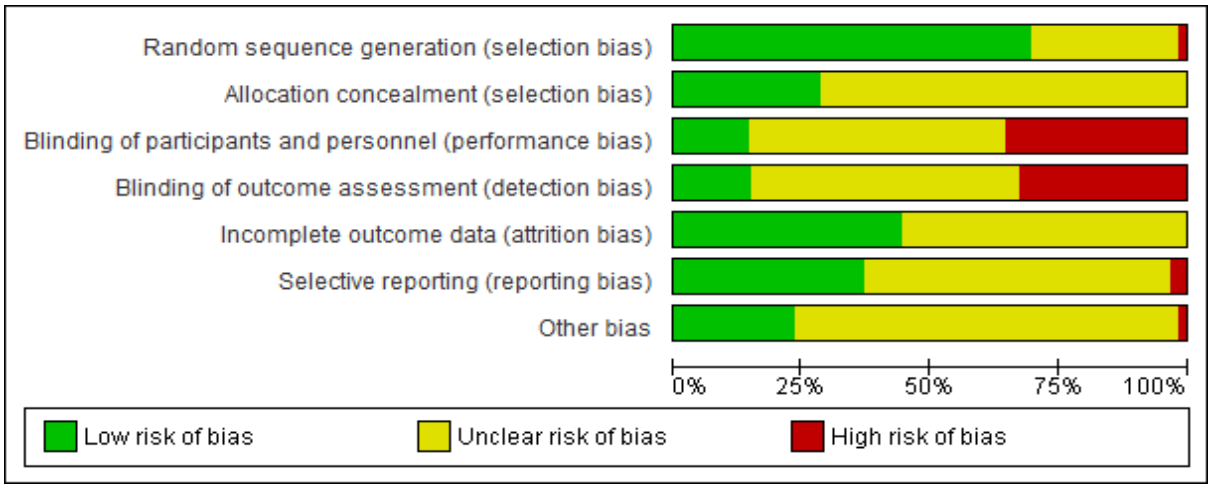
Zheng et al(775)	RCT	China	Rotterdam	Age: 27.70 ± 3.41 BMI: 28.27 ± 4.85	Exena, Metf	12 weeks	FBG,FI
Ziaee et al(776)	RCT	Iran	Rotterdam	Age: 25.28±4.38 BMI: 26.13 ±3.03	Metf, Piog	12 weeks	HOMA-IR

RCT: randomised clinical trial, **N/A**: not available, **FBG**: fasting blood glucose, **FI**: fasting insulin, **HOMA-IR:** the homeostatic model assessment of insulin resistance**, NIH**: national institute for health, **NICHD**: national institute of child health and development. **Metf:** metformin, **Saxa:** saxagliptin,**Piog**: pioglitazone, **Rosig**: rosiglitazone,**Atrova:** atorvastatin, **Simva**: simvastatin, **WHO:** world health Organisation, **Lira**: liraglutide, **USA:** United States of America, **UK**: United Kingdom, **HOMA-β: the** homeostatic model of the beta-cell.

4.3.5 Risk of bias assessment

The overall RoB is presented in Figure 4-2**.** One RCT was judged to have a high risk of selection bias due to an inappropriate method used to generate sequences (679). Twenty-one RCTs were judged to have a high risk of performance bias due to lack of blinding the participants (610-612, 615, 617, 619, 620, 623, 632, 662, 663, 665, 666, 668, 669, 672, 686, 690, 695). Nineteen RCTs were judged to have a high risk of detection bias due to lack of blinding outcome assessors (607, 610-612, 615, 617, 619, 623, 632, 665, 666, 668, 669, 672, 686, 690, 695). Two RCTs were judged to have a high risk of selective reporting (622, 634). Low RoB was judged for the majority of domains among the included RCTs. However, an unclear RoB was also judged due to insufficient reporting.

Figure 4-2: Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies



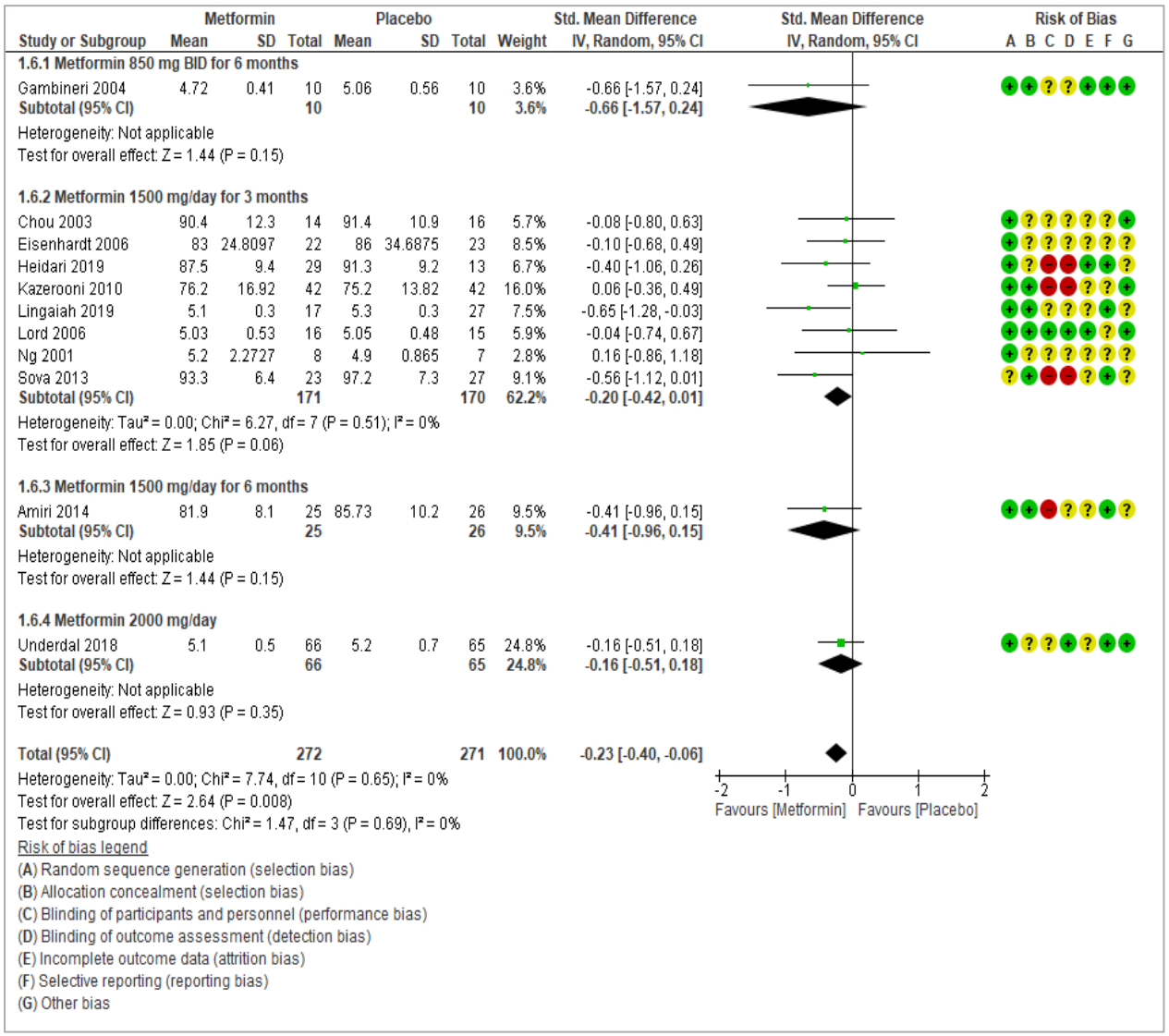
4.3.6 Effects of interventions on the insulin resistance outcomes

4.3.6.1 Fasting Blood Glucose (FBG)

4.3.6.1.1 Metformin versus placebo

In one RCT, metformin 850 mg BID for six months was associated with an insignificant reduction in the mean FBG (SMD: -0.66; 95% CI: -1.57, 0.24). In eight RCTs and compared with placebo, metformin 1500 mg QD for three months was associated with a significant reduction in the mean FBG (SMD: -0.20; 95% CI: -0.42, 0.01). In one RCT, metformin 1500 mg QD for six months was associated with an insignificant reduction in the mean FBG (SMD: -0.41; 95% CI: -0.96, 0.15). In one RCT, metformin 2000 mg QD was associated with an insignificant reduction in the mean FBG (SMD: -0.16; 95% CI: -0.51, 0.18). Overall, regardless of the administered dosage and duration, metformin was associated with a significant reduction in the mean FBG (SMD: -0.23; 95% CI: -0.40, -0.06, I^2 = 0%) in women who received metformin compared with women who received placebo (Figure 4-3) (low-grade evidence, table 6).

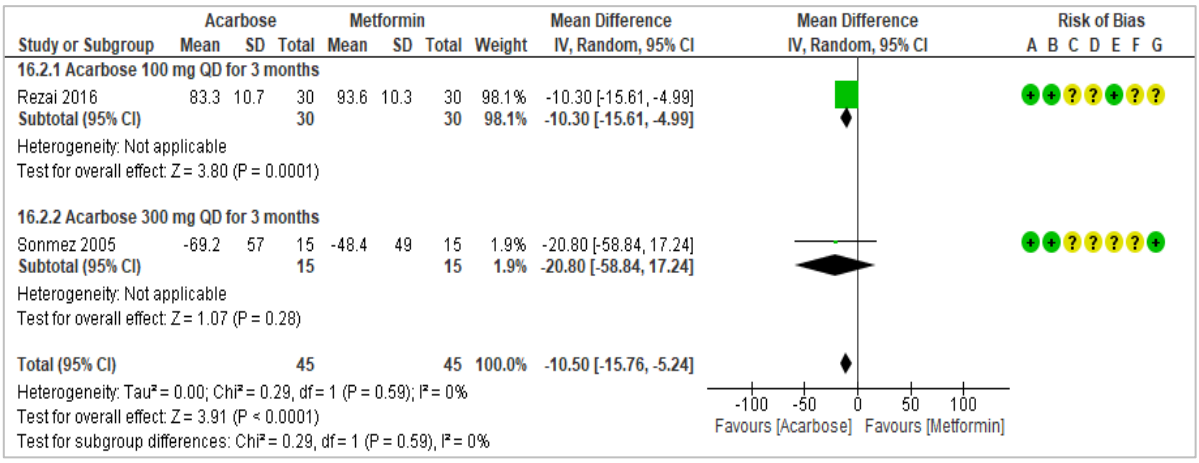
Figure 4-3: Forest plot of Metformin versus placebo on FBG



4.3.6.1.2 Acarbose versus Metformin

In one RCT, Acarbose 100 mg QD for three months significantly reduced the mean FBG (MD: -10.30 mg/dL; 95% CI: -15.61, -4.99) compared with metformin. In one RCT Acarbose 300 mg QD for three months had no effect on the mean FBG (MD: -20.80 mg/dL; 95% CI: -58.84, 17.24). However, in the two RCTs, regardless of the dosage, frequency, and duration, acarbose showed a significant reduction in the mean FBG (MD: -10.50 mg/dL; 95% CI: -15.76, -5.24, I^2 = 0%) (Figure 4-4) (low grade evidence, table 6).

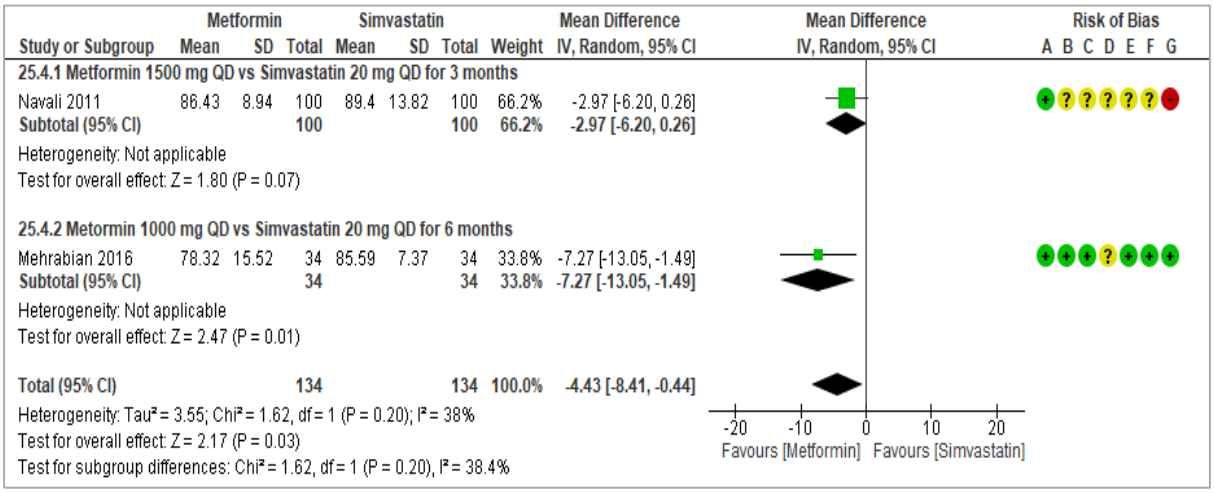
Figure 4-4: Forest plot of Acarbose versus Metformin on FBG (mg/dL)



4.3.6.1.3 Metformin versus Simvastatin

A significant reduction in the FBG level was also evident when metformin at various dosages was compared with Simvastatin 20 mg QD. In one RCT, metformin 1500 mg QD for three months significantly reduced the mean FBG (MD: -2.79 mg/dL; 95% CI: -6.20, 0.26). In one RCT, metformin 1000 mg QD for six months significantly reduced the mean FBG by 7.27 mg/dL (95%CI: -13.05, -1.49). Overall, regardless of the dosage and duration, metformin significantly reduced the mean FBG compared to simvastatin (MD: -4.43 mg/dL; 95% CI: -8.41, -0.44, I^2 = 38%) (Figure 4-5) (very low-grade of evidence, table 6).

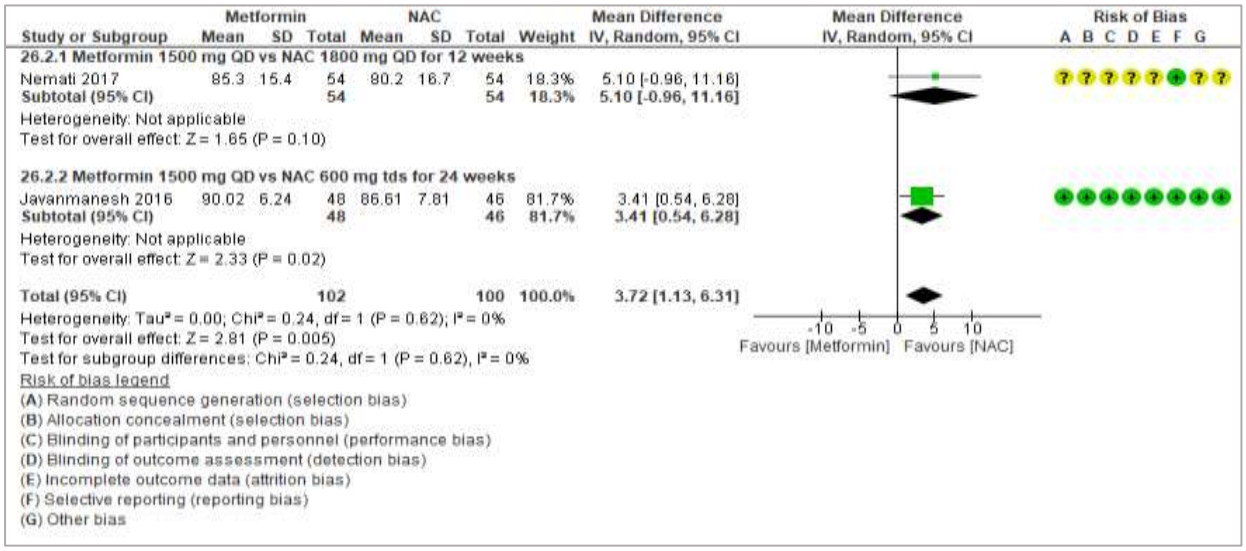
Figure 4-5: Forest plot of Metformin versus Simvastatin on FBG (mg/dL)



4.3.6.1.4 Metformin versus N-Acetylcysteine (NAC)

There was a significant increase in the mean FBG when Metformin was compared with NAC. In one RCT, when metformin 1500 mg QD was compared with NAC 1800 mg QD for 12 weeks, metformin significantly increased the mean FBG level (MD: 5.10 mg/dL; 95% CI: -0.96, 11.16). In another RCT, metformin 1500 mg QD was compared with NAC 600 mg TDS for 24 weeks and showed a significant increase in the mean FBG level (MD: 3.41 mg/dL; 95% CI: 0.54, 6.28). Overall, metformin significantly increased the mean FBG level (MD: 3.72 mg/dL; 95% CI: 1.13, 6.31, I^2 = 0%) compared with NAC (Figure 4-6) (very low-grade of evidence, table 6).

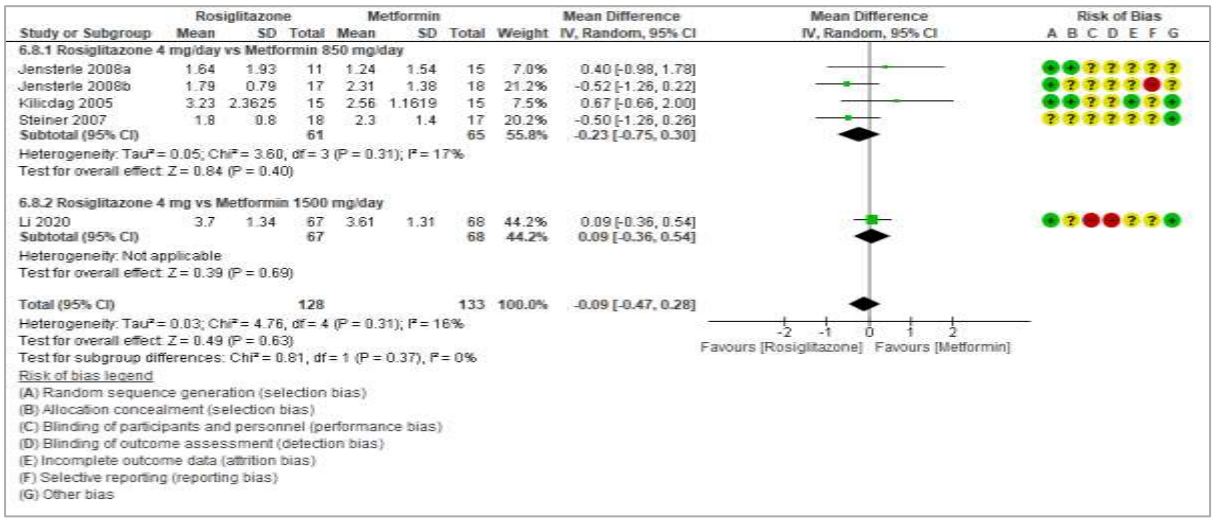
Figure 4-6: Forest plot of Metformin versus NAC on FBG (mg/dL)



4.3.6.1.5 Rosiglitazone versus Metformin

In four RCTs rosiglitazone 4 mg QD compared with metformin 850 mg QD has no effect on the mean FBG (MD: -0.23 mmol/L; 95% CI: -0.75, 0.30). In one RCT compared rosiglitazone 4 mg QD with metformin 1500 mg QD has no effect on the mean FBG (MD: 0.09 mmol/L; 95% CI: -036, 0.54). Overall, rosiglitazone 4 mg compared with various dosage of metformin has no effect on the mean FBG (MD: -0.09 mmol/L; 95% CI: -0.47, 0.28, I^2 = 0%) (Figure 4-7) (low grade evidence, table 6).

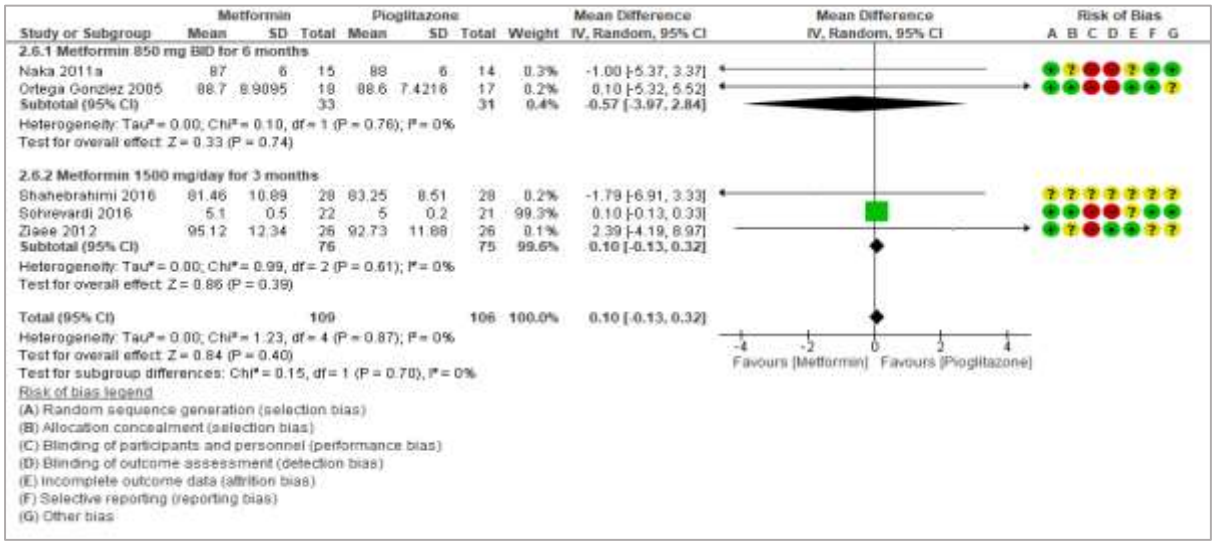
Figure 4-7: Forest plot of Rosiglitazone versus Metformin on FBG (mmol/L)



4.3.6.1.6 Metformin versus Pioglitazone

When metformin 850 mg BID for six months was compared with pioglitazone in two RCTs, it showed no effect on the mean FBG (MD: -0.57 mg/dl; 95% CI: -3.97, 2.84). In four RCTs, metformin 1500 mg QD for three months was compared with pioglitazone showed no effect on the mean FBG (MD: 0.10 mg/dl; 95% CI: -0.13, 0.32). Overall, metformin at various dosage had no effect on the mean FBG when compared with pioglitazone (MD: 0.10 mg/dl; 95% CI: -0.13, 0.32, I^2 = 0%) (Figure 4-8) (low grade evidence, table 6).

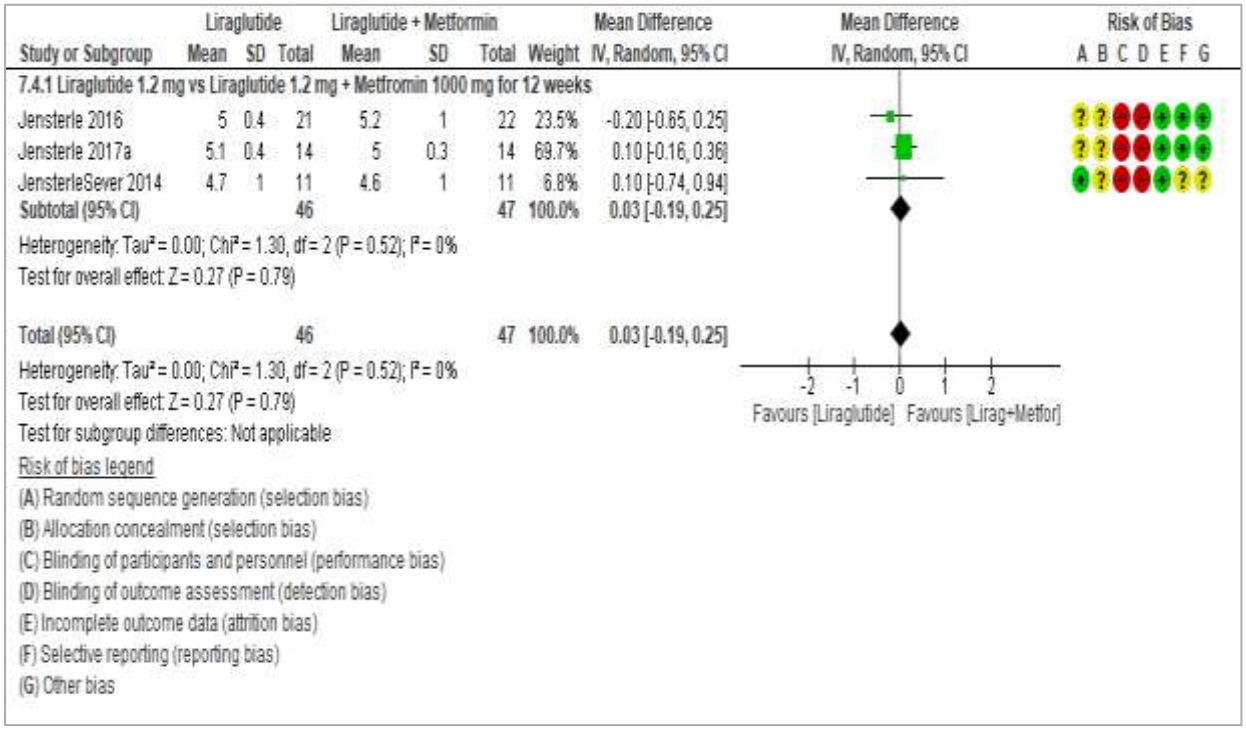
Figure 4-8: Forest plot of Metformin versus Pioglitazone on FBG (mg/dL)



4.3.6.1.7 Liraglutide versus Liraglutide + Metformin

In three RCTs compared liraglutide 1.2 mg QD with liraglutide 1.2 mg QD added to metformin 1000 mg QD for 12 weeks showed no effect on the mean FBG (MD: 0.03 mmol/L; 95% CI: -0.19, 0.25, I^2 = 0%) (Figure 4-9) (low grade evidence, table 6).

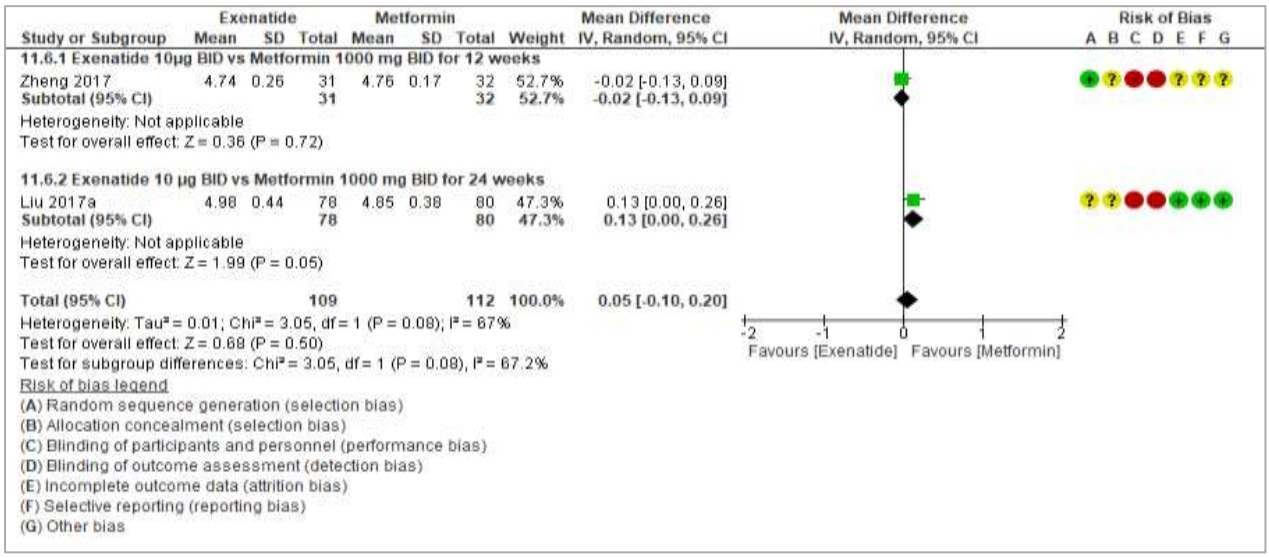
Figure 4-9: Forest plot of Liraglutide versus Liraglutide + Metformin on FBG (mmol/L)



4.3.6.1.8 Exenatide versus Metformin

In one RCT compared exenatide 10 µg BID with metformin 1000 mg BID for 12 weeks showed no effect on the mean FBG (-0.02 mmol/L; 95% CI: -0.13, 0.09). In one RCT exenatide 10 µg BID compared with metformin 1000 mg BID for 24 weeks showed increase in the mean FBG (MD: 0.13 mmol/L; 95% CI: 0.00, 0.26). Overall, exenatide 10 µg BID compared with metformin 1000 mg BID for various duration has no effect on the mean FBG (MD: 0.05 mmol/L; 95% CI: -0.10, 0.20, I^2 = 67.2%) (Figure 4-10) (very low-grade evidence, table 6).

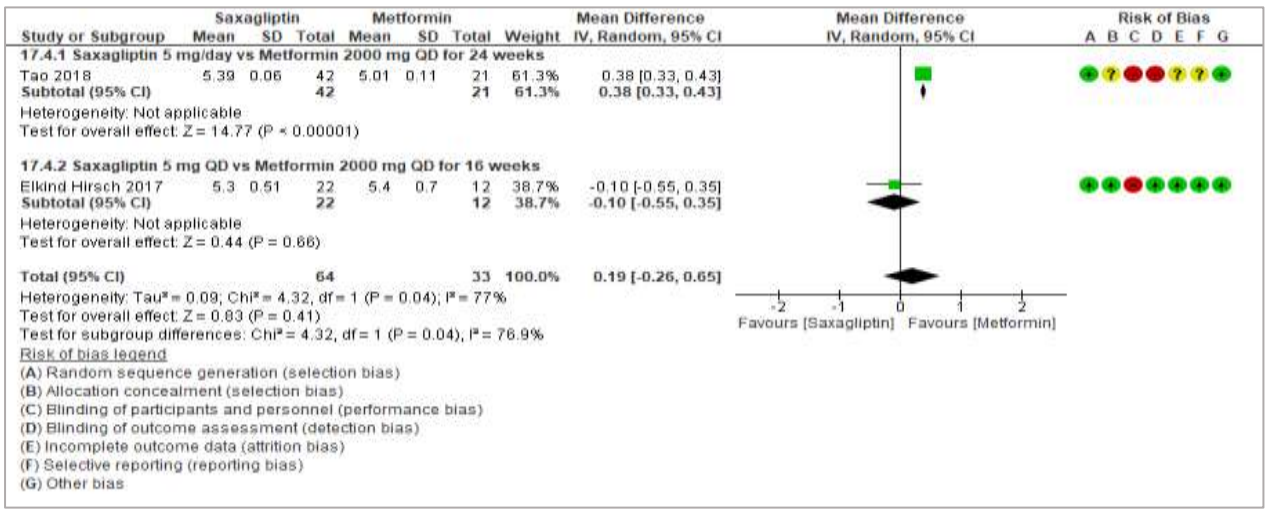
Figure 4-10: Forest plot of Exenatide versus Metformin on FBG (mmol/L)



4.3.6.1.9 Saxagliptin versus Metformin

One RCT compared saxagliptin 5 mg QD compared with metformin 2000 mg QD for 24 weeks has increased the mean FBG (0.38 mmol/L; 95% CI: 0.33, 0.43). One RCT, saxagliptin 5 mg QD compared with metformin 2000 mg QD for 16 weeks, has no effect on the mean FBG (MD: -0.10 mmol/L; 95%CI: -0.55, 0.35). Overall, saxagliptin 5 mg QD compared with metformin 2000 mg QD has no effect on the mean FBG (MD: 0.19 mmol/L; 95% CI: -0.26, 0.65, I^2 = 76.9%) (Figure 4-11) (very low-grade evidence, table 6).

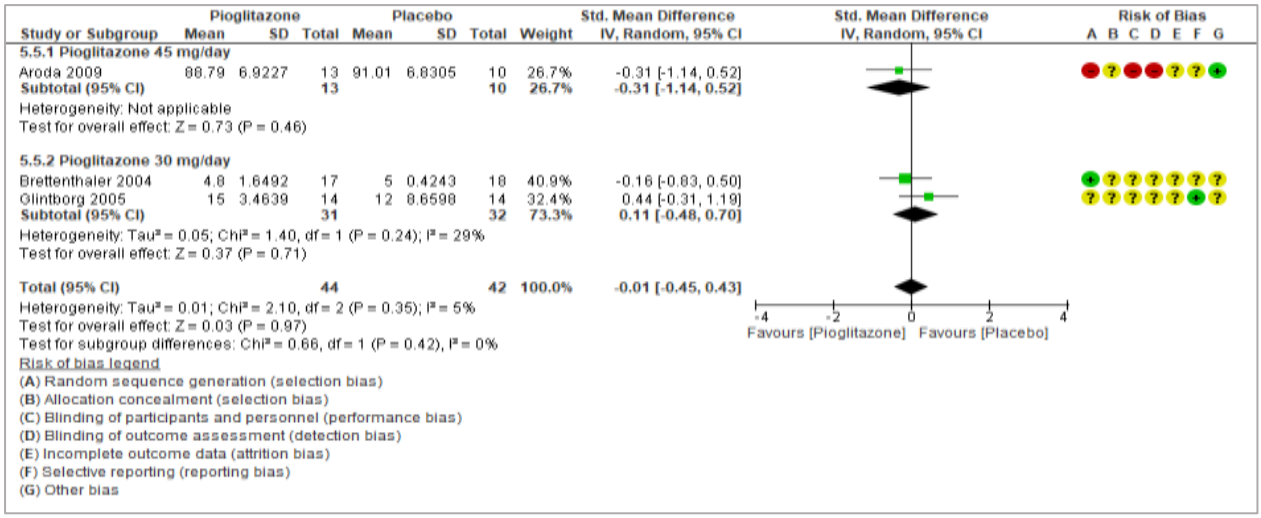
Figure 4-11: Forest plot of Saxagliptin versus Metformin on FBG (mmol/L)



4.3.6.1.10 Pioglitazone versus placebo

In one RCT, pioglitazone 45 mg QD showed no effect on the mean FBG compared with placebo (SMD: -0.31; 95% CI: -1.14, 0.52). In two RCTs, pioglitazone 30 mg QD showed no effect on the mean FBG compared with placebo (SMD: 0.11; 95% CI: -0.48, 0.70). Overall, pioglitazone of various dosage has no effect on the mean FBG compared with placebo (SMD: -0.01; 95% CI: -0.45, 0.43, I^2 = 0%) (Figure 4-12) (low grade evidence, table 6).

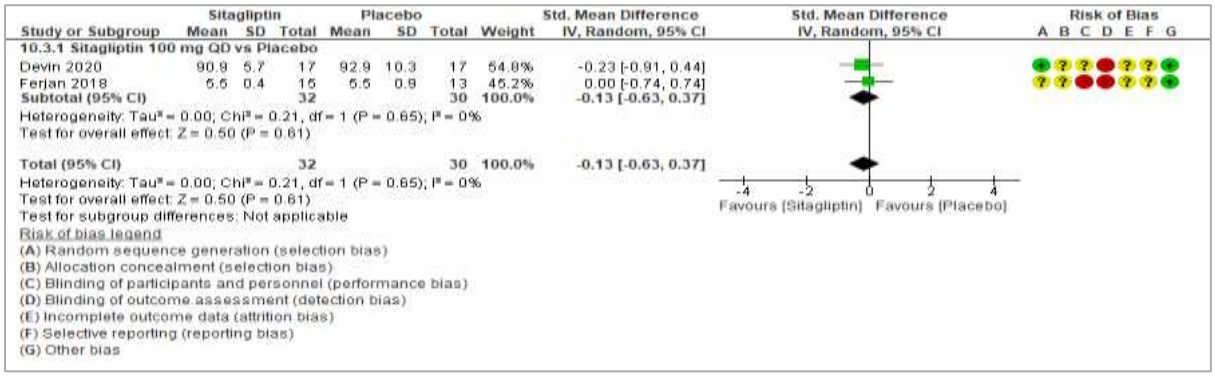
Figure 4-12: Forest plot of Pioglitazone versus placebo on FBG



4.3.6.1.11 Sitagliptin versus placebo

In two RCTs sitagliptin 100 mg QD has no effect on the mean FBG compared with placebo (SMD: -0.13; 95% CI: -0.63, 0.37, I^2 = 0%) (Figure 4-13) (low grade evidence, table 6).

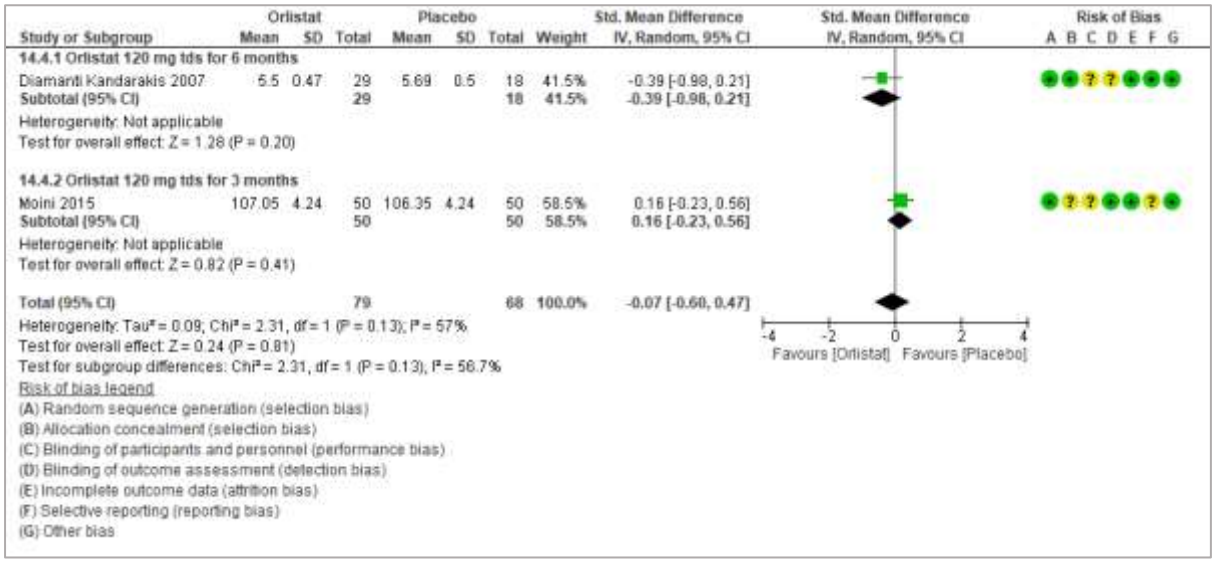
Figure 4-13: Forest plot of Sitagliptin versus placebo on FBG



4.3.6.1.12 Orlistat versus placebo

Orlistat 120 mg TDS for six months in one RCT has no effect on the mean FBG compared with placebo (SMD: -0.39; 95% CI: -0.98, 0.21). One RCT compared orlistat 120 mg TDS with placebo for three months has no effect on the mean FBG (SMD: 0.16; 95% CI: -0.23, 0.56). Overall, orlistat 120 mg TDS for various duration has no effect on the mean FBG compared with placebo (SMD: -0.07; 95% CI: -0.60, 0.47, I^2 = 56.7%) (Figure 4-14) (low grade evidence, table 6).

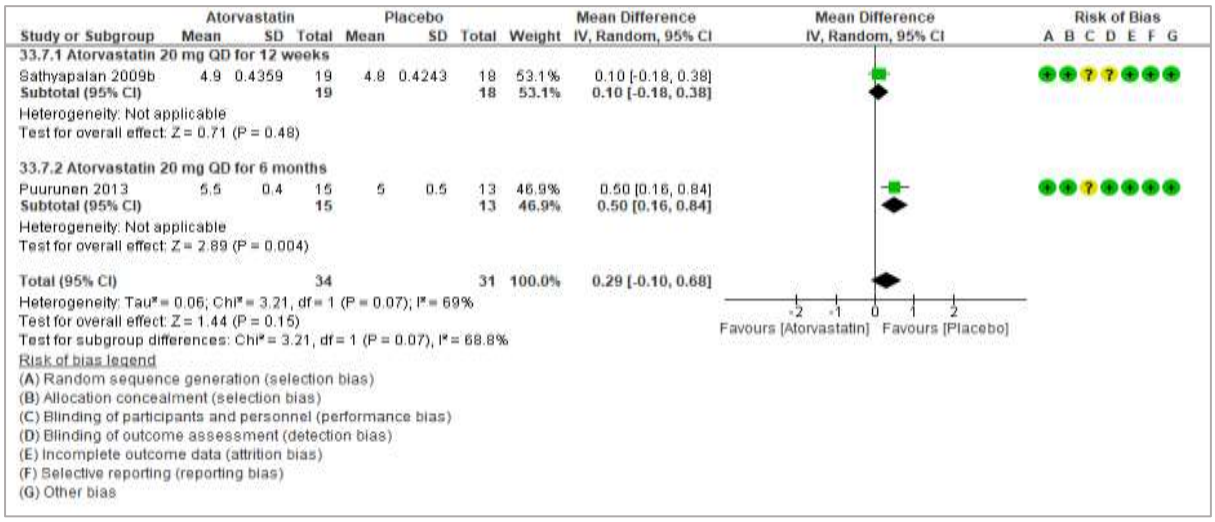
Figure 4-14: Forest plot of Orlistat versus placebo on FBG



4.3.6.1.13 Atorvastatin versus placebo

In one RCT, atorvastatin 20 mg QD for 12 weeks has no effect on the mean FBG compared with placebo (MD: 0.10 mmol/L;95% CI: -0.18, 0.38). In one RCT, atorvastatin 20 mg QD for six months has significantly increased the mean FBG compared with placebo (MD: 0.50 mmol/L; 95% CI: 0.16, 0.84). Overall, atorvastatin 20 mg QD for various duration has no effect on the mean FBG compared with placebo (MD: 0.29 mmol/L; 95% CI: -0.10, 0.68, I^2 = 68.8%) (Figure 4-15) (very low-grade evidence, table 6).

Figure 4-15: Forest plot of Atorvastatin versus placebo on FBG (mmol/L)

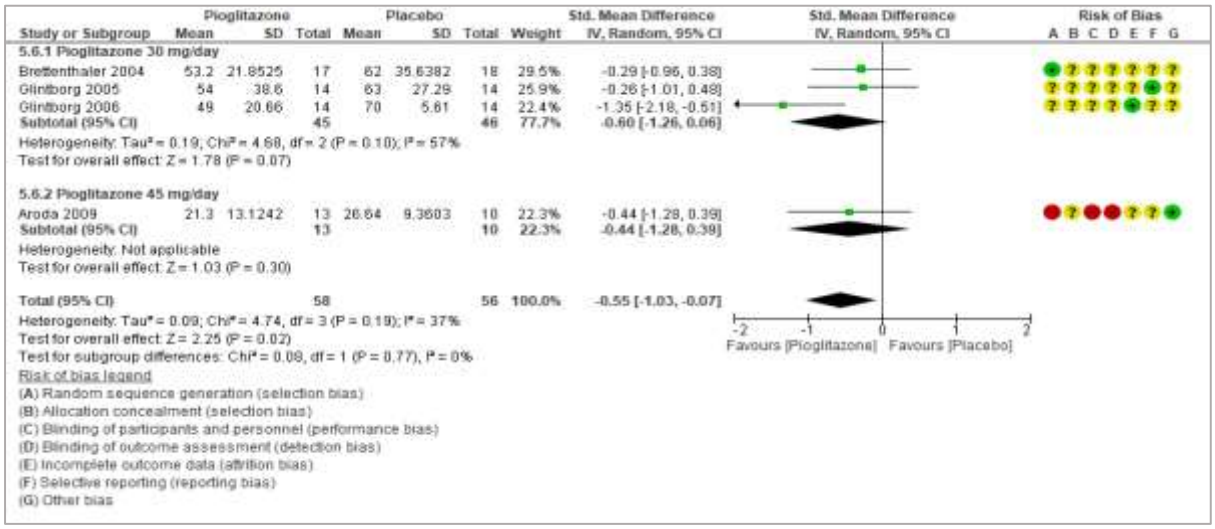


4.3.6.2 Fasting Insulin (FI)

4.3.6.2.1 Pioglitazone versus placebo

In three RCTs, pioglitazone 30 mg QD significantly reduced the mean FI (SMD: -0.60; 95% CI: -1.26,0.06) compared with placebo. In one RCT, pioglitazone 45 mg QD insignificantly reduced the mean FI (SMD: -0.44; 95% CI: -1.28,0.39). Overall, pioglitazone in various dosages significantly reduced the mean FI (SMD: -0.55; 95% CI: -1.03, -0.07, I^2= 37%) (Figure 4-16) (very low-grade of evidence, table 6).

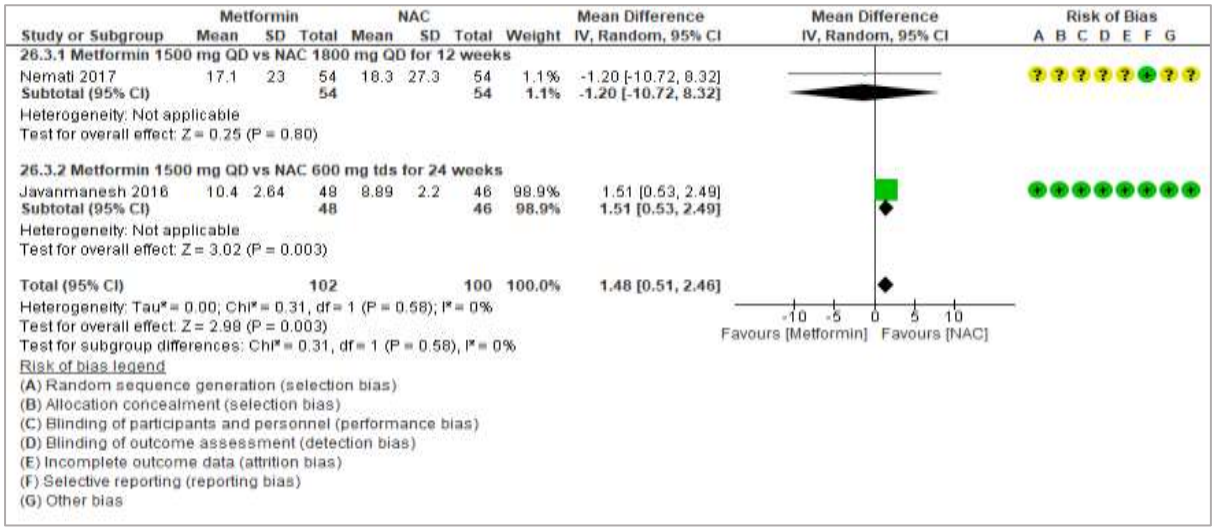
Figure 4-16: Forest plot of Pioglitazone versus placebo on FI



4.3.6.2.2 Metformin versus NAC

In one RCT, NAC 1800 mg QD showed no significant effect in the mean FI compared with Metformin 1500 mg QD for 12 weeks (MD: -1.20 pmol/L; 95% CI: -10.72, 8.32). One RCT compared NAC 600 mg QD with Metformin 1500 mg QD for 24 weeks showed a significant increase in the mean FI (MD: 1.51 pmol/L; 95% CI: 0.53, 2.49). Overall, metformin compared with NAC significantly increased the mean FI (MD: 1.48 pmol/L; 95% CI: 0.51, 2.46, I^2 = 0%) (Figure 4-17) (low grade evidence, table 6).

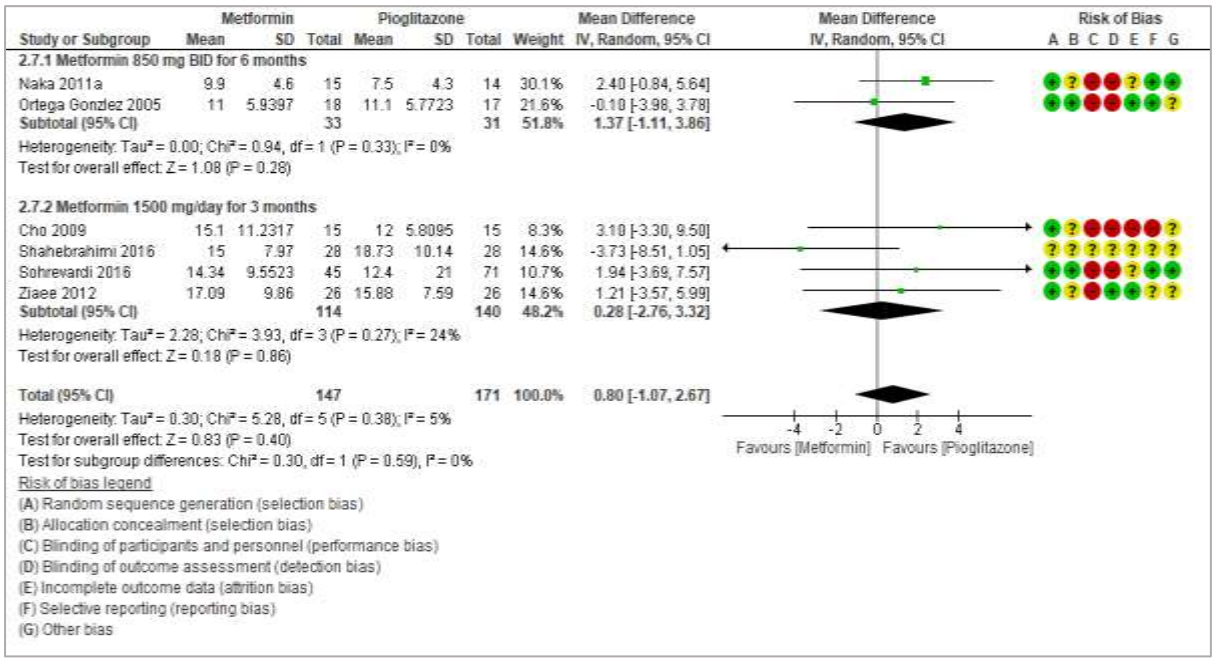
Figure 4-17: Forest plot of Metformin versus NAC on FI (pmol/L)



4.3.6.2.3 Metformin versus Pioglitazone

Two RCTs compared metformin 850 mg BID with pioglitazone for six months showed no effect on the mean FI (MD: 1.37 pmol/L; 95% CI: -1.11, 3.86). In four RCTs, metformin 1500 mg QD with pioglitazone for three months showed no effect on the mean FI (MD: 0.28 pmol/L; 95% CI: -2.76, 3.32). Overall, metformin at various dosage and for various duration compared with pioglitazone has no effect on the mean FI (MD: 0.80 pmol/L; 95% CI: -1.07, 2.67, I^2 = 0%) (Figure 4-18) (low grade evidence, table 6).

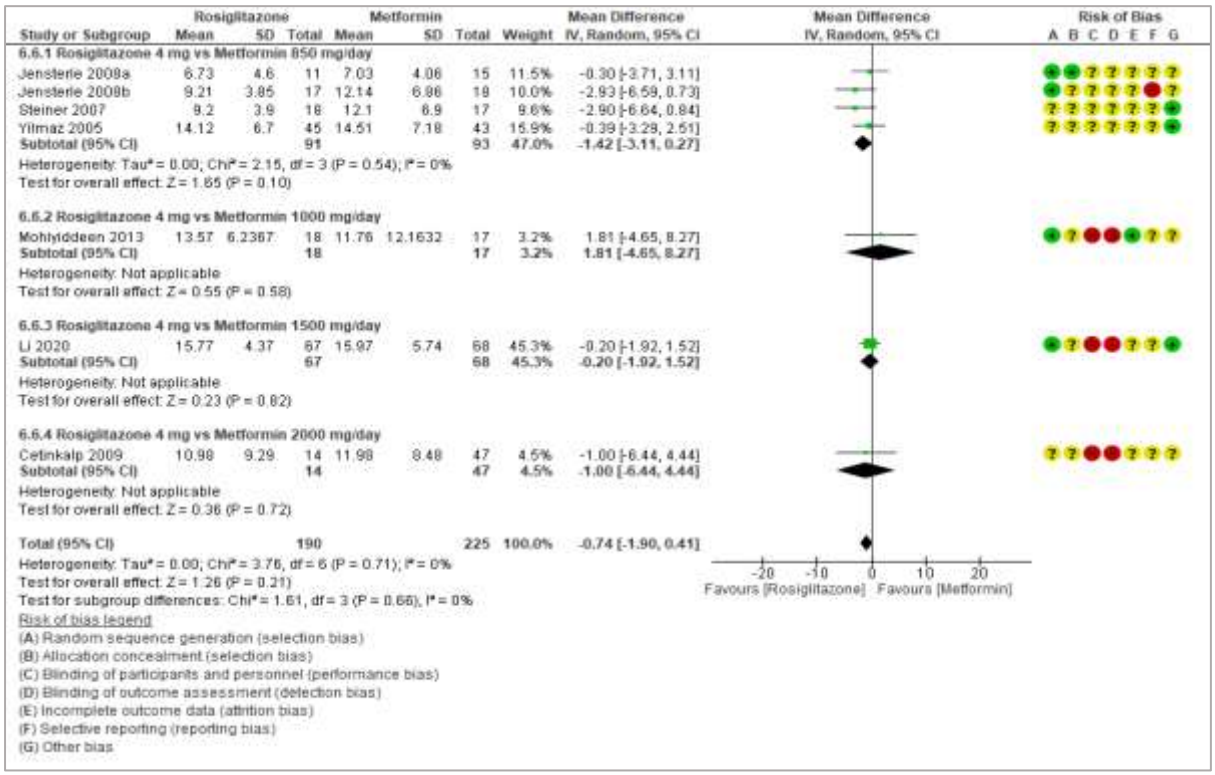
Figure 4-18: Forest plot of Metformin versus Pioglitazone on FI (pmol/L)



4.3.6.2.4 Rosiglitazone versus Metformin

In four RCTs, rosiglitazone 4 mg QD compared with metformin 850 mg QD has no effect on the mean FI (MD: -1.42 pmol/L; 95% CI: -3.11, 0.27). In one RCT, rosiglitazone 4 mg QD compared with metformin 1000 mg QD has no effect on the mean FI (MD: 1.81 pmol/L; 95% CI: -4.65, 8.27). In one RCT, rosiglitazone 4 mg QD compared with metformin 1500 mg QD has no effect on the mean FI (MD: -0.20 pmol/L; 95% CI: -1.92, 1.52). One RCT compared rosiglitazone 4 mg QD with metformin 2000 mg QD has no effect on the mean FI (MD: -1.00 pmol; 95% CI: -6.44, 4.44). Overall, rosiglitazone 4 mg compared with the various dosage on metformin has no effect on the mean FI (MD: -0.74 pmol/L; 95%CI: -1.90, 0.41, I^2 = 0%) (Figure 4-19) (low grade evidence, table 6).

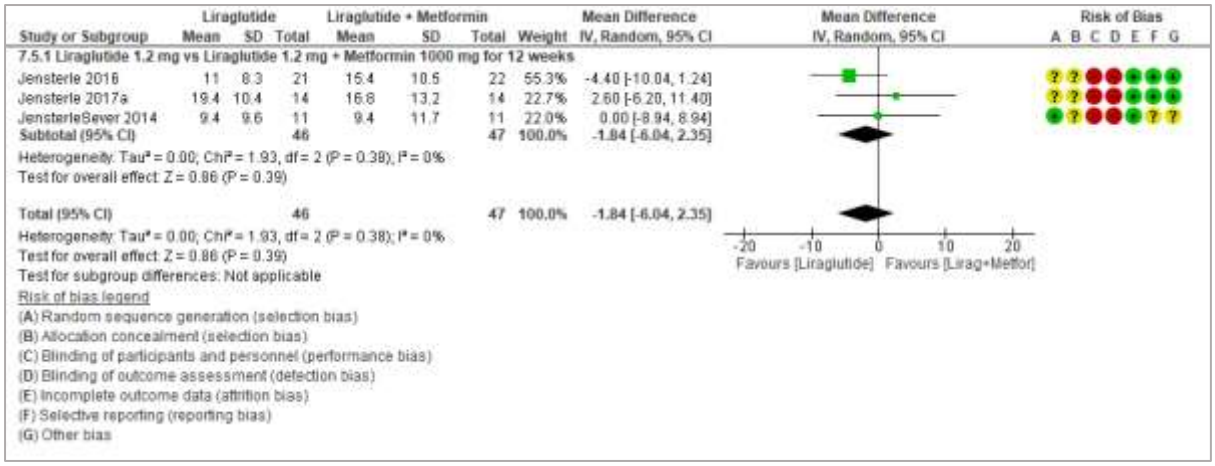
Figure 4-19: Forest plot of Rosiglitazone versus Metformin on FI (pmol/L)



4.3.6.2.5 Liraglutide versus Liraglutide + Metformin

Three RCTs compared liraglutide 1.2 mg QD with liraglutide 1.2 mg QD added to metformin 1000 mg QD for 12 weeks has no effect on the mean FI (MD: -1.84 pmol/L; 95% CI: -6.04, 2.35, I^2 = 0%) (Figure 4-20) (low grade evidence, table 6).

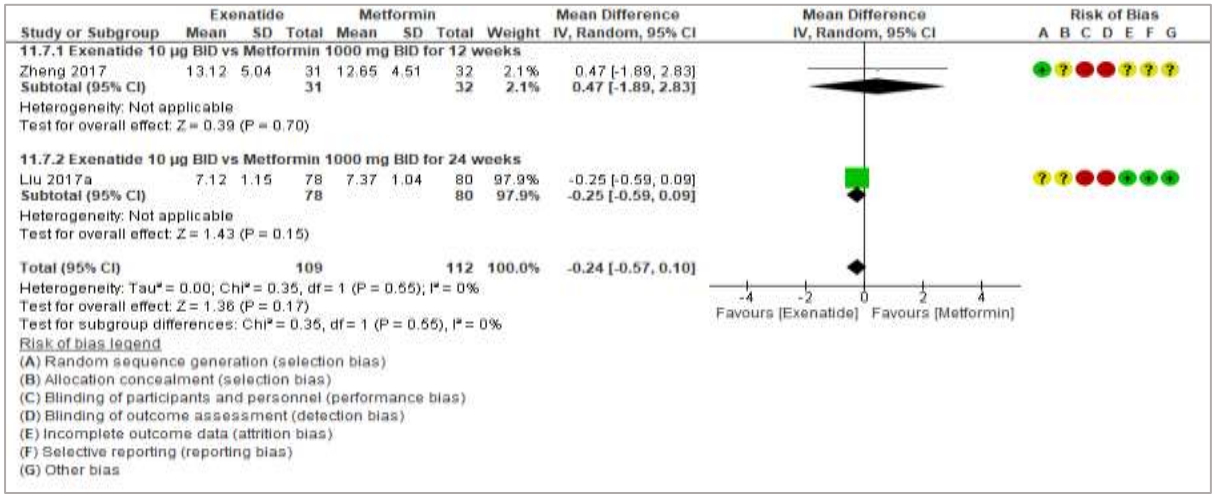
Figure 4-20: Forest plot of Liraglutide versus Liraglutide + Metformin on FI (pmol/L)



4.3.6.2.6 Exenatide versus Metformin

In two RCTs, exenatide 10 µg BID compared with metformin 1000 mg BID for duration of 12 and 24 weeks showed no effect on the mean FI (MD: -0.24 pmol/L; 95% CI: -0.57, 0.10, I^2 = 0%) (Figure 4-21) (low grade evidence, table 6).

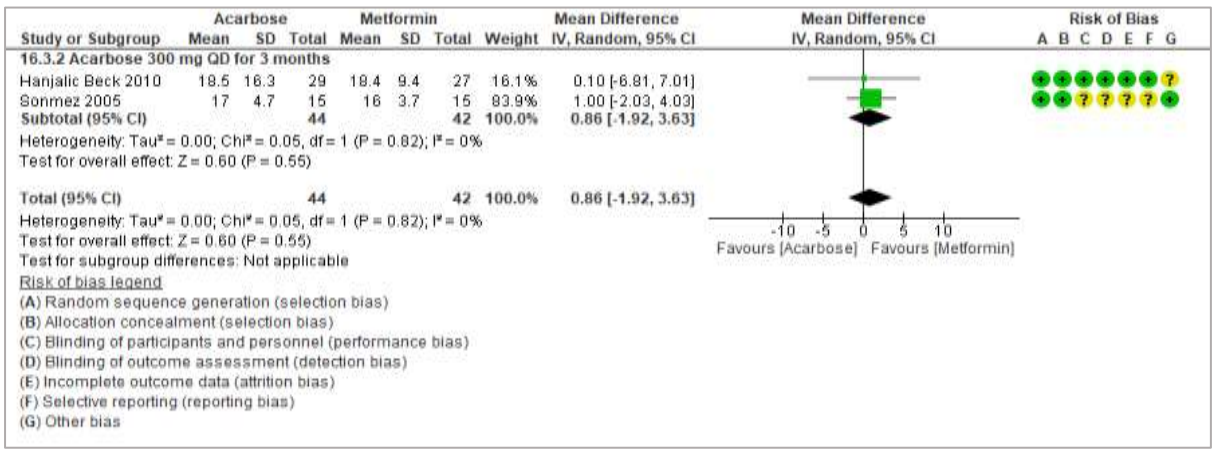
Figure 4-21: Forest plot of Exenatide versus Metformin on FI (pmol/L)



4.3.6.2.7 Acarbose versus Metformin

In two RCTs compared acarbose 300 mg QD for three months compared with metformin has no effect on the mean FI (MD: 0.86 pmol/L; 95% CI: -1.92, 3.63, I^2 = 0%) (Figure 4-22) (low grade evidence, table 6).

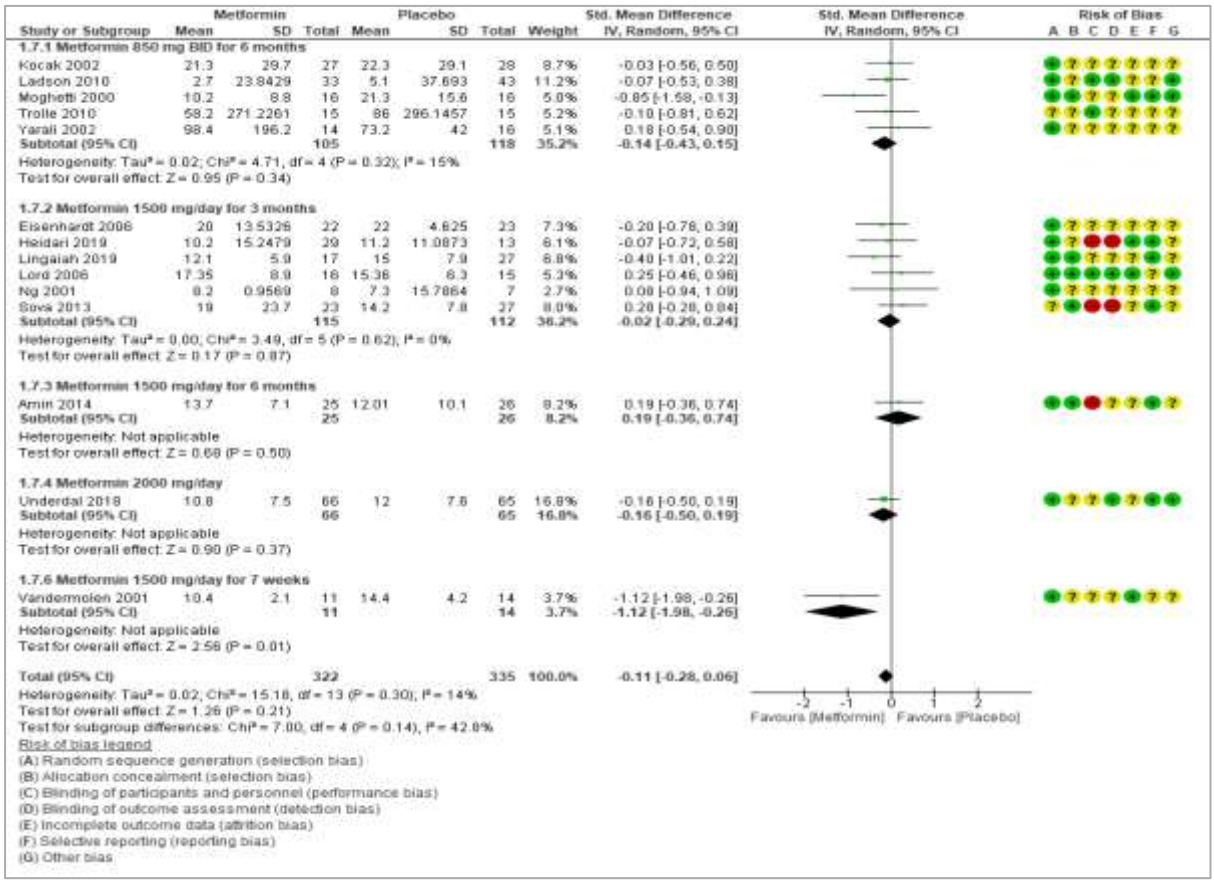
Figure 4-22: Forest plot of Acarbose versus Metformin on FI (pmol/L)



4.3.6.2.8 Metformin versus placebo

In five RCTs, metformin 850 mg BID for six months has no effect on the mean FI compared with placebo (SMD: -0.14; 95% CI: -0.43, 0.15). In six RCTs, metformin 1500 mg for three months compared with placebo has no effect on the mean FI (SMD: -0.02; 95%CI: -0.29, 0.24). One RCT of metformin 1500 mg QD for six months has no effect on the mean FI (SMD: 0.19; 95%CI: -0.36, 0.74). In one RCT, metformin 2000 mg QD has no effect on the mean FI compared with placebo (SMD: -0.16;95%CI: -0.50, 0.19). In one RCT, metformin 1500 mg QD for seven weeks compared with placebo has no effect on the mean FI (SMD: -1.12; 95%CI: -1.98, -0.26). Overall, metformin at various dosage has no effect on the mean FI compared with placebo (SMD: -0.11; 95% CI: -0.28, 0.06, I^2 = 0%) (Figure 4-23) (moderate grade evidence, table 6).

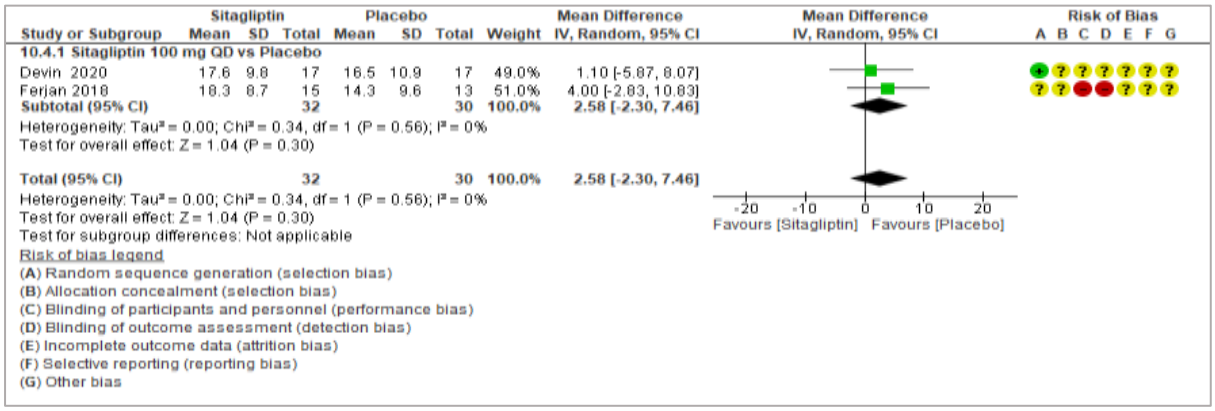
Figure 4-23: Forest plot of Metformin versus placebo on FI



4.3.6.2.9 Sitagliptin versus placebo

In two RCTs sitagliptin 100 mg QD compared with placebo has no effect on the mean FI (MD: 2.58 pmol/L; 95% CI: -2.30, 7.46, I^2 = 0%) (Figure 4-24) (low grade evidence, table 6).

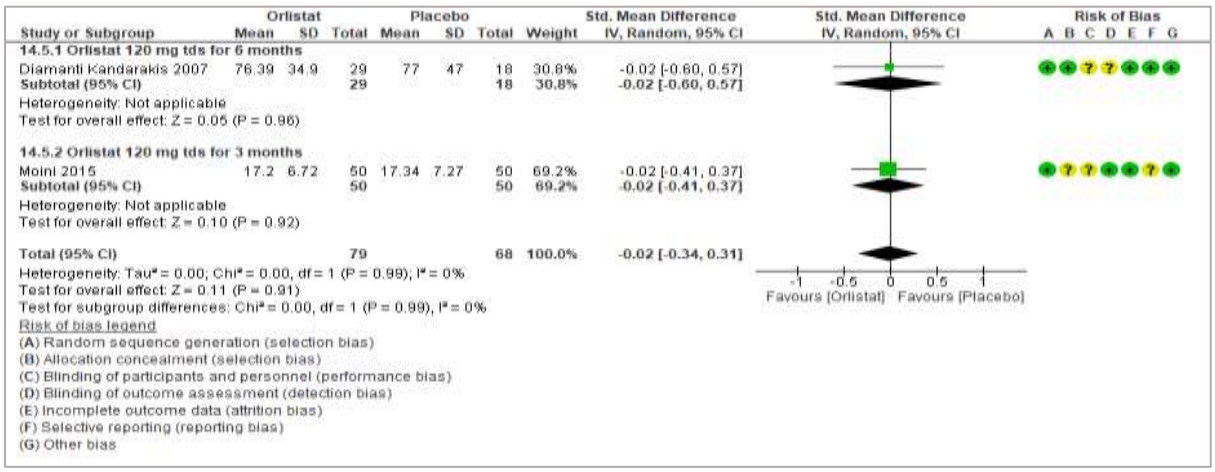
Figure 4-24: Forest plot of Sitagliptin versus placebo on FI (pmol/L)



4.3.6.2.10 Orlistat versus placebo

In one RCT, orlistat 120 mg TDS for six months compared with placebo has no effect on the mean FI (SMD: -0.02; 95%CI: -0.60, 0.57). In one RCT, orlistat 120 mg TDS for three months has no effect on the mean FI compared with placebo (SMD: -0.02; 95%CI: -0.41, 0.37). Overall, orlistat 120 mg TDS for various duration has no effect on the mean FI compared with placebo (SMD: -0.02; 95% CI: -0.34, 0.31, I^2 = 0%) (Figure 4-25) (low grade evidence, table 6).

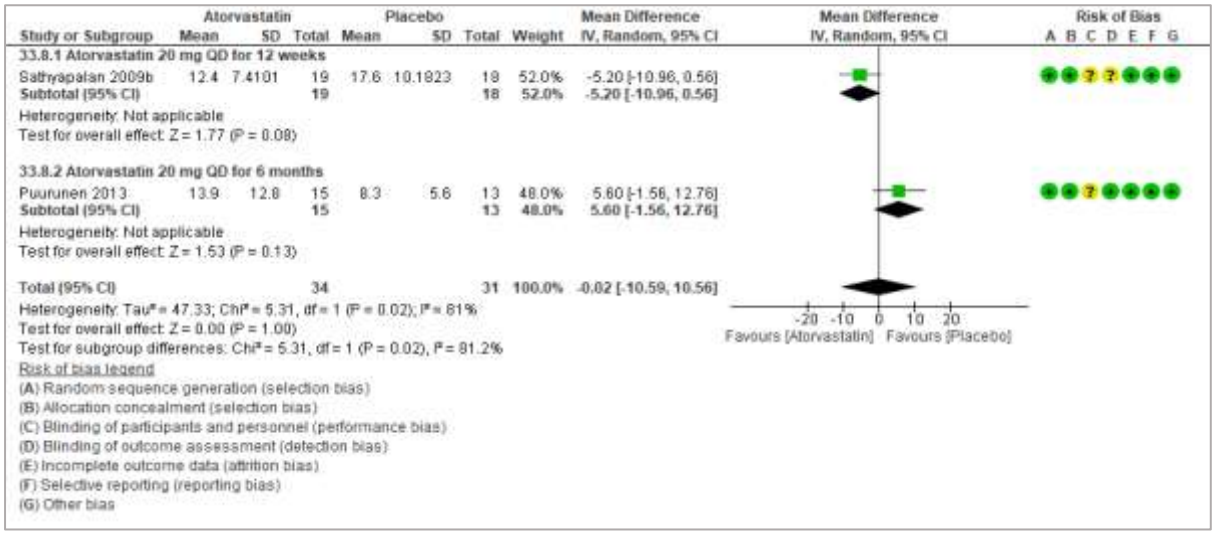
Figure 4-25: Forest plot of Orlistat versus placebo on FI



4.3.6.2.11 Atorvastatin versus placebo

In one RCT, atorvastatin 20 mg QD for 12 weeks has no effect on the mean FI compared with placebo (MD: -5.20 pmol/L; 95% CI: -10.96, 0.56). In one RCT, atorvastatin 20 mg QD for six months has no effect on the mean FI compared with placebo (MD: 5.60 pmol/L; 95% CI: -1.56, 12.76). Overall, atorvastatin 20 mg QD for various duration has no effect on the mean FI compared with placebo (MD: -0.02 pmol/L; 95% CI: -10.59, 10.56, I^2 = 81.2%) (Figure 4-26) (very low-grade evidence, table 6).

Figure 4-26: Forest plot of Atorvastatin versus placebo on FI (pmol/L)

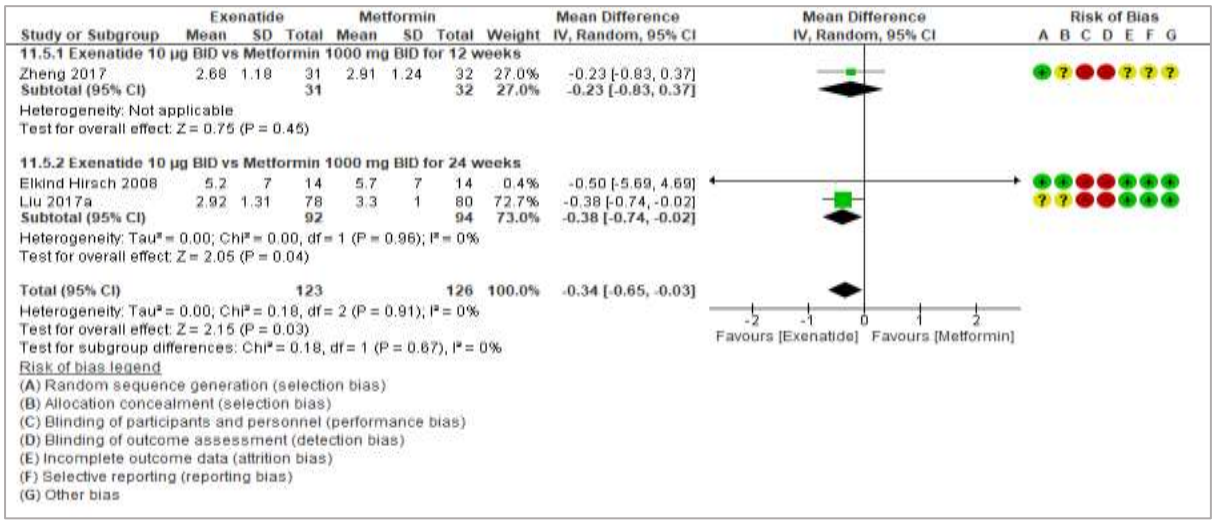


4.3.6.3 HOMA-IR

4.3.6.3.1 Exenatide versus Metformin

In one RCT, exenatide 10 µg BID compared with Metformin 1000 mg BID for 12 weeks showed insignificantly but the lower mean level of HOMA-IR (MD: -0.23; 95% CI: -0.83, 0.37). However, in one RCT comparing exenatide 10 µg BID with metformin 1000 mg BID for 24 weeks, a significant reduction in the mean HOMA-IR was observed (MD: -0.38; 95% CI: -0.74, -0.02). Overall, exenatide significantly reduced the mean HOMA-IR (MD: -0.34; 95% CI: -0.65, -0.03, I^2= 0%) compared with metformin (Figure 4-27) (low grade of evidence, table 6).

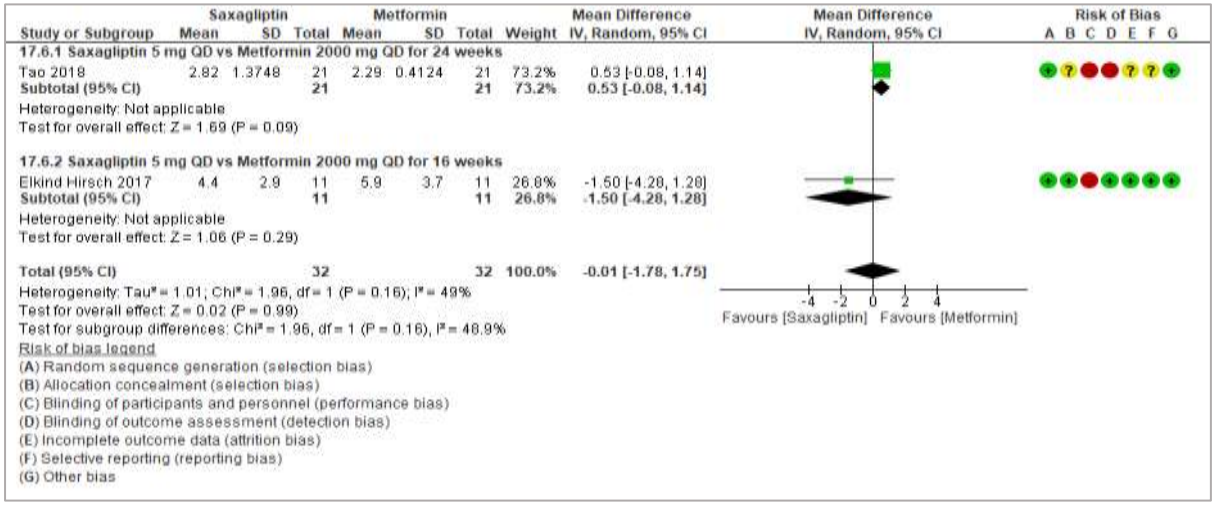
Figure 4-27: Forest plot of Exenatide versus Metformin on HOMA-IR



4.3.6.3.2 Saxagliptin versus Metformin

In one RCT, saxagliptin 5 mg QD compared with metformin 2000 mg QD for 24 weeks significantly increased the mean HOMA-IR (MD: 0.53; 95% CI: -0.08, 1.14). In one RCT, saxagliptin 5 mg QD compared with metformin 2000 mg for 16 weeks has no effect on the mean HOMA-IR (MD: -1.50; 95% CI: -4.28, 1.28). Overall, saxagliptin 5 mg QD for various duration has no effect on the mean HOMA-IR compared with metformin 2000 mg QD (MD: -0.01; 95%CI: -1.78, 1.75, I^2= 48.9%) (Figure 4-28) (very low-grade evidence, table 6).

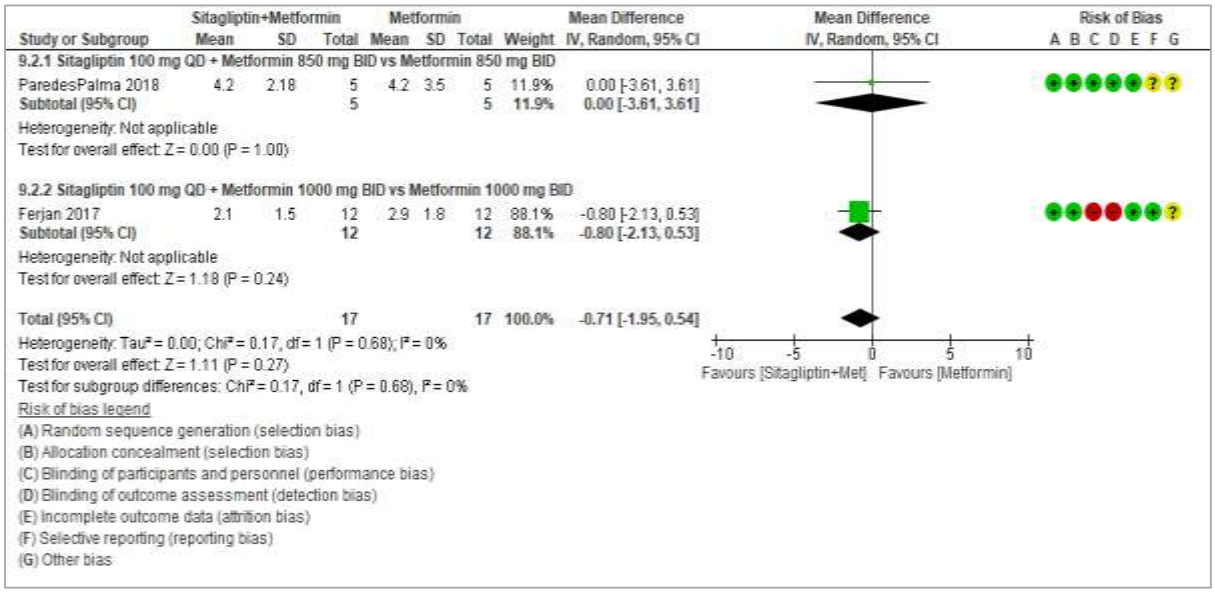
Figure 4-28: Forest plot of Saxagliptin versus Metformin on HOMA-IR



4.3.6.3.3 Sitagliptin + Metformin versus Metformin

In one RCT, sitagliptin 100 mg QD added to metformin 850 mg BID compared with metformin 850 alone has no effect on the mean HOMA-IR (MD: 0.00; 95% CI: -3.61, 3.61). Another RCT compared sitagliptin 100 mg QD added to metformin 1000 mg BID compared with metformin 1000 mg BID alone has no effect on the mean HOMA-IR (MD:-0.80; 95% CI: -2.13, 0.53). Overall, sitagliptin 100 mg QD added to various dosages of metformin and compared with metformin alone has no effect on the mean HOMA-IR (MD: -0.71; 95% CI:-1.95, 0.54, I^2= 0%) (Figure 4-29) (low-grade evidence, table 6).

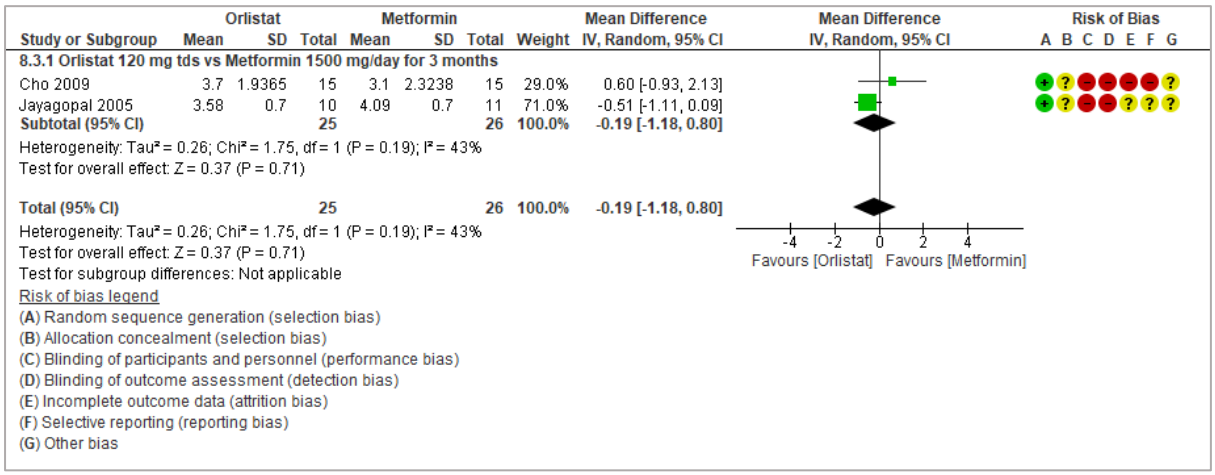
Figure 4-29: Forest plot of Sitagliptin + Metformin versus Metformin on HOMA-IR



4.3.6.3.4 Orlistat versus Metformin

Two RCTs compared orlistat 120 mg TDS with metformin 1500 mg QD for three months showed no effect on the mean HOMA-IR (MD: -0.19; 95%CI: -1.18, 0.80, I^2= 43%) (Figure 4-30) (very low-grade evidence, table 6).

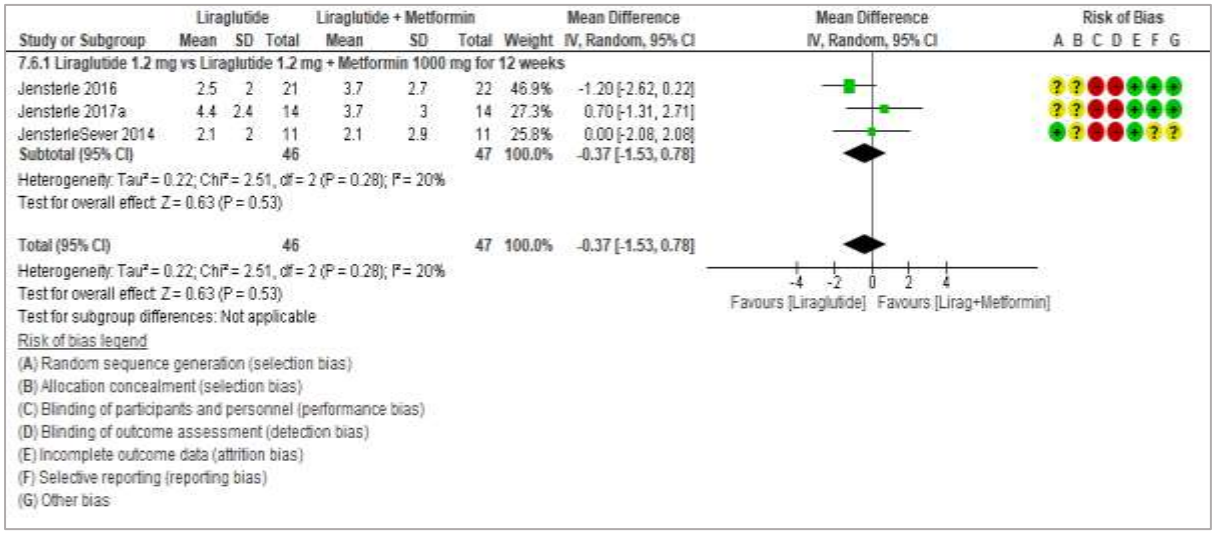
Figure 4-30: Forest plot of Orlistat versus Metformin on HOMA-IR



4.3.6.3.5 Liraglutide versus Liraglutide + Metformin

In three RCTs, liraglutide 1.2 mg QD compared with liraglutide 1.2 mg QD and added to metformin 1000 mg QD for 12 weeks has no effect on the mean HOMA-IR (MD: -0.37; 95% CI: -1.53, 0.78, I^2= 20%) (Figure 4-31) (low-grade evidence, table 6).

Figure 4-31: Forest plot of Liraglutide versus Liraglutide + Metformin on HOMA-IR

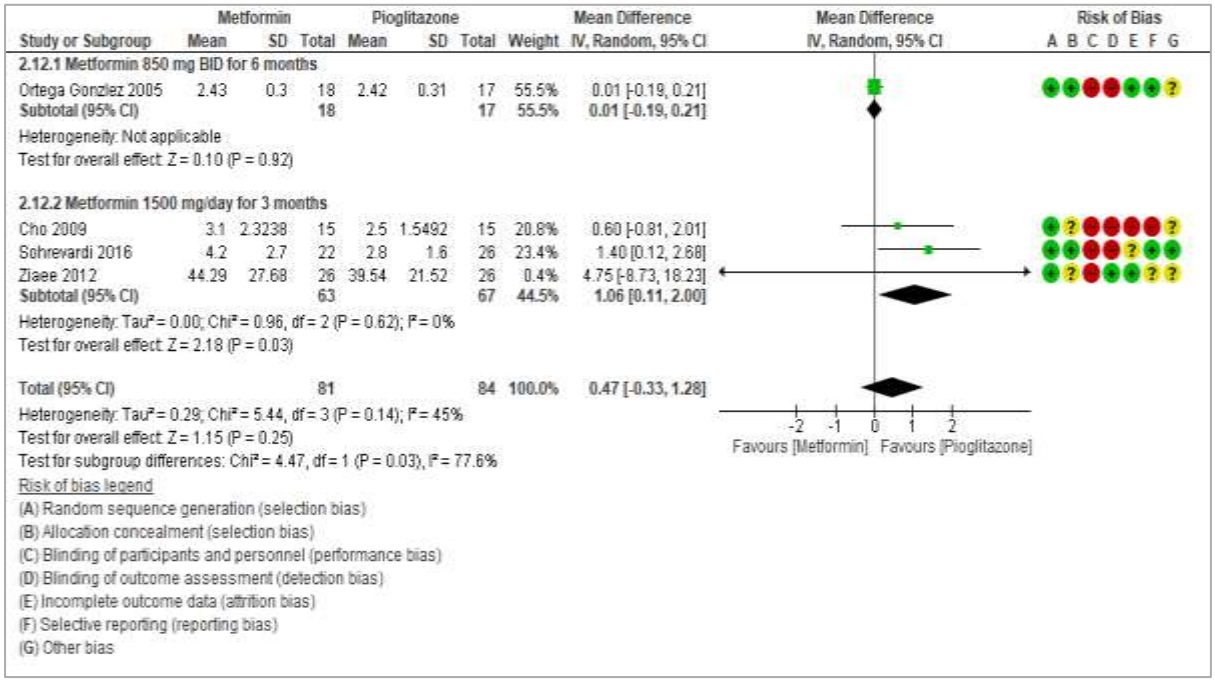


4.3.6.3.6 Metformin versus Pioglitazone

In one RCT, compared metformin 850 mg BID with pioglitazone for six months has no effect on the mean HOMA-IR (MD: 0.01; 95% CI: -0.19, 9.21). On the other hand, three RCTs

compared metformin 1500 mg QD with pioglitazone for three months showed a significant increase in the mean HOMA-IR (MD: 1.06; 95% CI: 0.11, 2.00). Overall, metformin of various dosages has no effect on the mean HOMA-IR when compared with pioglitazone (MD: 0.47; 95% CI: -0.33, 1.28, I^2= 77.6%) (Figure 4-32) (very low-grade evidence, table 6).

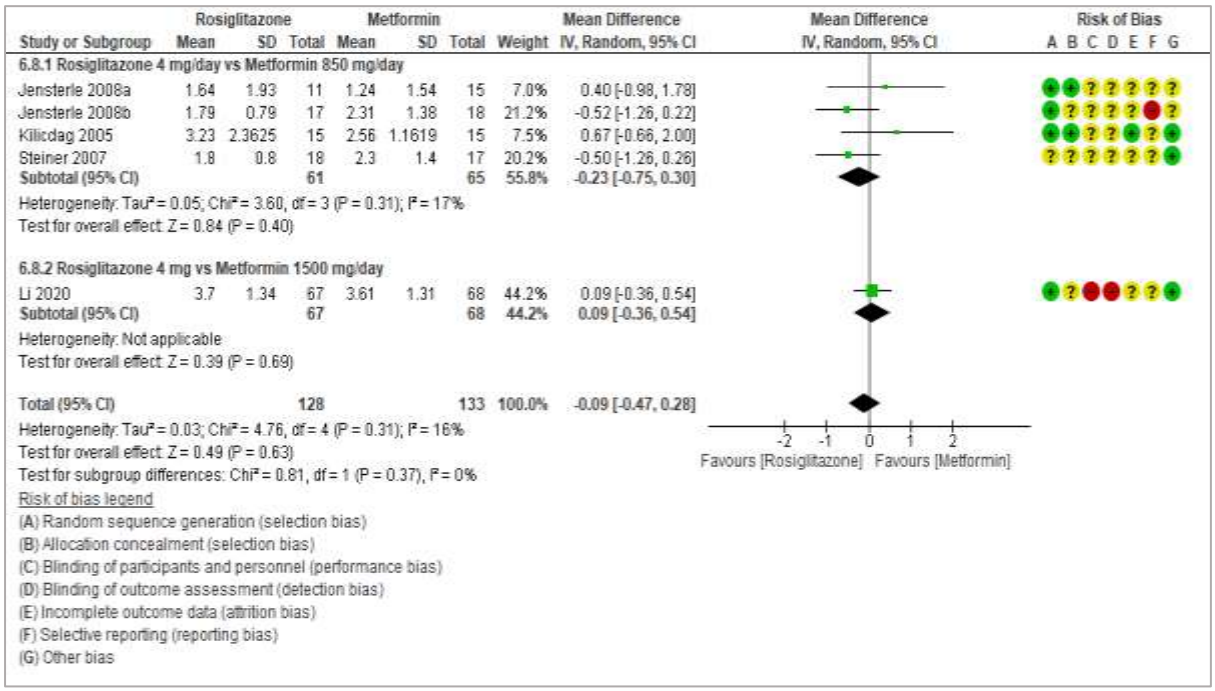
Figure 4-32: Forest plot of Metformin versus Pioglitazone on HOMA-IR



4.3.6.3.7 Rosiglitazone versus Metformin

Four RCTs compared rosiglitazone 4 mg QD with metformin 850 mg QD showed no effect on the mean HOMA-IR (MD: -0.23; 95% CI: -0.75, 0.30). One RCT compared rosiglitazone 4 mg QD with metformin 1500 mg QD showed no effect on the mean HOMA-IR (MD: 0.09; 95%CI: -0.36, 0.54). Overall, rosiglitazone 4 mg QD compared with metformin of various dosages has no effect on the mean HOMA-IR (MD: -0.09; 95% CI: -0.47, 0.28, I^2= 0%) (Figure 4-33) (low-grade evidence, table 6).

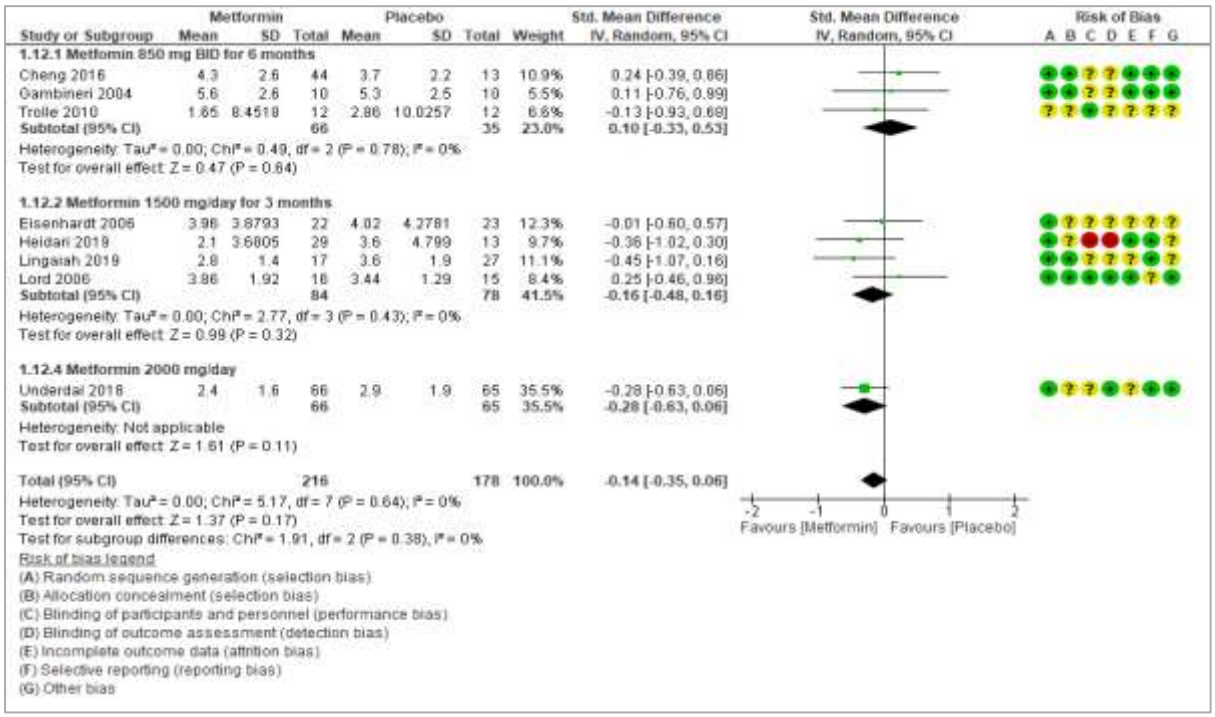
Figure 4-33: Forest plot of Rosiglitazone versus Metformin on HOMA-IR



4.3.6.3.8 Metformin versus placebo

Three RCTs compared metformin 850 mg BID for six months with placebo showed no effect on the mean HOMA-IR (SMD: 0.10; 95%CI: -0.33, 0.53). In four RCTs, metformin 1500 mg QD for three months compared with placebo has no effect on the mean HOMA-IR (SMD: -0.16; 95% CI: -0.48, 0.16). One RCT compared metformin 2000 mg QD with placebo showed no effect on the mean HOMA-IR (SMD: -0.28; 95%CI: -0.63, 0.06). Overall, regardless of the administered dosage, metformin has no effect on the mean HOMA-IR compared with placebo (SMD: -0.14; 95%CI: -0.35, 0.06, I^2= 0%) (Figure 4-34) (moderate grade evidence, table 6).

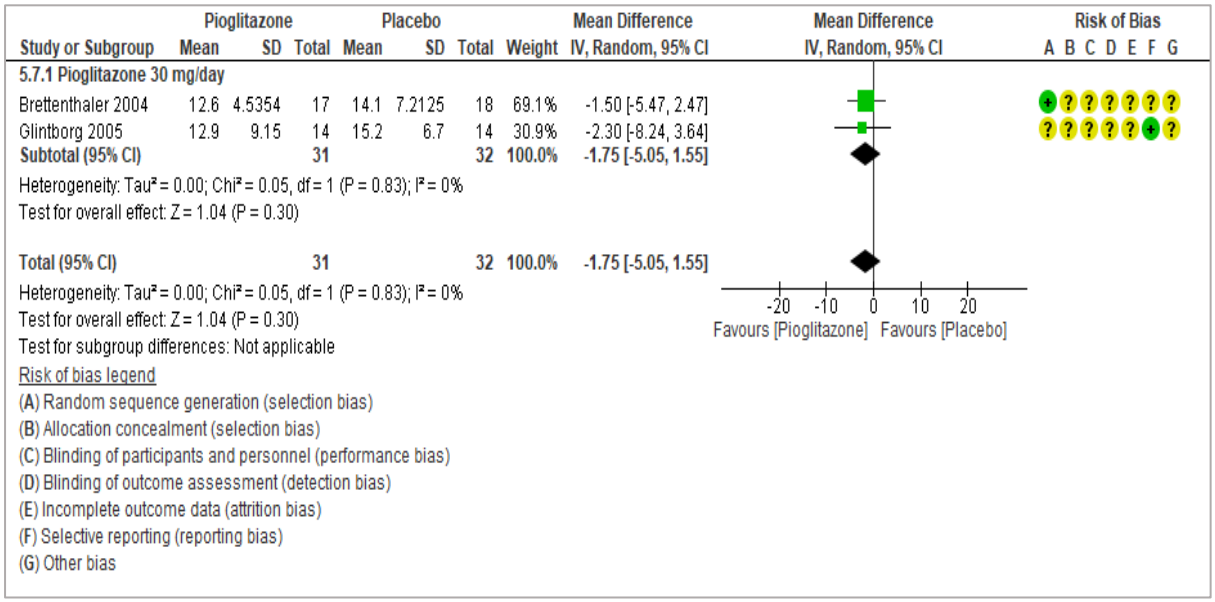
Figure 4-34: Forest plot of Metformin versus placebo on HOMA-IR



4.3.6.3.9 Pioglitazone versus placebo

In two RCTs pioglitazone 30 mg QD compared with placebo has no effect on the mean HOMA-IR (MD: -1.75; 95% CI: -5.05, 1.55, I^2= 0%) (Figure 4-35) (low grade evidence, table 6).

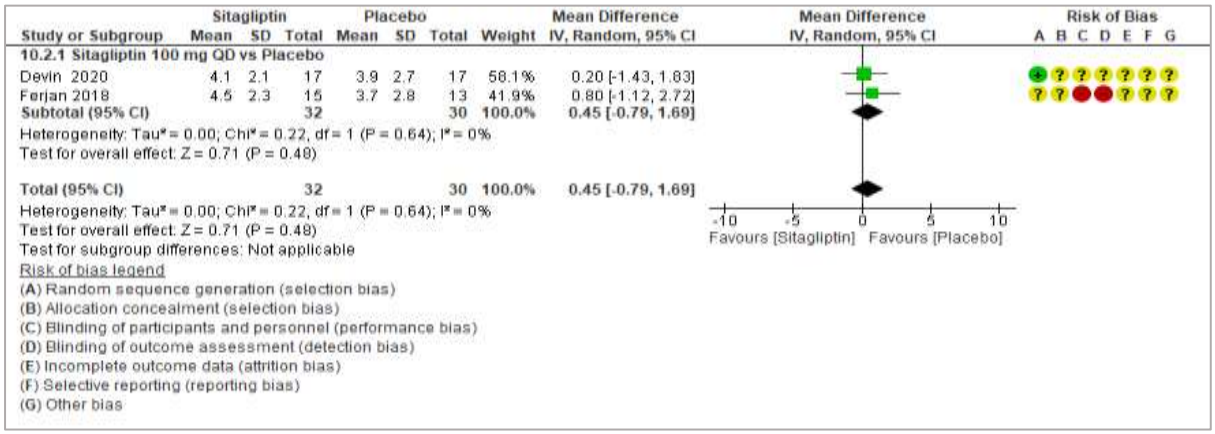
Figure 4-35: Forest plot of Pioglitazone versus placebo on HOMA-IR



4.3.6.3.10 Sitagliptin versus placebo

In two RCTs compared sitagliptin 100 mg QD with placebo has no effect on the mean HOMA-IR (MD: 0.45; 95%CI: -0.79, 1.69, I^2= 0%) (Figure 4-36) (low grade evidence, table 6).

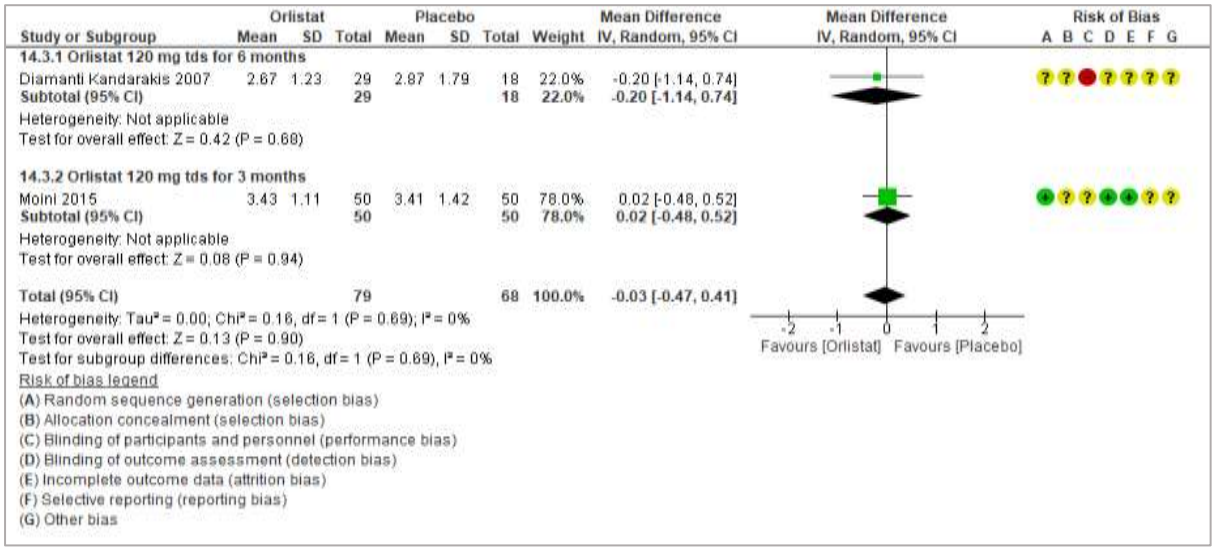
Figure 4-36: Forest plot of Sitagliptin versus placebo on HOMA-IR



4.3.6.3.11 Orlistat versus placebo

Orlistat 120 mg TDS for various durations has no effect on the mean HOMA-IR compared with placebo (MD: -0.03; 95%CI: -0.47, 0.41, I^2= 0%) (Figure 4-37) (low grade evidence, table 6).

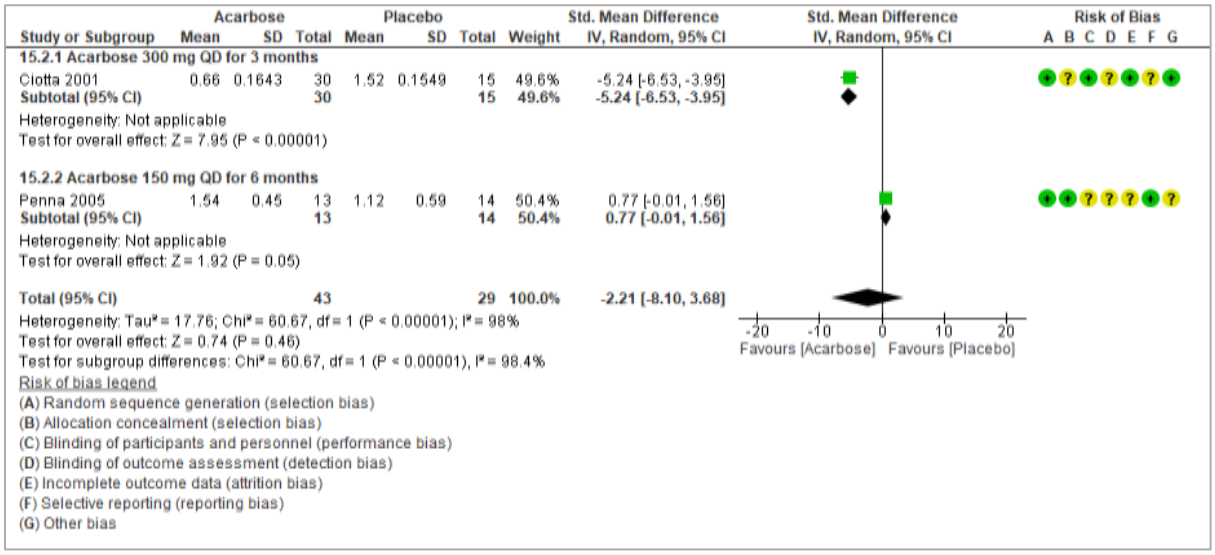
Figure 4-37: Forest plot of Orlistat versus placebo on HOMA-IR



4.3.6.3.12 Acarbose versus placebo

One RCT compared acarbose 300 mg QD with placebo for three months showed no effect on the mean HOMA-IR (SMD: -5.24; 95%CI: -6.53, -3.95). However, one RCT of acarbose 150 mg QD for six months compared with placebo significantly increased the mean HOMA-IR (SMD: 0.77; 95%CI: -0.01, 1.56). Overall, acarbose of various dosages for various duration has no effect on the mean HOMA-IR compared with placebo (SMD: -2.21; 95%CI: -8.10, 3.68, I^2= 98.4%) (Figure 4-38) (very low-grade evidence, table 6).

Figure 4-38: Forest plot of Acarbose versus placebo on HOMA-IR

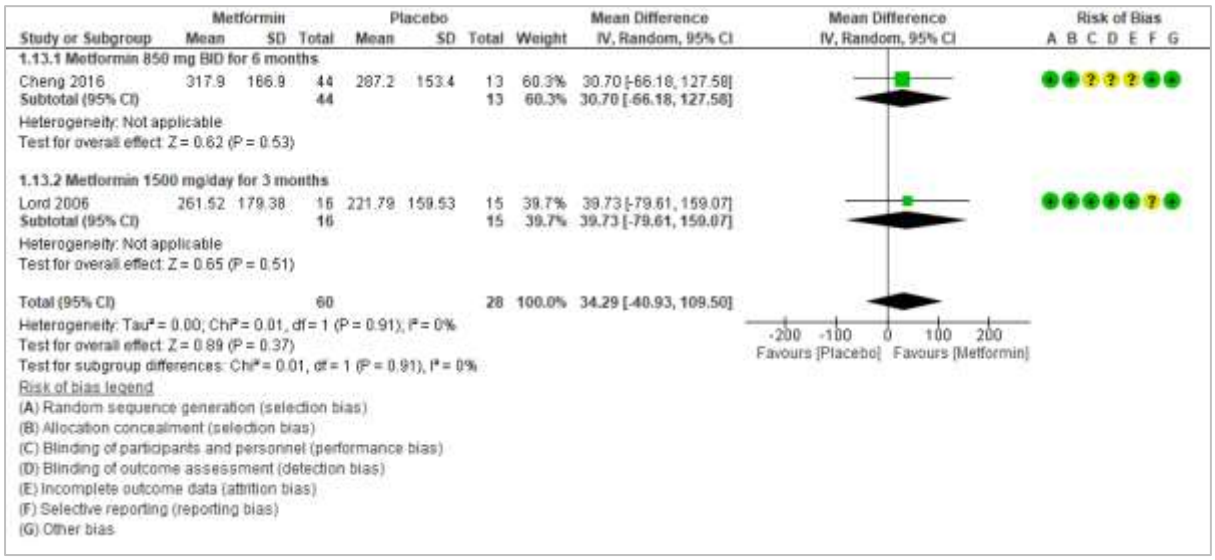


4.3.6.4 HOMA-B

4.3.6.4.1 Metformin versus placebo

One RCT compared metformin 850 mg BID for six months with placebo showed no effect on the mean HOMA-B (MD: 30.70; 95% CI: -66.18, 127.58). In one RCT, metformin 1500 mg for three months also showed no effect on the mean HOMA-B (MD: 39.73; 95% CI: -79.61, 159.07) compared with placebo. Overall, metformin was not associated with changes in mean HOMA-B level (MD: 34.29; 95% CI: -40.93, 109.50, I^2= 0%) compared with placebo (Figure 4-39) (low-grade evidence, table 6).

Figure 4-39: Forest plot of Metformin versus placebo on HOMA-B



4.3.7 Sensitivity analysis

The impact of each study on heterogeneity and the strength of the summary was assessed using sensitivity analysis. Small sample-sized trials and those with overall high RoB were removed from the analysis while observing their effects on the cumulative results. Thus, no significant effect was found, and hence no trial was removed from the meta-analysis.

4.3.8 Assessment of publication bias

For the effect of metformin versus placebo on fasting blood glucose and fasting insulin, we have assessed for publication bias as there were more than 10 RCTs. The funnel plot of RevMan showed no significant asymmetry, which reflects the low chance of publication bias (Figure 4-40).

Figure 4-40: Funnel plot of comparison metformin versus placebo

Figure 4-41: Fasting Blood glucose

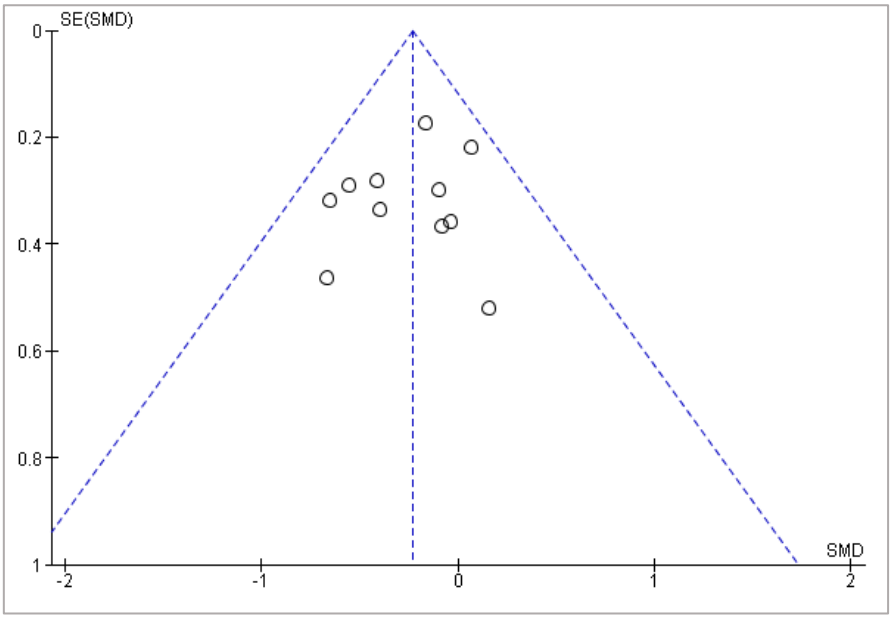
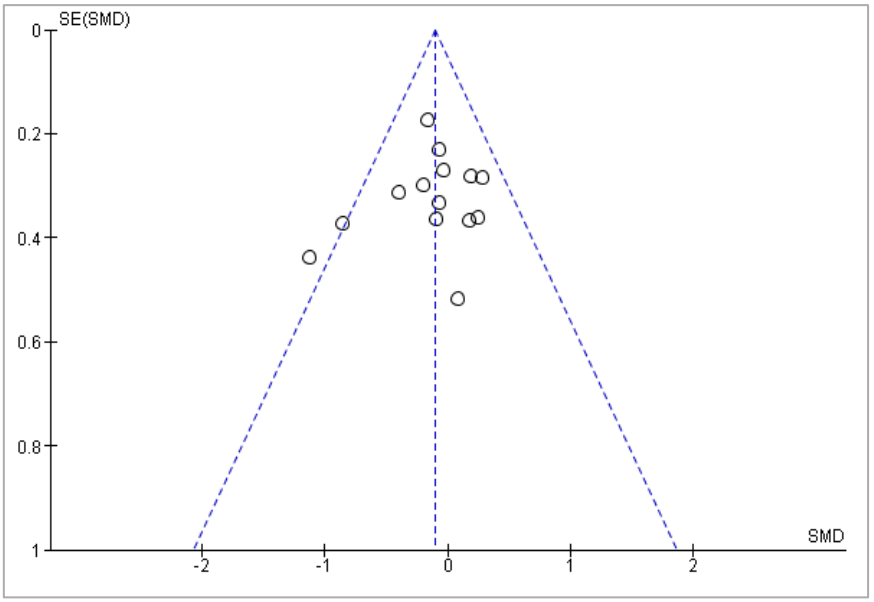


Figure 4-42: Fasting insulin



SE: standard error, **SMD:** standardised mean difference.

Table 6: Summary of findings for the outcomes on insulin resistance

Patient or population: PCOS **Setting:** **Intervention: First treatment (T1)** **Comparison: Second treatment (T2)**					
Outcome	**№ of participants (studies)**	**Certainty of the evidence (GRADE)**	**Relative effect (95% CI)**	**Anticipated absolute effects**	
				Assumed risk	
				Risk difference with intervention	**Risk difference with comparison**
<u>Meformin versus placebo</u> FBG FI HOMA-IR HOMA-B	 429 (9 RCTs) 657 (14 RCTs) 394 (8 RCTs) 88 (2 RCTs)	 ⊕⊕◯◯ LOW a,b ⊕⊕⊕◯ MODERATE c ⊕⊕⊕◯ MODERATE a,c ⊕⊕◯◯ LOW b,d	 - - - -	 The mean fasting Blood glucose was 4.9- 97.2 The mean fasting insulin was 5.1-73.2 The mean HOMA-IR was 2.9-5.3 The mean HOMA-B was 221.79-287.2	**(T1 minus T2)** SMD 0.16 lower (0.32 lower to 0.01 lower) MD 2.2 lower (3.62 lower to 0.77 lower) MD 0.31 lower (0.74 lower to 0.11 higher) MD 34.29 higher (40.93 lower to 109.5 higher)
<u>Metformin versus Pioglitazone</u> FBG FI HOMA-IR	 215 (5 RCTs 318 (6 RCTs) 165 (4 RCTs)	 ⊕⊕◯◯ LOW a,b ⊕⊕◯◯ LOW a,b,c ⊕◯◯◯ VERY LOW a,b,d	 - - -	 The mean fasting Blood glucose was 83.25-92.73 The mean fasting insulin was 7.5-18.73 The mean HOMA-IR was 2.42-39.54	**(T1 minus T2)** MD 0.1 higher (0.13 lower to 0.32 higher) MD 0.8 higher (1.07 lower to 2.67 higher) MD 0.47 higher (0.33 lower to 1.28 higher)
<u>Pioglitazone versus placebo</u> FBG FI HOMA-IR	 86 (3 RCTs) 114 (4 RCTs) 63 (2 RCTs)	 ⊕⊕◯◯ LOW a,b ⊕◯◯◯ VERY LOW a,b,c ⊕⊕◯◯ LOW a,b	 - - -	 The mean fasting Blood glucose was 5- 91.01 The mean fasting insulin was 5.61-35.6 The mean HOMA-IR was 14.1-15.1	**(T1 minus T2)** MD 0.11 lower (1.28 lower to 1.07 higher) MD 11.47 lower (20.2 lower to 2.74 lower) MD 1.75 lower (5.05 lower to 1.55 higher)
<u>Rosiglitazone versus Metformin</u> FBG FI HOMA-IR	 131 (4 RCTs) 131 (4 RCTs) 96 (3 RCTs)	 ⊕⊕◯◯ LOW a,b ⊕⊕◯◯ LOW a,b ⊕⊕◯◯ LOW a,b	 - - -	 The mean fasting Blood glucose was 1.24-3.61 The mean fasting insulin was 7.3-15.97 The mean HOMA-IR was 1.24-3.61	**(T1 minus T2)** MD 0.05 higher (0.03 lower to 0.13 higher) MD 1.59 lower (3.57 lower to 0.38 higher) MD 0.39 lower (0.89 lower to 0.1 higher)
<u>Liraglutide versus Liraglutide +Metformin</u> FBG FI HOMA-IR	 93 (3 RCTs) 93 (3 RCTs) 93 (3 RCTs)	 ⊕⊕◯◯ LOW a,b ⊕⊕◯◯ LOW a,b ⊕⊕◯◯ LOW a,b	 - - -	 The mean fasting Blood glucose was 4.6- 5.2 The mean fasting insulin was 9.4-16.8 The mean HOMA-IR was 2.1-3.7	**(T1 minus T2)** MD 0.03 higher (0.19 lower to 0.25 higher) MD 1.84 lower (6.04 lower to 2.35 higher) MD 0.37 lower (1.53 lower to 0.78 higher)

Orlistat versus Metformin HOMA-IR	51 (2 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean HOMA-IR was 3.1-4.09	**(T1 minus T2)** MD 0.19 lower (1.18 lower to 0.8 higher)
Sitagliptin + Metformin versus Metformin HOMA-IR	34 (2 RCTs)	⊕⊕◯◯ LOW a,b	-	The mean HOMA-IR was 2.9-4.2	**(T1 minus T2)** MD 0.71 lower (1.95 lower to 0.54 higher)
Sitagliptin versus placebo FBG FI HOMA-IR	 62 (2 RCTs) 62 (2 RCTs) 62 (2 RCTs)	 ⊕⊕◯◯ LOW a,b ⊕⊕◯◯ LOW a,b ⊕⊕◯◯ LOW a,b	 - - -	 The mean fasting Blood glucose was 5.5- 92.9 The mean fasting insulin was 14.3-16.5 The mean HOMA-IR was 3.7-3.9	**(T1 minus T2)** MD 0.02 lower (0.54 lower to 0.51 higher) MD 2.58 higher (2.3 lower to 7.46 higher) MD 0.45 higher (0.79 lower to 1.69 higher)
Exenatide versus Metformin **F**BG FI HOMA-IR	 221 (2 RCTs) 221 (2 RCTs) 221 (2 RCTs)	 ⊕◯◯◯ VERY LOW a,b,c ⊕⊕◯◯ LOW a,b ⊕⊕◯◯ LOW a,b	 - - -	 The mean fasting Blood glucose was 4.76-4.85 The mean fasting insulin was 1.04-4.51 The mean HOMA-IR was 2.91-5.7	**(T1 minus T2)** MD 0.05 higher (0.1 lower to 0.2 higher) MD 0.24 lower (0.57 lower to 0.1 higher) MD 0.34 lower (0.65 lower to 0.03 lower)
Orlistat versus placebo **F**BG FI HOMA-IR	 147 (2 RCTs) 147 (2 RCTs) 147 (2 RCTs)	 ⊕⊕◯◯ LOW a,b ⊕⊕◯◯ LOW a,b ⊕⊕◯◯ LOW a,b	 - - -	 The mean fasting Blood glucose was 5.69-106.35 The mean fasting insulin was 17.34-77 The mean HOMA-IR was 2.87-3.41	**(T1 minus T2)** MD 0.14 lower (0.55 lower to 0.28 higher) MD 0.15 lower (2.87 lower to 2.58 higher) MD 0.03 lower (0.47 lower to 0.41 higher)
Acarbose versus placebo HOMA-IR	 72 (2 RCTs)	 ⊕◯◯◯ VERY LOW a,b,c	 -	 The mean HOMA-IR was 1.12-1.52	**(T1 minus T2)** MD 0.23 lower (1.49 lower to 1.02 higher)
Acarbose versus Metformin **F**BG FI	 90 (2 RCTs) 86 (2 RCTs)	 ⊕⊕◯◯ LOW a,b ⊕⊕◯◯ LOW a,b	 - -	 The mean fasting Blood glucose was - 48.4-93.6 The mean fasting insulin was 16-18.4	**(T1 minus T2)** MD 10.5 lower (15.76 lower to 5.24 lower) MD 0.86 higher (1.92 lower to 3.63 higher)
Saxagliptin versus Metformin **F**BG HOMA-IR	 97(2 RCTs) 64(2 RCTs)	 ⊕◯◯◯VERY LOW a,b,c ⊕◯◯◯VERY LOW a,b,c	 - -	 The mean fasting Blood glucose was 5.01-5.4 The mean HOMA-IR was 2.9-5.9	**(T1 minus T2)** MD 0.19 higher (0.26 lower to 0.65 higher) MD 0.01 lower (1.78 lower to1.75 higher)
Metformin versus Simvastatin FBG	 268 (2 RCTs)	 ⊕◯◯◯ VERY LOW a,b,c	 -	 The mean fasting Blood glucose was 89.4-85.59	**(T1 minus T2)** MD 4.43 lower (8.41 lower to 0.44 lower)
Metformin versus NAC **F**BG FI	 202 (2 RCTs) 202 (2 RCTs)	 ⊕◯◯◯ VERY LOW a,b ⊕◯◯◯ VERY LOW a,c	 - -	 The mean fasting Blood glucose was 80.2-86.61 The mean fasting insulin was 8.89-18.3	**(T1 minus T2)** MD 3.72 higher (1.13 higher to 6.31 higher) MD 1.48 higher (0.51 higher to 2.46 higher)
Atorvastatin versus placebo **F**BG FI	 65 (2 RCTs) 65 (2 RCTs)	 ⊕◯◯◯ VERY LOW a,b,c ⊕◯◯◯ VERY LOW a,b,c	 - -	 The mean fasting Blood glucose was 4.8- 5 The mean fasting insulin was 8.3-17.6	**(T1 minus T2)** MD 0.5 higher (0.16 higher to 0.84 higher) MD 5.6 higher (1.56 lower to 12.76 higher)

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **MD:** Mean difference; **RCT:** randomised clinical trials; **BMI:** body mass index; **WHR**: waist to hip ratio; **WC:** waist circumference; **T1:** first treatment, **T2:** second treatment

GRADE Working Group grades of evidence.
High certainty: We are very confident that the true effect lies close to the estimate of the effect.

Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

Explanations
a. Two studies have an unclear risk of bias across five or more domains. One study has a high risk of performance bias. Thus, we downgraded one level.
b. A small number of participants with a wide confidence interval. So, we downgraded one level.
c. There is no overlapping of confidence interval between the studies, which could mean there are small studies with negative results been unreported. Thus, we downgraded one level.
d. unclear risk of bias across more than five domains. one study has a high-performance bias. Thus, we downgraded one level.

4.4 Discussion

This systematic review has collected the current evidence supporting the effect of various pharmacological interventions on insulin resistance. To our knowledge, this is the first systematic review to report on the effect of various pharmacological interventions on insulin resistance in women with PCOS. When metformin was administered at various doses compared with placebo, there was a significant reduction in the mean FBG and FI. This was also evident when metformin was compared with simvastatin and acarbose. The result also showed a significant increase in the mean FBG and FI when metformin was compared with NAC. On the other hand, exenatide significantly reduced HOMA-IR compared with metformin. The strength of evidence for these data ranged from very low to moderate, and therefore, care should be applied when interpreting these findings.

Metformin is a widely used drug that exerts its action by targeting various organs via multiple molecular mechanisms. For instance, it acts on the liver to reduce hepatic glucose production by opposing glucagon action and activating the activated protein kinase (AMPK), enhancing insulin sensitivity by modulating lipid metabolism (655,697). The current systematic review showed significant reductions in FBG and FI with metformin at various doses and when administered for both long and short duration compared with placebo. These results are in accord with what has been reported in a non-randomised cohort study of 108 insulin resistant and obese women with PCOS who received Metformin 1500 mg QD for six months (698). However, in a meta-analysis of RCTs evaluating the effects of metformin on the metabolic, hormonal, and clinical outcomes in women with PCOS, no effects on FBG, FI and HOMA-IR were found (699). However, there was a significantly high level of heterogeneity amongst those studies.

Furthermore, a recent systematic review and meta-analysis of RCTs evaluating the effect of metformin in overweight women with PCOS reported that although there was a significant effect on the anthropometric indices, no effect was seen on the parameters of insulin resistance (638). Therefore, considering these previous findings, it appears that metformin alone has a variable effect on the parameters of insulin resistance in women with PCOS. In the present review, we reported a significant reduction in FI with pioglitazone compared with placebo and metformin. However, data from a meta-analysis assessed the effect of metformin versus thiazolidinediones in women with PCOS showed no changes in insulin sensitivity (700). We also found a significant increase in FBG when metformin was compared with NAC. However, a recent meta-analysis of RCTs that compared the efficacy of metformin versus NAC showed no significant changes in insulin resistance parameters (701). Therefore, this review did not establish any significant effect on HOMA-B with various pharmacological interventions used to manage PCOS.

This current review followed a comprehensive and systematic search of the relevant databases and grey sources that only included RCTs and randomised crossover trials. Observational studies and non-randomised clinical trials were excluded to reduce the risk of bias. We applied a language filter, and only trials reported in the English language were included, and therefore several clinical trials in foreign languages may not have been retrieved. Assessing such trials requires sophisticated translation, which is challenging and could affect this review's methodology. The majority of the trials were of a smaller sample size. The statistical power used to calculate sample size and detect the meaningful differences between the groups were not fully reported. All the trials were of short duration and reported

baseline and immediate post-intervention data. Therefore, the long-term effect of the different pharmacological interventions in women with PCOS is not clear.

This systematic review acknowledges the poor quality of the included clinical trials, which is also reflected in the summary of evidence of the GRADE score. Due to the nature of the clinical trials, there was a significantly high level of heterogeneity and performance bias among the included studies. Although a simple logistical approach could have been taken by blinding the outcome assessors, there was a significantly high level of detection bias. Reporting and selection bias were inadequately reported amongst the trials, so the judgment of unclear risk of bias was made in nearly 75% of the included trials. Disproportionately, only 20% of the trials reported information of the method used to blind the participants and the outcome assessor and 49% were judged to have an unclear risk of attrition bias. Around 25% of the included trials had a high performance and detection bias risk. For the insulin resistance outcomes, the grade of evidence was rated from very low to moderate due to the unclear or high risk of performance bias. In addition, for 16 RCTs (27.58%), no clear PCOS diagnostic criteria were detailed; this was due to incomplete reporting considered while assessing the overall risk of bias as one of the main limitations of the included RCTs.

Based on our findings, it is clear that there is a lack of robust clinical trials assessing the different pharmacological interventions in PCOS management. Furthermore, trials examining the clinical effectiveness of these interventions are of low or very low quality. Therefore, the available data are not suitable for drawing definite conclusions and recommendations for clinical practice. Furthermore, these trials are of small sample sizes that undermined the statistical power used to calculate the meaningful effects of the outcomes. Therefore, further clinical trials with robust design are needed to make better-informed decisions and

recommendations and draw guidelines for the various pharmacological interventions used in women with PCOS.

4.5 Conclusion

In conclusion, data pooled in this meta-analysis showed that pharmacological interventions including metformin, pioglitazone, acarbose and exenatide reduce FBG, FI and HOMA-IR. However, some other therapeutic agents have no effect on insulin resistance parameters. Even though data presented in this systematic review and meta-analysis are drawn mainly from clinical trials, caution should be taken when interpreting these results. The majority of the interventions showed modest effects with wide confidence intervals that indicate significant uncertainties. Therefore, further clinical trials with rigorous methodology and sufficient power are needed for each pharmacological intervention.

5 Chapter 5: Impact of pharmacological interventions on anthropometric indices in women with polycystic ovary syndrome: a systematic review and meta-analysis of randomised controlled trials

5.1 Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous and complex endocrine disorder affecting women of reproductive age, with a prevalence ranging from 8% to 13 % (646,647). PCOS is characterised by clinical and biochemical evidence of excess androgen levels (manifested as acne and hirsutism), menstrual irregularities and sonographic polycystic ovarian morphology (702). Additionally, metabolic disorders such as insulin resistance (IR) and impaired glucose tolerance are common in women with PCOS, leading to an increased risk of type 2 diabetes mellitus (T2DM) (506). Moreover, PCOS associated with a range of other complications, including infertility, increased body weight, increased risk of cardiovascular disease (CVD) and endometrial cancer (703-705).

Increased body weight is a prominent feature of PCOS, and around 50% of women with PCOS are either overweight or obese (706). Obesity exacerbates PCOS features such as excessive hair growth, infertility and pregnancy complications, aggravating IR, culminating in an increased metabolic risk associated with PCOS (78). Therapeutic approaches, including lifestyle modifications through dietary interventions and physical activity, are the cornerstone in PCOS management (707). There are also differing pharmacotherapeutic interventions, including insulin sensitisers (metformin and thiazolidinediones), which can improve IR and peripheral glucose uptake (391,393). However, these therapeutic options are primarily licensed to treat other conditions such as T2DM and their effectiveness in PCOS remains

unclear in the literature. There are also significant gaps between the available evidence and the evidence-based treatment options (702). This might often lead to the delay in offering satisfactory treatment options and the clinical inertia around treating PCOS (702). Therefore, this systematic review and meta-analysis aimed to evaluate and analyse the available evidence on different therapeutic options' effectiveness in treating PCOS and improving anthropometric outcomes.

5.2 Methods and materials

5.2.1 Protocol and registration

The protocol and the registration of this systematic review and meta-analysis are explained in chapter 2, section 2.1.1.1.

5.2.2 Eligibility criteria for the included studies

The eligibility criteria, including inclusion/exclusion criteria, is explained in chapter 2, section 2.1.1.2.

5.2.3 Literature search

The search strategy, including the search in the medical databases, is explained in chapter 2, section 2.1.1.3.

5.2.4 Study selection

The study selection, including screening titles and abstracts of the retrieved studies, is explained in chapter 2, section 2.1.1.4.

5.2.5 Data extraction

The method used to extract information from the included studies is explained in chapter 2, section 2.1.1.5.

5.2.6 Risk of bias assessment in the included studies

The RoB assessment for the included studies is explained in chapter 2, section 2.1.1.6.

5.2.7 GRADE scoring

The robustness of evidence for each chosen outcome is assessed using the GRADE scoring system and is explained in chapter 2, section 2.1.1.7.

5.2.8 Statistical analysis

The statistical method used to estimate the pooled effects of the various interventions is explained in chapter 2, section 2.1.1.12.

5.2.9 Assessment of heterogeneity

Heterogeneity for the outcomes across each RCT was evaluated and explained in chapter 2, section 2.1.1.8.

5.2.10 Subgroup analysis

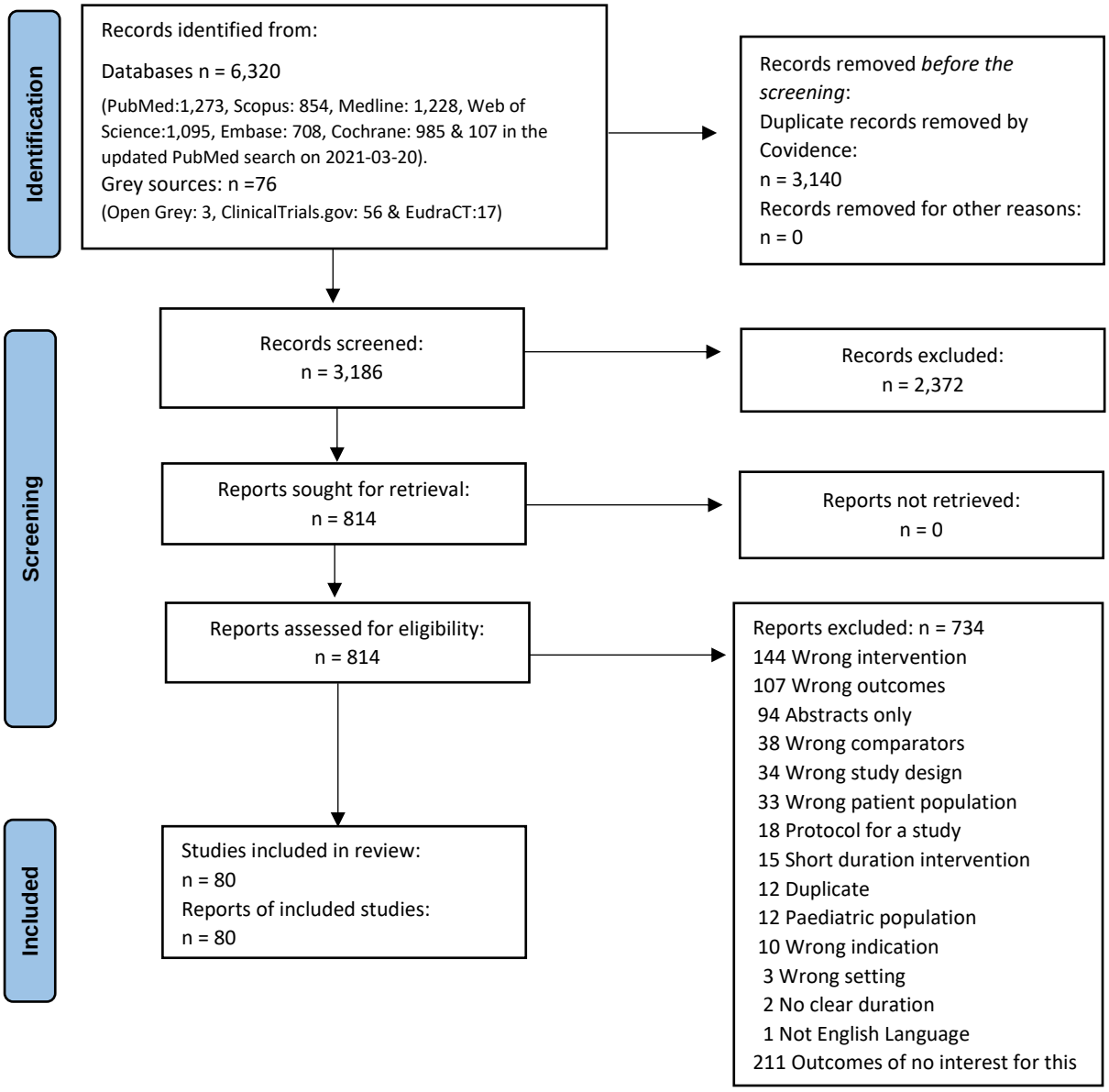
Subgroup analysis was conducted for the included RCTs and explained in chapter 2, section 2.1.1.9.

5.3 Results

5.3.1 Search results

In total, 6,326 records were identified, of which 3,186 studies were screened for eligibility based on titles and abstracts after removing duplicates. A total of 814 full-text articles were retrieved for detailed assessment for eligibility, of which 80 RCTs were found eligible and included in this study (Figure 5-1). No additional eligible studies were identified in the hand screening of the included papers.

Figure 5-1: PRISMA flow diagram



5.3.2 Characteristics of the included RCTs

The 80 RCTs were published between 2000 and 2020 and included (4,028 participants, both PCOS and control) that met the inclusion criteria and were included in the meta-analysis. Forty-two trials (607, 608, 610-613, 616-621, 659, 661-663, 665-668, 670-678, 681, 686, 691, 696, 708-716) diagnosed PCOS based on the Rotterdam 2003 criteria (30), ten trials (609, 621-623, 679, 680, 683, 684, 717-719) used the National Institute of Health 1990 (NIH, NICHD) criteria, one trial (720) used the Androgen Excess Society 2006 (AES) criteria (38), and no diagnostic criteria were specified for the remainder of the trials (Table 7).

5.3.2.1 Interventions and comparisons details

Twenty-one RCTs (26.3%) assessed the effect of metformin compared with placebo (607, 610, 616, 627-629, 663, 670, 671, 673, 687-689, 709, 711, 712, 714-716, 721-723). Six RCTs (7.5%) compared metformin with pioglitazone (615, 620, 631, 632, 677, 686). Two RCTs (2.5%) evaluated liraglutide compared with metformin (719, 724). Five RCTs (6.3%) examined pioglitazone compared with placebo (608, 633, 637, 679, 725). Nine RCTs (11.3%) compared rosiglitazone with metformin (612, 659, 672, 678, 681, 685, 691, 726, 727). Three RCTs (3.8 %) examined liraglutide compared to liraglutide added to metformin (621, 623, 668). Three RCTs (3.8%) compared orlistat with metformin (686, 695, 708). Two RCTs (2.5%) compared sitagliptin added to metformin with metformin alone (665, 692). Two RCTs (2.5%) evaluated sitagliptin with placebo (661,666). Three RCTs (3.8%) assessed exenatide compared to metformin (611, 619, 696). Two RCTs (2.5%,) compared orlistat with placebo (662, 674). Two RCTs (2.5%) compared acarbose versus placebo (693, 694). Three RCTs (3.8%) compared acarbose to metformin (676, 680, 684). Two RCTs (2.5%) compared saxagliptin alone and with metformin plus saxagliptin (609,617). Two RCTs (2.5%) compared metformin with simvastatin (634, 683). Two RCTs (2.5%) compared metformin with NAC (667, 675). Two RCTs (2.5%)

examined atorvastatin with placebo (636). Three RCTs (3.8%) compared spironolactone and placebo (713, 717, 718). Five RCTs (6.3%) assessed rosiglitazone with placebo (710, 720, 728-730) (Table 7).

5.3.2.2 Outcomes measured

All RCTs assessed the outcomes at baseline and post-intervention at various follow up times**.** Thirty-one trials (610, 611, 615, 616, 618, 619, 621, 623, 627, 630-632, 659, 663, 668, 672, 673, 677, 688, 691, 695, 708, 710, 711, 716, 719, 720, 724, 728) reported on changes in body weight. Seventy-nine RCTs reported on changes in BMI as the primary outcome (607-613, 615-617, 619-623, 627-629, 631-634, 636, 637, 659, 661-663, 665-668, 670-681, 683-688, 691-694, 696, 708-720, 722-726, 729, 730). Twenty-three RCTs reported on changes in WC (609, 610, 616-618, 621-623, 628, 631, 637, 668, 672, 677-679, 711, 719, 724, 726). Thirty-four RCTs reported on changes in WHR (607, 610, 611, 615, 616, 619, 628, 631-633, 671-673, 678, 679, 687, 689, 710, 711, 714, 720, 725, 730) (Table 7).

Table 7: Characteristics of the included RCTs

Author	Study design	Country	POCS diagnostic Criteria	Participants characteristics (PCOS) Mean±SD	Interventions	Durations	Outcomes
Amiri et al(607)	RCT	Iran	Rotterdam	Age:25.6±4.02	Metf, Flu, Metf+ Flu, Placebo	6 months	BMI, WHR, WC, FBG,LDL,HDL, TG
J.Ahmad et al(727)	RCT	India	NIH	Age: 22.81± 4.52	Rosig, Metf	12 months	WHR, BMI
Aroda et al(679)	RCT	USA	NIH	Age: 27.87 ±0.87	Piog, Placebo	6 months	Wt, BMI, WHR, WC, FBG,FI
Ashraf Ganie et al(717)	RCT	India	NIH	Age: 22.9 ±5.3	Spironolactone versus Met	3 months	BMI,FBG,FI, WHR
Batista et al(720)	RCT	Brazil	AES-2006	Age: 24.5±4.33	Rosig, placebo	12 weeks	FBG.FI,HOMA-IR
Brettenthaler et al(608)	RCT	Switzerland	Rotterdam	Age: 30.2± 1.4	Piog, placebo	3 months	BMI,WHR,FBG, FI, HOMA-IR
Cataldo et al (728)	RCT	USA	NICHD	Age: 29.3 ± 1.5	Rosig 2mg, 4mg, 8 mg	12 weeks	BMI, WHR, Wt
Cetinkalp et al(659)	RCT	Turkey	Rotterdam	Age: N/A	Met, Rosigl , ECA	4 months	FBG,FI, Wt, BMI, HOMA-IR,TC,
Cheng et al(660)	RCT	Australia	Rotterdam	Age: 26 ± 4	Metf, placebo	6 months	Wt, BMI, WC, WHR, LDL,HDL,HOMA-IR, HOMA-B
Cho et al(686)	RCT	UK	Rotterdam	Age: 26·4 ± 1·5	Metf, Orlistat, Piog	12 weeks	HOMA-IR, BMI
Chou et al(689)	RCT	Brazil	N/A	Age:24±5	Metf, placebo	3 months	BMI,WHR,FBG, FI, TG,TC,HDL,LDL
Ciotta et al(693)	RCT	Italy	N/A	Age:20.5±0.6	Acarbose, Placebo	3 months	BMI, HOMA-IR
Dereli et al(729)	RCT	Turkey	NICHD	Age: 31.4 ± 0.9	Rosig 2mg, 4 mg	8 months	BMI, WHR
Devin et al(661)	RCT-cross over	USA	Rotterdam	Age: N/A	Sitag, placebo	4 weeks	FBG, BMI,WHR,WC, LDL.HDL, TC
Diamanti-Kandarakis et al(662)	RCT	Greece	Rotterdam	Age: 27·52 ± 5·77	Orli, placebo	6 months	BMI,WHR,HOMA-IR
Eisenhardt et al(663)	RCT	Germany	Rotterdam	Age: 27.0±0	Metf,placebo	12 weeks	FBG.FI,HOMA-IR
Elkind-Hirsch et al(664)	RCT	USA	Rotterdam	Age: 28.2 ± 1.1	Exen, Metf,Exen+Metf	24 weeks	Wt, BMI, CRP, TG, TC,HDL,LDL, FBG
Elkind-Hirsch et al(609)	RCT	USA	NIH	Age: 29.9± 7	Sax, Metf, Sax+Metf	16 weeks	FBG,FI, HDL,TG, LDL,HOMA-IR

Ferjan et al(666)	RCT	Slovenia	Rotterdam	Age: 34.3 ± 6.8	Metf, Metf+Sitag	12 weeks	HOMA-IR , Wt ,BMI,WC TC,TG,LDL, HDL,
Ferjan et al(665)	RCT	Slovenia	Rotterdam	Age: 35.0 ± 7.2	Sitag, placebo	12 weeks	HOMA-IR, HOMA-B, FBG
Gambineri et al(627)	RCT	Italy	N/A	Age: 27·1 ± 3·6	Plac, Metfo, Flut, Metf + Flut	6 months	FBG,FI,HOMA-IR, Wt, BMI,
Glintborg et al(637)	RCT	USA	N/A	Age: 32±0	Piog, placebo	16 weeks	FI, HOMA-IR
Glintborg et al(633)	RCT	USA	N/A	Age: 32±0	Piog,plcebo	16 weeks	BMI,WHR, WC, FI
Glintborg et al(725)	RCT	Denmark	N/A	Age: N/A	Piog, placebo	16 weeks	BMI, CRP, LDL
Ghandi et al(708)	RCT	Iran	Rotterdam	Age: 27±4.92	Orlistat, Metf	3 months	BMI,WC, TC, TG
Ganie et al(718)	RCT	USA	NIH	Age: 22.6 ±5.0	Spironolactone, Metf	6 months	WHR,BMI,FBG,FI
Hanjalic-Beck et al(680)	RCT	Germany	NIH	Age: N/A	Metf, Acarbose	12 weeks	BMI, FBG,FI
Heidari et al(610)	RCT	USA	Rotterdam	Age: 32.4±7.5	Metf, placebo	3 months	BMI,WC,WHR, Weight FBG, FI
Javanmanesh et al(667)	RCT	Iran	Rotterdam	Age: 29.75 ± 4.90	Metf, NAC	24 weeks	BMI, FBG,FI, LDL, TC,TG, HDL, HOMA-IR
Jayagopal et al(695)	RCT	UK	N/A	Age: 27 ±0.9	Orlistat, Metf	3 months	FBG, FI, TC,TG, HDL
Jensterle et al(833)	RCT	Slovenia	NIH	Age: 27.6±7.2	Metf, Rosi	6 months	BMI,WC, TC,TG,LDL,HDL ,FBG, FI
Jensterle et al(778)	RCT	Slovenia	Rotterdam	Age: 30.7 ± 7.9	Metfo, Rosi, Lira	6 months	Wt, BMI,WC,FI, FBG, HOMA-IR
Jensterle et al(821)	RCT	Slovenia	Rotterdam	Age: 33.1 ± 6.1	Met+Lira, Lira	12 weeks	BMI, Wt, WC, FBG,FI, HOMA-IR
Jensterle et al(587)	RCT	Slovenia	Rotterdam	Age: 34.4 ± 6.5	Met+Lira,Lira	12 weeks	Wt ,BMI, WC, FI,FBG
Jensterle Sever et al(779)	RCT	Slovenia	NIH	Age: 31.3±7.1	Lira,Metf, Lira+Metf	12 weeks	FBG,BMI,WC,FI,TC,TG,HDL,LDL
Jensterle et al(681)	RCT	Slovenia	NIH	Age: 23.5±0.7	Metf,Rosi	6 months	FBG,FI,BMI, HOMA-IR
Jensterle et al(622)	RCT	Slovenia	NIH	Age: 23.1±3.7	Metf, Rosi	6 months	WC,BMI, FI,FBG,TC, TG,
Kilicdag et al(691)	RCT	Turkey	Rotterdam	Age:24.13 ±1.42	Metf, Rosi	3 months	BMI, FI,FBG,TC, TG, HOMA-IR
Kazerooni et al(822)	RCT	Iran	Rotterdam	Age: 25.6± 4.32	Metf, Simva,placebo	12 weeks	BMI, FI,FBG,TC, TG, HDL,LDL
Kocak et al(823)	RCT	Turkey	Rotterdam	Age: 26.2 ±3.7	Metf, placebo	2 months	BMI, FI,FBG,WHR
Karimzadeh et al(709)	RCT	Iran	Rotterdam	Age: 28.81±3.18	Metf, placebo	3months	BMI, FI,FBG,TC, TG, HDL,LDL

Lam et al(710)	RCT	China	Rotterdam	Age: N/A	Rosi, placebo	12 months	WC,BMI, FI,FBG,TC, TG
Li et al(824)	RCT	China	Rotterdam	Age: 25.95± 4.36	Rosi, Metformin	6 months	WC,BMI, FI,FBG,TC, TG, WHR, LDL,HDL
Lingaiah et al(825)	RCT	Finland	Rotterdam	Age: 27.6 ±4.0	Metf, placebo	3 months	BMI, WC ,WHR,FI,FBG
Liu et al(767)	RCT	China	Rotterdam	Age: 27.69 ± 3.80	Metf, Exena	24 weeks	FI,FBG, HOMA-IR WC,BMI,TC, TG, WHR, LDL,HDL
Lord et al(784)	RCT	UK	N/A	Age: 27.76 ±4.89	Metf, placebo	3 months	WC,BMI, FI,FBG,TC, TG, WHR, LDL,HDL, HOMA-IR
Legro et al(726)	RCT	USA	N/A	Age: 28.0 ±4.0	Metf,Rosi	3 months	WC,BMI, FI,FBG, WHR
Morin-Papunen et al(711)	RCT	Finland	Rotterdam	Age: 28.4 ± 3.9	Metf,placebo	3months	Wt, WC,BMI,WHR
Morteza Taghavi et al(712)	RCT	Iran	Rotterdam	Age: N/A	Metf, placebo	6 months	BMI
Mehrabian et al(835)	RCT	Iran	NIH	Age: 29.18±8.28	Metf, Flut, Simva	6 months	WC,CRP,BMI,FBG,TG,HDL
Mohiyiddeen et al(768)	RCT	UK	Rotterdam	Age: 29.0 ±1.0	Metf,Rosig	3 months	WC,BMI, FI,FBG,TC, TG, WHR, LDL,HDL
Moini et al(826)	RCT	Iran	Rotterdam	Age: 27.42 ± 3.31	Orlistat, placebo	3 months	WC,BMI, FI,FBG,TC, TG, WHR, LDL,HDL
Muneyyirci-Delale et al(713)	RCT	USA	Rotterdam	Age: N/A	Metf, Spironolactone	12 weeks	BMI,TC, TG,
Naka et al(842)	RCT	Greece	N/A	Age: 23.3± 4.9	Metf,Piogl	6 months	WC,BMI, FI,FBG,TC, TG, WHR, LDL,HDL
Navali et al(790)	RCT	Iran	N/A	Age:26.43±4.67	Metf, Simva	3 months	BMI, FI,FBG,TC, TG, WHR, LDL,HDL
Nemati et al(827)	RCT	Iran	Rotterdam	Age: N/A	Metf, NAC	12 weeks	BMI,FBG,FI
Ng et al(785)	RCT	China	N/A	Age:30.5±0	Metf, placebo	3 months	BMI,FBG,FI,TC,TG
Ortega-González et al(788)	RCT	Mexico	N/A	Age: 28.8 ±0.9	Metf, Piogl	6 months	Wt, BMI,WHR ,FBG, FI
Palomba et al(722)	RCT	Italy	N/A	Age: 24.3 ± 3.1	Metf, placebo	24 months	BMI,LDL
Paredes Palma et al(845)	RCT	Mexico	N/A	Age: N/A	Metf, Sitag	N/A	BMI, HOMA-IR
Penna et al(847)	RCT	Brazil	N/A	Age: 26.69 ±1.46	Acarbose, placebo	6 months	BMI, FI
Puurunen et al(792)	RCT	Finland	N/A	Age: 40.5 ±5.9	Atorva, placebo	6 months	BMI, WHR,LDL, HDL
Rautio et al(730)	RCT	Finland	N/A	Age: 29.1 ± 1.2	Rosig, placebo	4 months	BMI, WHR, Wt
Romualdi et al(714)	RCT	Italy	Rotterdam	Age: 24.7 ±4.4	Metf, placebo	6 months	BMI,WHR,LDL,HDL,TC

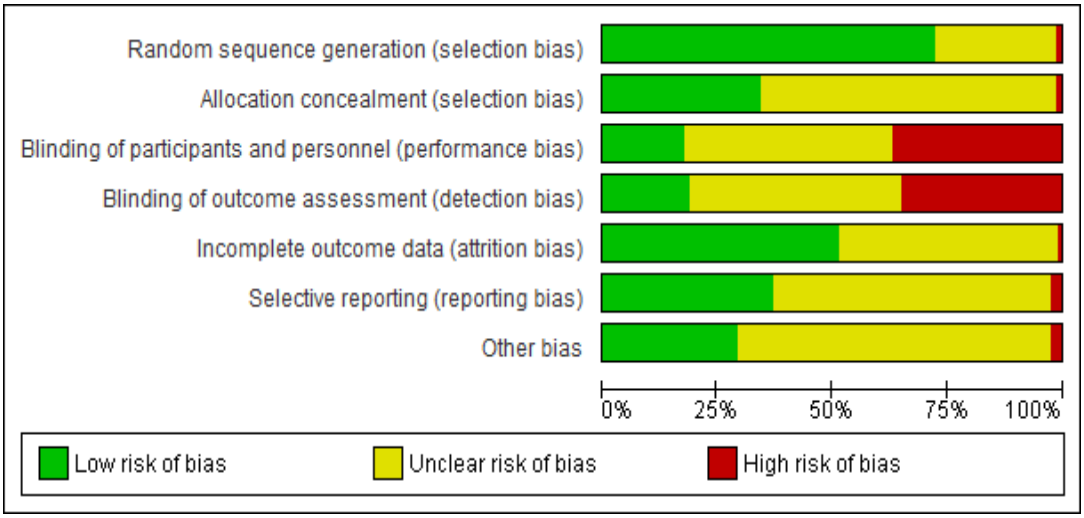
Rezai et al(828)	RCT	Iran	Rotterdam	Age: 26.3±4	Metf, Acarbose	3 months	BMI,FBG,HDL,TG,TC.LDL
Sathyapalan et al(769)	RCT	UK	Rotterdam	Age: 27.7± 1.4	Atorvas, placebo	12 weeks	Wt,BMI,WC,WHR, HOMA-IR, FBG,FI
Shahebrahimi et al(829)	RCT	Iran	Rotterdam	Age: 27.5 ± 3.68	Metf, Piog	3 months	Wt, BMI,WC, FBG, LDL,HDL,TG
Sohrevardi et al(771)	RCT	Iran	Rotterdam	Age: N/A	Metf,Piog, Metf+Piog	3 months	Wt, BMI, WHR,HOMA-IR, FBG, FI
Sönmez et al(836)	RCT	Turkey	NIH	Age: 26.13 ±5.08	Metf, Acarbose	3 months	BMI, Wt, FBG,FI
Sova et al(772)	RCT	Finland	Rotterdam	Age: : 27.7 ±4.0	Metf, placebo	3 months	Wt, WC, WHR,BMI,FBG,FI
Steiner et al(837)	RCT	Germany	NIH	Age: 22.9±4.5	Metf, Rosig	6 months	BMI,HOMA-IR, FBG,FI
Tao et al(773)	RCT	China	Rotterdam	Age: 30 ± 5	Saxag, Metf	24 weeks	Wt, BMI,WC,WHR, LDL,HDL,TG, HOMA-IR
Trolle et al(786)	RCT	Denmark	N/A	Age: 31±0	Metf, placebo	6 months	Wt,WHR,FBG,FI,HOMA-IR, LDL,HDL
Underdal et al(774)	RCT	Denmark	Rotterdam	Age: 29.5 ±3.9	Metf, placebo	N/A	Wt,BMI,WC,WHR
Vanky et al(715)	RCT	Norway	Rotterdam	Age: 28.9 ±4.8	Metf, placebo	36 weeks	BMI, DHEAS
Vandermolen et al(840)	RCT	USA	N/A	Age: 29 6 ±1.2	Metf, placebo	7 weeks	Wt ,BMI, FBG,FI
Yarali et al(839)	RCT	Turkey	N/A	Age:29.7±5.6	Metf, placebo	6 weeks	WHR,BMI,FBG,FI
Yilmaz et al(830)	RCT	Turkey	Rotterdam	Age: 24.67+4.60	Metf, Rosig	24 weeks	FBG,FI,BMI,WHR
Zahra et al(716)	RCT	Pakistan	Rotterdam	Age: 25.8 ± 6.1	Metf, placebo	3 months	Wt, BMI,FBG,FI,HOMA-IR
Zheng et al(775)	RCT	China	Rotterdam	Age: 27.70 ± 3.41	Exena, Metf	12 weeks	Wt, BMI ,WHR,FBG,FI,HDL,LDL, TG, TC
Ziaee et al(776)	RCT	Iran	Rotterdam	Age: 25.28±4.38	Metf, Piog	12 weeks	BMI,HOMA-IR,HDL,LDL,TG

RCT: randomised clinical trial, **N/A**: not available, **BMI**: body mass index, **Wt:** weight, **WHR:** waist to hip ratio, **WC**: waist circumference, **FBG**: fasting blood glucose, **FI**: fasting insulin, **HDL**: high-density lipoprotein**, LDL:** Low-density lipoprotein, **TG:** triglycerides, **TC**: total cholesterol, **HOMA-IR:** the homeostatic model of insulin resistance**, NIH**: national institute for health, **NICHD**: national institute of child health and development. **Metf:** Metformin, **Saxa:** Saxagliptin, **Piog**: Pioglitazone, **Rosig**: Rosiglitazone,**Atrova:** Atorvastatin, **Simva**: Simvastatin, **WHO:** world health organisation, **CRP:** C-reactive protein, **Lira**: Liraglutide, **USA:** United States of America, **UK**: United Kingdom.

5.3.3 Risk of bias assessment

The overall RoB is illustrated in (Figure 5-2). Twenty-six RCTs were judged to have low RoB with regard to selection bias for using appropriate methods to generate their sequences for randomisation and allocation concealment (607, 609, 615, 628, 632, 665, 667, 670, 680, 683, 684, 691-694, 696, 709-711, 714, 715, 717, 722, 723). One trial (679) was judged to have a high risk of selection bias. Five trials were categorised as having an unclear RoB across all six assessed RoB domains due to insufficient information (637, 675, 677, 712, 716). Due to the nature of the trials (open-label), thirty-five trials were judged to have a high risk of performance bias (607, 609-612, 615, 617, 619-623, 632, 659, 662, 665, 666, 668, 672, 681, 686, 689, 695, 696, 708, 717-719, 724). The remainder of the trials were judged to have an unclear risk of performance bias due to a lack of a clear statement about whether the outcome assessors were blinded to the participant's allocation and interventions. One trial was judged to have a high risk of reporting bias due to selective data reporting (689).

Figure 5-2: Risk of bias graph: review of authors' judgements about each risk of bias item presented as percentages across all included studies.

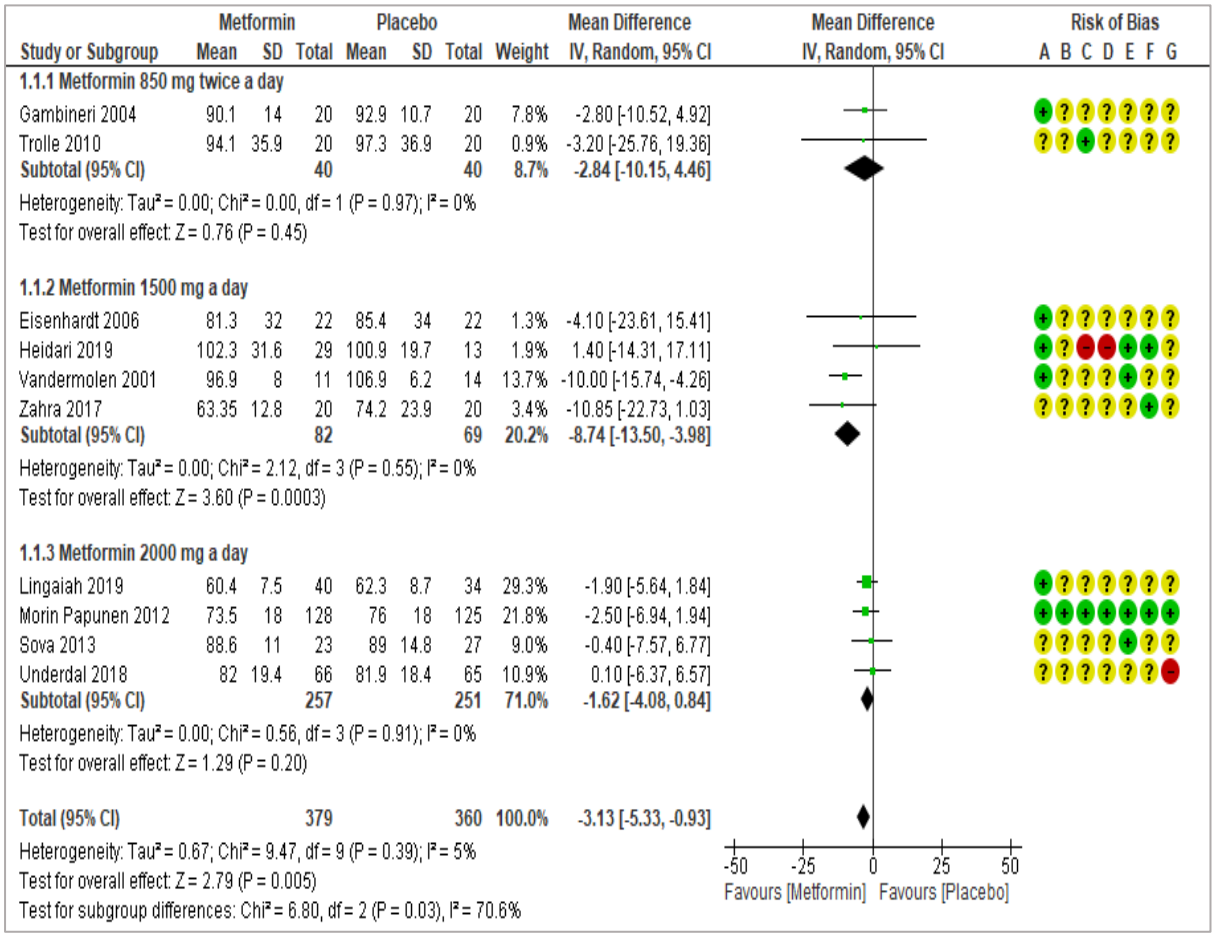


5.3.4 Body weight

5.3.4.1 Metformin versus placebo

In four RCTs, metformin 1500 mg once a day (QD) significantly reduced the mean body weight by nearly 9 kgs (95% CI: -13.50,3.98). In two RCTs compared placebo, metformin 850 mg twice a day (BID) was associated with no significant change in the mean body weight by 2.84 kgs (95% CI: -10.15 ,4.46). In four RCTs, metformin 2000 mg QD was associated with no significant change in the mean body weight by 1.6 kgs (95% CI: -4.08, 0.84). Overall, regardless of the administered dosage, metformin was associated with a significant reduction in the mean body weight by 3.13 kgs (95 %CI:-5.33, -0.93, I^2= 5%) compared with placebo. (Figure 5-3) (moderate grade evidence, table 8).

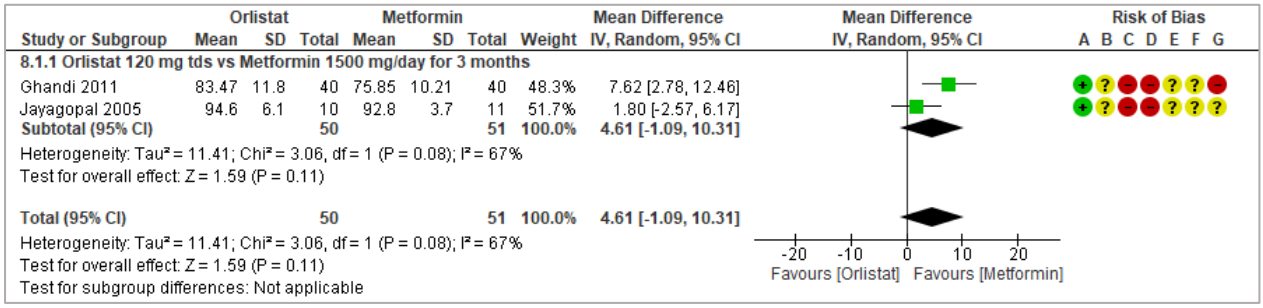
Figure 5-3: Forest plot of Metformin versus placebo on body weight (Kg)



5.3.4.2 Metformin versus Orlistat

In two RCTs, Metformin 1500 mg QD for three months compared with orlistat 120 mg three times a day (TDS) insignificantly increased body weight by 4.61 kgs (95% CI: -1.09,10.31, I^2= 67%) (Figure 5-4) (very low-grade evidence, table 8).

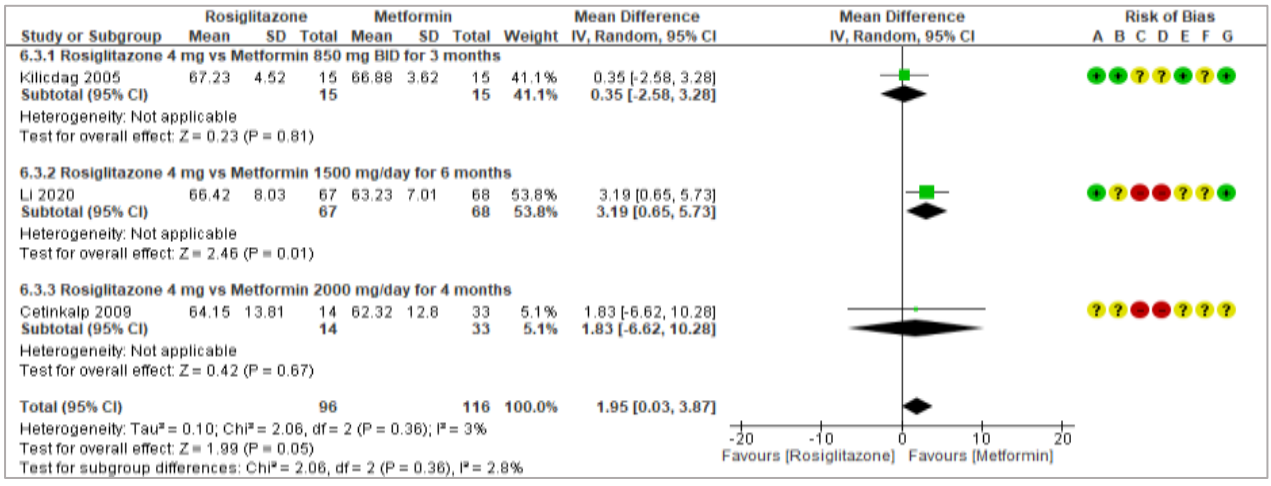
Figure 5-4: Forest plot of Metformin versus Orlistat on body weight (Kg)



5.3.4.3 Rosiglitazone versus Metformin

Three RCTs compared metformin with rosiglitazone showed a significant increase in the mean body weight by 1.95 kgs (95% CI: 0.03, 3.87, I^2*= 3%*) with rosiglitazone. This significant increase was mainly driven by the RCTs that administered 1500 mg/day of metformin compared to 4 mg/day of rosiglitazone for six months (MD in body weight (3.19 kgs: 95%; 0.65, 5.73) (Figure 5-5) (very low-grade evidence, table 8).

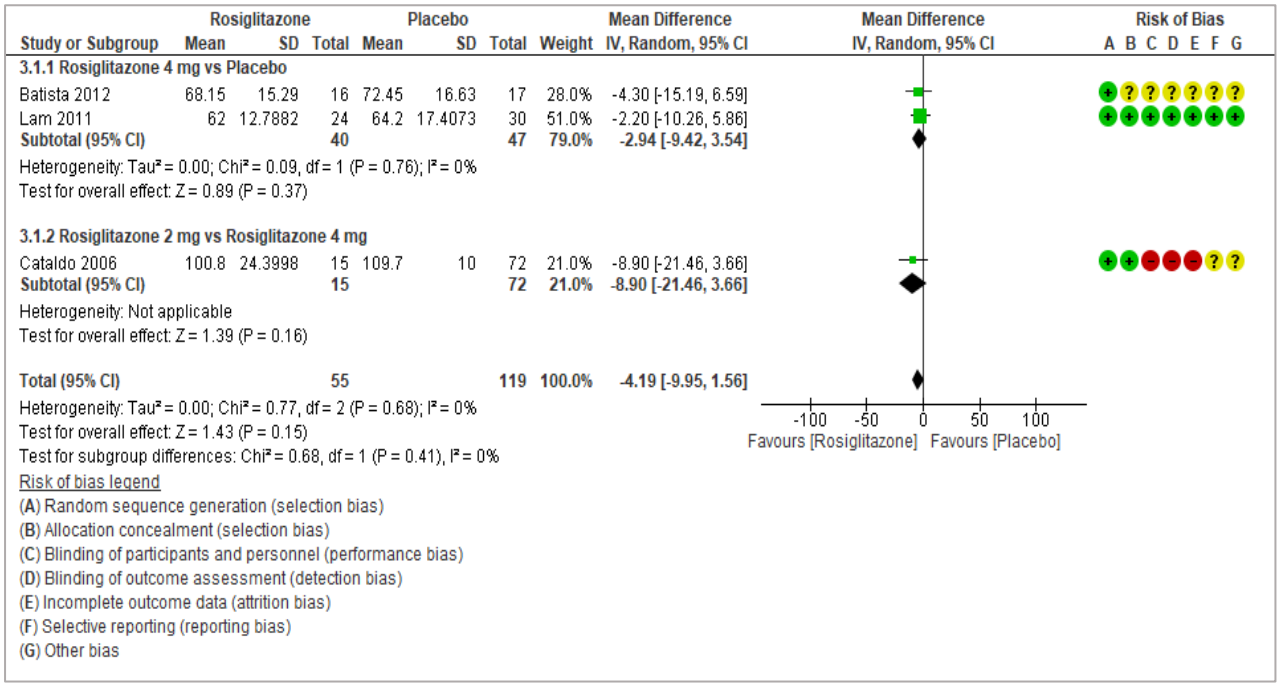
Figure 5-5: Forest plot of Metformin versus Rosiglitazone on body weight (Kg)



5.3.4.4 Rosiglitazone versus placebo

In two RCTs, rosiglitazone 4 mg QD showed no effect on the mean body weight compared with placebo (MD: -2.94 kgs; 95% CI: -9.42, 3.54). In one RCT compared two doses of rosiglitazone 2 mg QD and rosiglitazone 4 mg QD showed no effect on the mean body weight (MD: -8.90 kgs; 95% CI: -21.46, 3.66). Overall, rosiglitazone of different dosage has no effect on the mean body weight (MD: -4.19 kgs; 95% CI: -9.95,1.56, I^2= *0%*) (Figure 5-6) (very low-grade evidence, table 8).

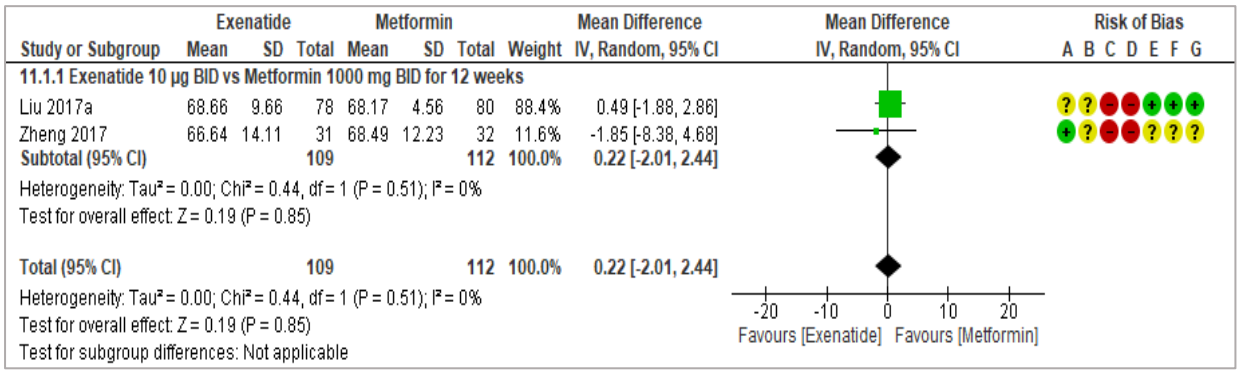
Figure 5-6: Forest plot of Rosiglitazone versus placebo on body weight (Kg)



5.3.4.5 Exenatide versus Metformin

In two RCTs exenatide 10 µg BID compared with metformin 1000 mg BID for 12 weeks has no effect on the mean body weight (MD: 0.22 kgs; 95% CI: -2.01, 2.44, I^2= 0%) (Figure 5-7) (low grade evidence, table 8).

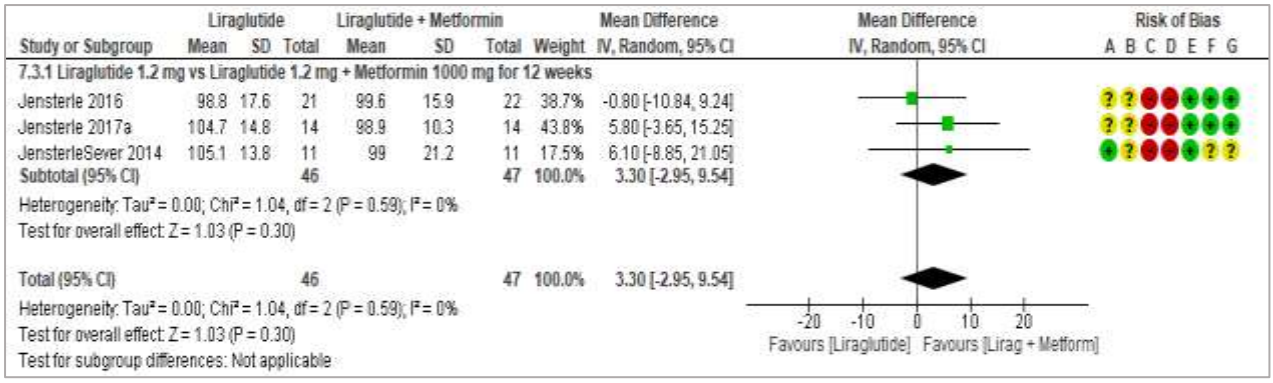
Figure 5-7: Forest plot of Exenatide versus Metformin on body weight (Kg)



5.3.4.6 Liraglutide versus Liraglutide + Metformin

In three RCTs, liraglutide 1.2 mg QD compared with liraglutide 1.2 mg QD added to metformin 1000 mg QD for 12 weeks showed no effect on the mean body weight (MD: 3.30 kgs; 95%CI: -2.95, 9.54, I^2= 0%) (Figure 5-8) (low- grade evidence, table 8).

Figure 5-8: Forest plot of Liraglutide versus Liraglutide + Metformin on body weight (Kg)



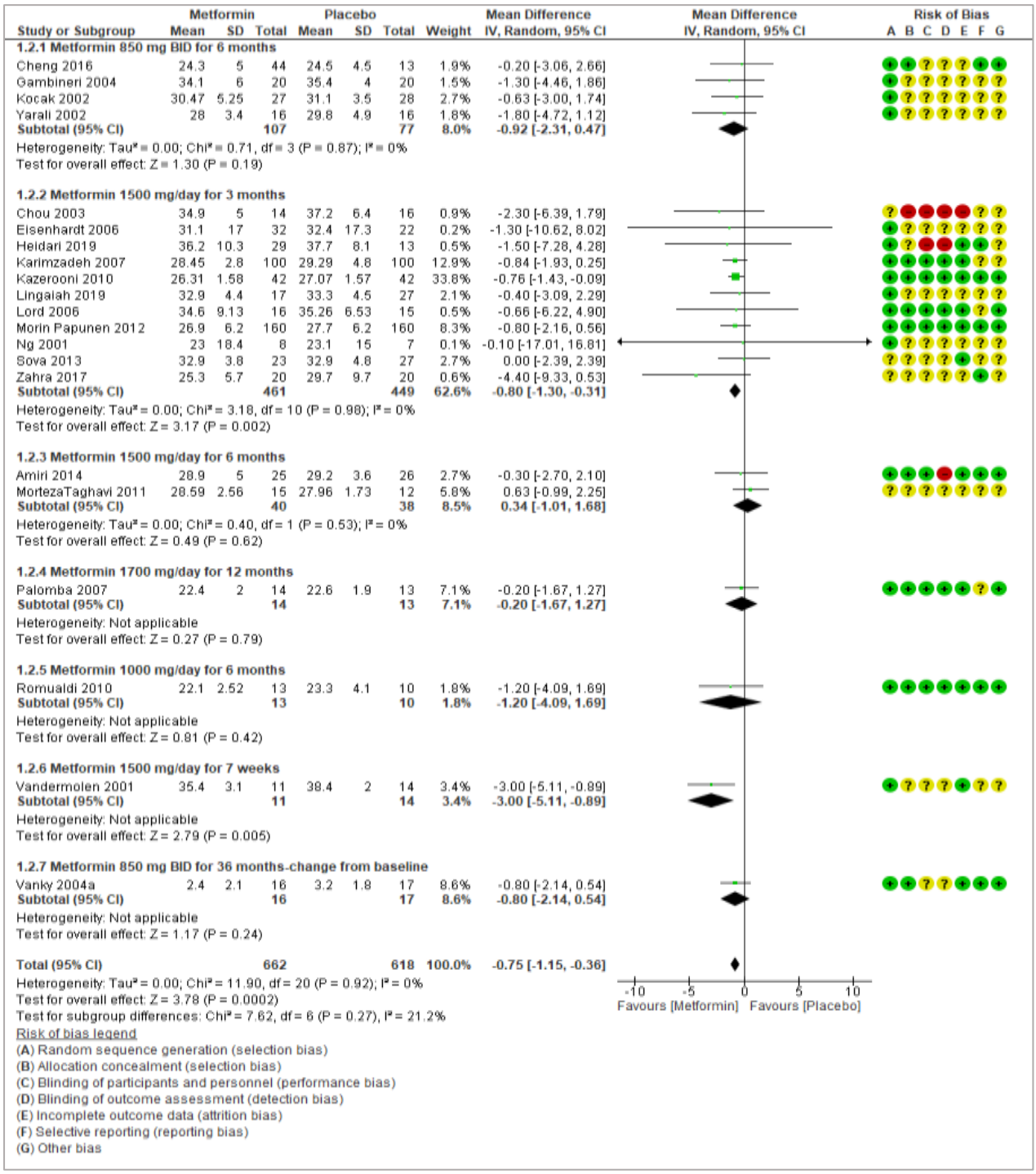
5.3.5 Body Mass Index (BMI)

5.3.5.1 Metformin versus placebo

In four RCTs, metformin 850 mg BID for six months was associated with no significant change in BMI by 0.94 kg/m² (95% CI: -2.31, 0.47, I^2= 0%) compared with placebo. The pooled effect estimates from 11 RCTs showed that metformin 1500 QD for three months was associated with a significant reduction in BMI by 0.80 kg/m² (95% CI: -1.30, -0.31, I^2= 0%). The pooled

effect estimates from two RCTs showed that metformin 1500 mg QD for six months was associated with no significant change in BMI by 0.34 kg/m² (95% CI: -1.01, 1.68, I^2= 0%). Individual studies used metformin 1700 mg QD for 12 months (MD: -0.20 kg/m²; 95% CI: -1.67, 1.27), metformin 1000 mg QD for 6 months (MD: -1.20 kg/m²; 95% CI: -4.09,1.69), and metformin 850 mg BID for 36 months (MD: -0.80 kg/m²; 95% CI: -2.14, 0.54) showed no significant change in BMI compared to placebo group. Whereas metformin 1500 mg QD for seven weeks was associated with a significant reduction in BMI by 3.0 kg/m² (95% CI: -5.11, -0.89). Overall, regardless of the dosage, duration, and frequency per day. The pooled effect estimates from 21 RCTs included 1,280 participants (662 in the intervention arm, 618 in the placebo arm) with PCOS showed that metformin was associated with a significant reduction in the BMI by 0.75 kg/m^2 (95% CI: -1.15, -0.36, I^2= 0%)(Figure 5-9) (moderate grade evidence, table 8).

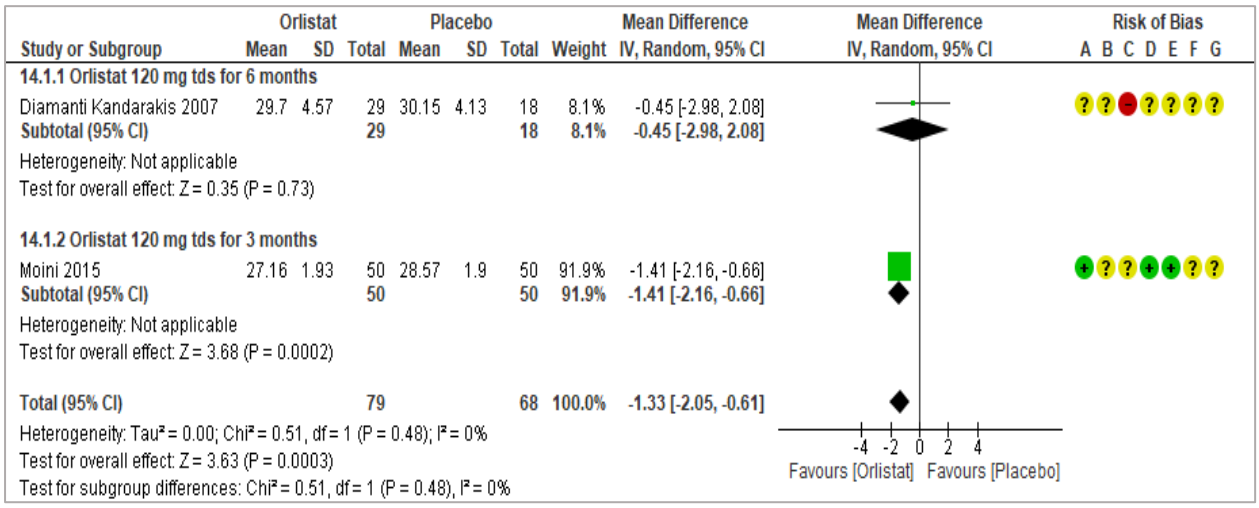
Figure 5-9: Forest plot of Metformin versus placebo on BMI (kg/m^2)



5.3.5.2 Orlistat versus placebo

Orlistat 120 mg TDS for six months in one RCT and three months in another RCT significantly reduced the mean BMI by 1.33 kg/m² (95% CI: -2.16 , 0.66, I^2= 0.0%) compared with placebo (Figure 5-10) (very low-grade evidence, table 8).

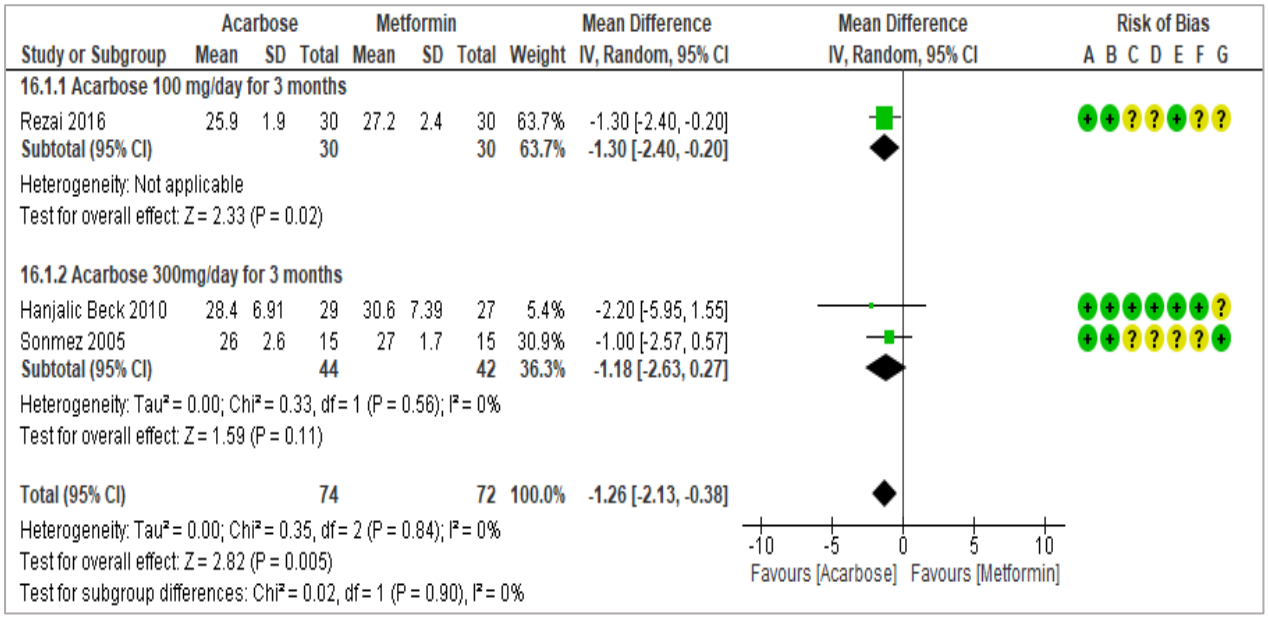
Figure 5-10: Forest plot of Orlistat versus placebo on BMI (kg/m^2)



5.3.5.3 Acarbose versus Metformin

In one RCT, acarbose 100 mg QD for three months was associated with a significant reduction in BMI. In two RCTs, acarbose 300 mg QD for three months was associated with no significant change in the BMI. However, in the three RCTs, regardless of the dosage, frequency, and duration, acarbose showed a significant reduction in the mean BMI by 1.26 kg/m²(95% CI: -2.13,-0.38, I^2*= 0%*) (Figure 5-11) (low-grade evidence, table 8).

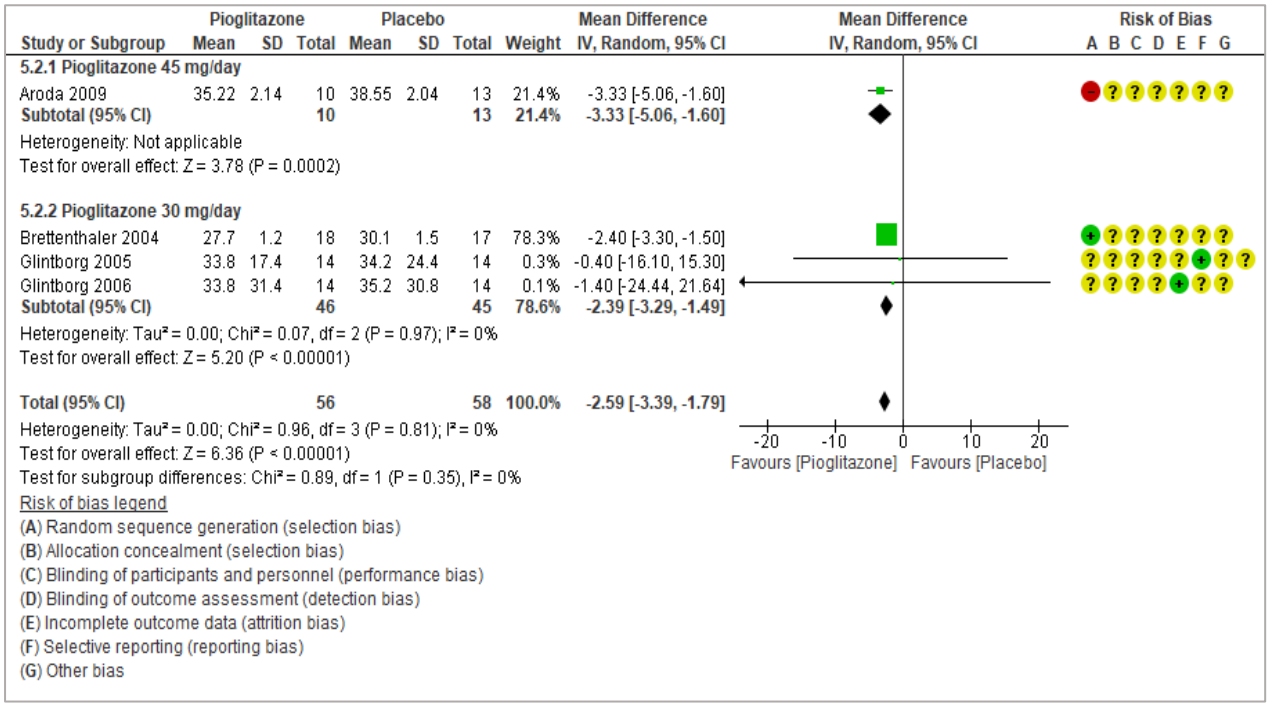
Figure 5-11: Forest plot of Acarbose versus Metformin on BMI (kg/m^2)



5.3.5.4 Pioglitazone versus placebo

The pooled effect estimate showed that there was a significant increase in the mean BMI between women who received pioglitazone 45mg QD (MD: 3.33 kg/m²; 95% CI: 1.60, 5.06) and pioglitazone 30 mg QD (MD: 2.38 kg/m²; 95% CI; 1.48, 3.28). However, regardless of the dosage, frequency, and duration, the mean BMI increased by 2.59 kg/m² (95% CI: 1.78, 3.38, I^2= 0%) in 56 women who received pioglitazone compared to 58 women who received placebo. (Figure 5-12) (low-grade evidence, table 8).

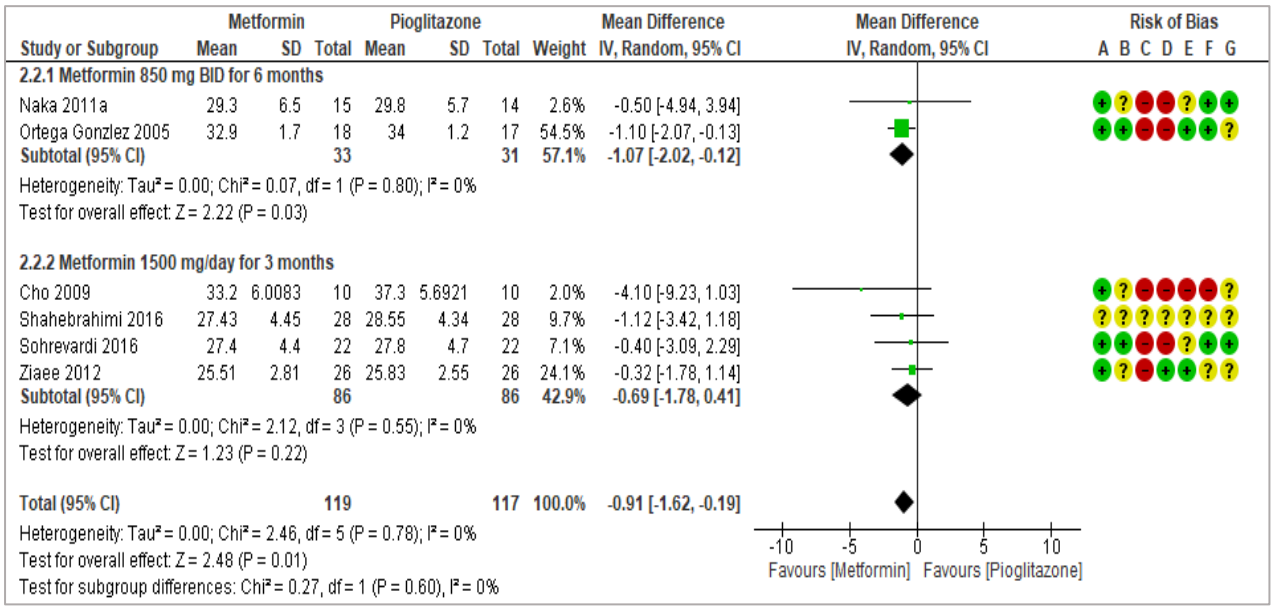
Figure 5-12: Forest plot of Pioglitazone versus placebo on BMI (kg/m^2)



5.3.5.5 Metformin versus Pioglitazone

Metformin 850 mg BID for six months in two RCTs showed a significant reduction in the mean BMI by 1.07 kg/m². In contrast, in four RCTs, metformin 1500 mg QD for three months showed no significant change in the BMI compared to women in the pioglitazone group. Overall, metformin at various dosages significantly reduced the mean BMI by 0.91 kg/m²(95% CI: -1.62, 0.19). (Figure 5-13) (very low-grade evidence, Table 8).

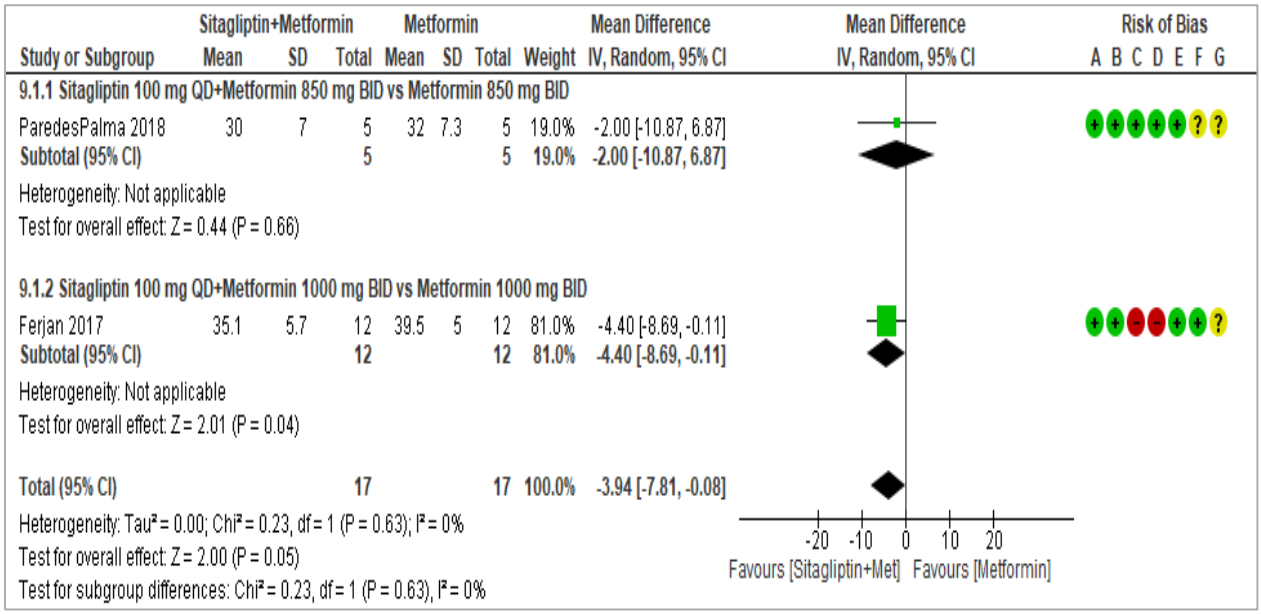
Figure 5-13: Forest plot of Metformin versus Pioglitazone on BMI (kg/m^2)



5.3.5.6 Sitagliptin + Metformin versus Metformin

In two RCTs, when Sitagliptin 100 mg was added to metformin at different doses, a significant reduction in the mean BMI by 3.94 kg/m²(95% CI: -7.81, 0.08, I^2= 0%) was observed (Figure 5-14) (very low-grade evidence, Table 8).

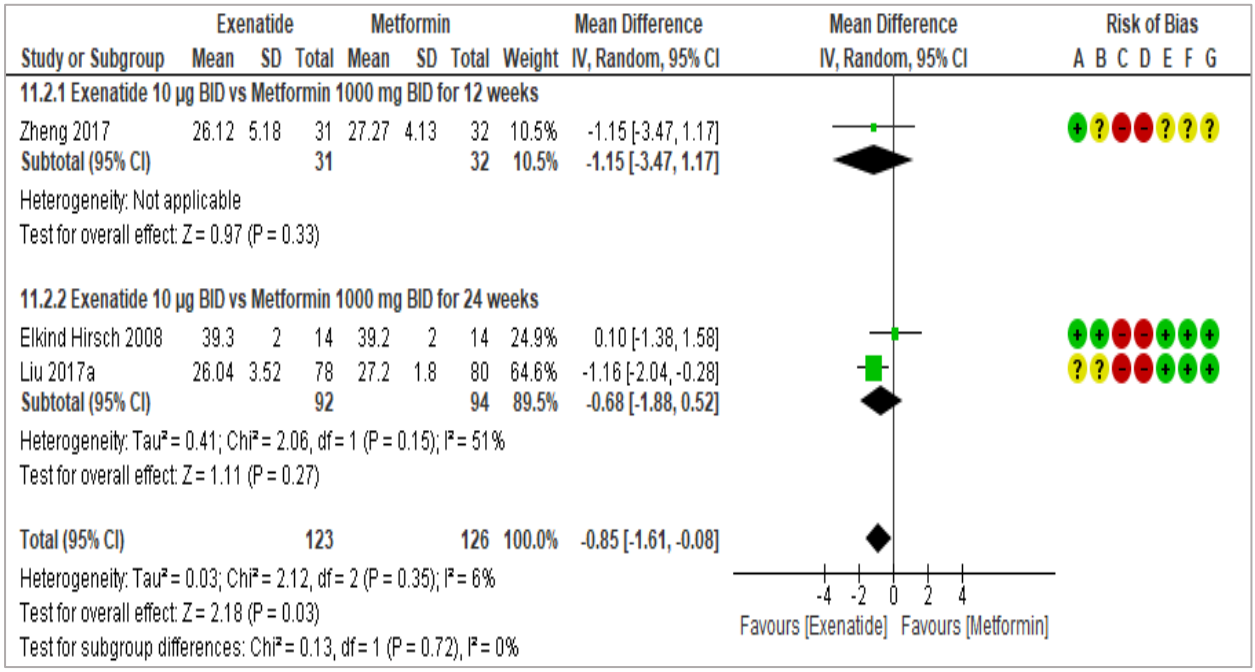
Figure 5-14: Forest plot of Sitagliptin + Metformin versus Metformin on BMI (kg/m^2)



5.3.5.7 Exenatide versus Metformin

In three RCTs, exenatide 10 ug showed a significant reduction in the mean BMI by 0.85 kg/m²(95% CI:- 1.61, 0.08, I^2= 0%) when compared with Metformin 1000 mg QD (Figure 5-15) (very low-grade evidence, Table 7).

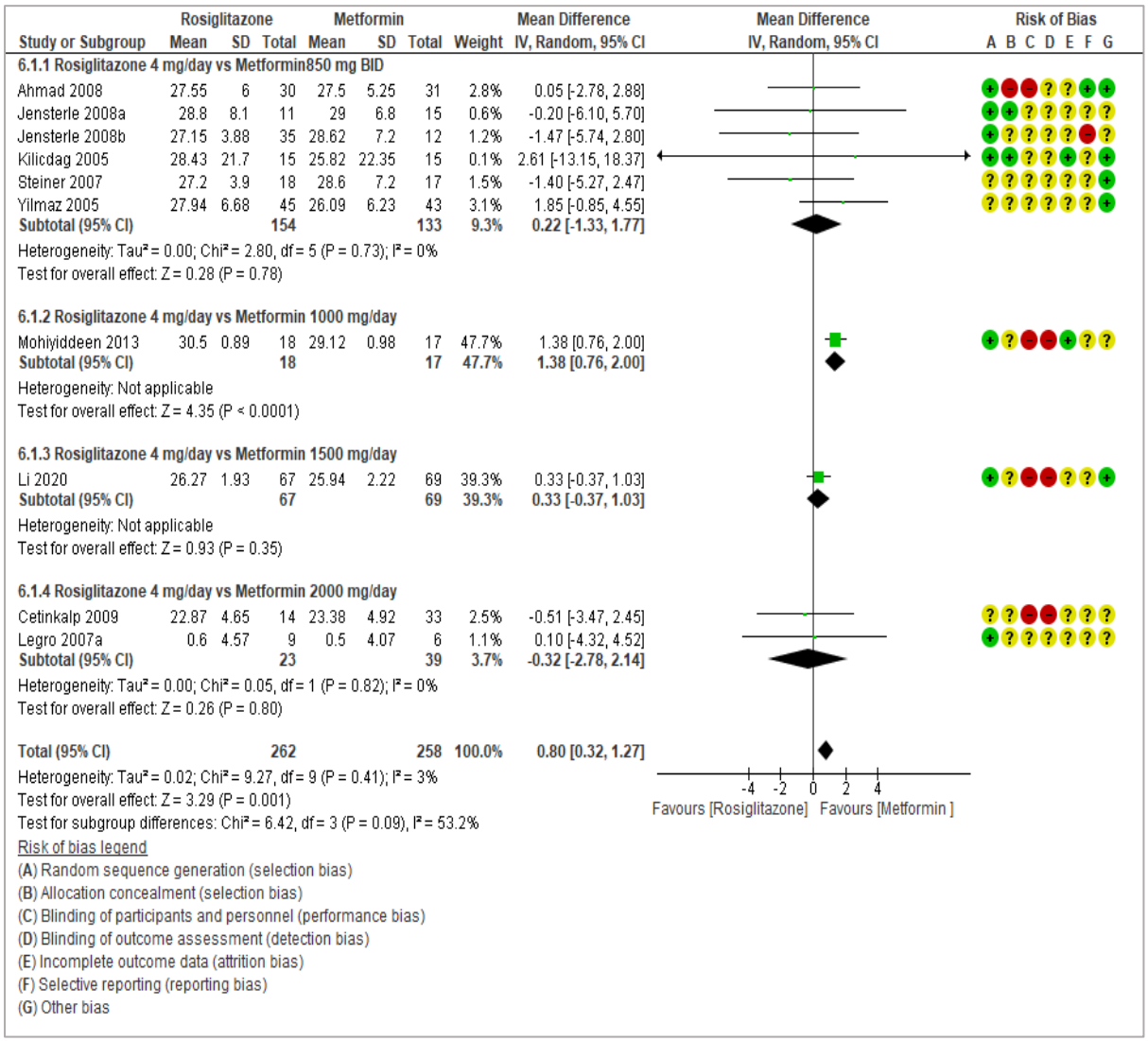
Figure 5-15: Forest plot of Exenatide versus Metformin on BMI ((kg/m^2)



5.3.5.8 Rosiglitazone versus Metformin

Compared with rosiglitazone 4 mg QD, metformin 850 mg BID, metformin 1500 mg QD, metformin 1000 mg QD and metformin 2000 mg QD were associated with no significant change in the BMI. However, regardless of the dosage, frequency, and duration, in 10 RCTs that included 262 women with PCOS in the metformin arm compared with 258 women in the rosiglitazone arm, rosiglitazone was associated with a significant increase in the mean BMI by 0.80 kg/m²(95% CI: 0.32, 1.27, I^2= 3.0%) (Figure 5-16) (moderate grade evidence, table 8).

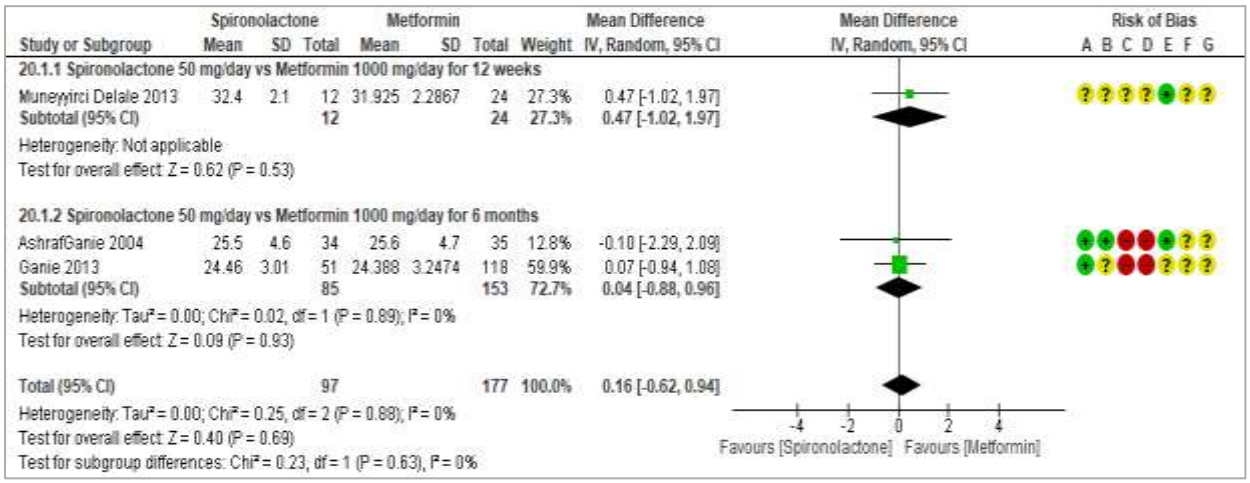
Figure 5-16: Forest plot of Rosiglitazone versus Metformin on BMI (kg/m^2)



5.3.5.9 Spironolactone versus Metformin

One RCT compared spironolactone 50 mg QD with metformin 1000 mg QD for 12 weeks showed no effect on the mean BMI (MD: 0.47 kg/m^2; 95% CI: -1.02, 1.97). Two RCTs compared spironolactone 50 mg QD with metformin 1000 mg QD for six months showed no effect on the mean BMI (MD: 0.04 kg/m^2; 95%CI: -0.88, 0.95). Overall, spironolactone 50 mg QD compared with metformin 1000 mg QD for various duration has no effect on the mean BMI (MD: 0.16 kg/m^2; 95%CI: -0.62, 0.94, I^2= 0%) (Figure 5-17) (very low-grade evidence, table 8).

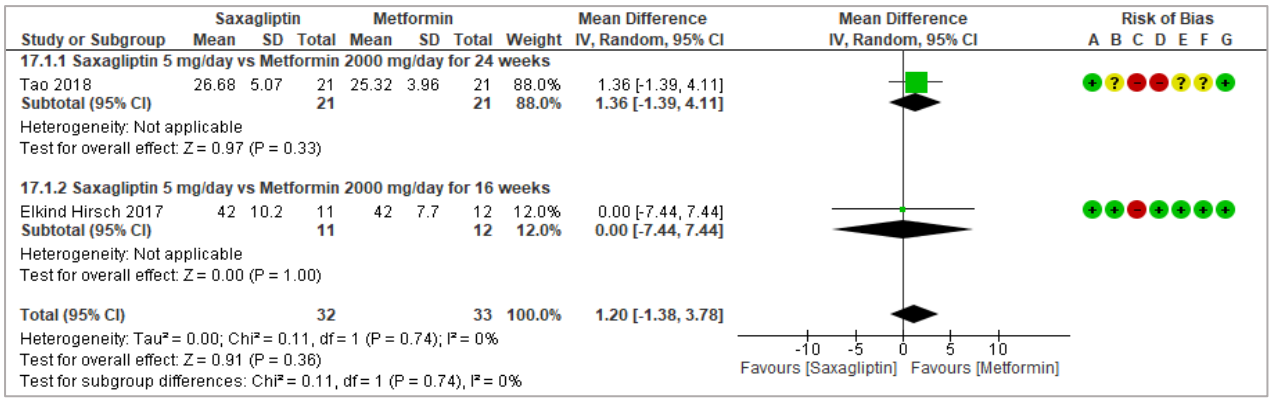
Figure 5-17: Forest plot of Spironolactone versus Metformin on BMI (kg/m^2)



5.3.5.10 Saxagliptin versus Metformin

In two RCTs compared saxagliptin 5 mg QD with metformin 2000 mg QD for various duration showed no effect on the mean BMI (MD: 1.20 kg/m²; 95% CI: -1.38, 3.78, I^2= 0%) (Figure 5-18) (very low-grade evidence, table 8).

Figure 5-18: Forest plot of Saxagliptin versus Metformin on BMI (kg/m^2)

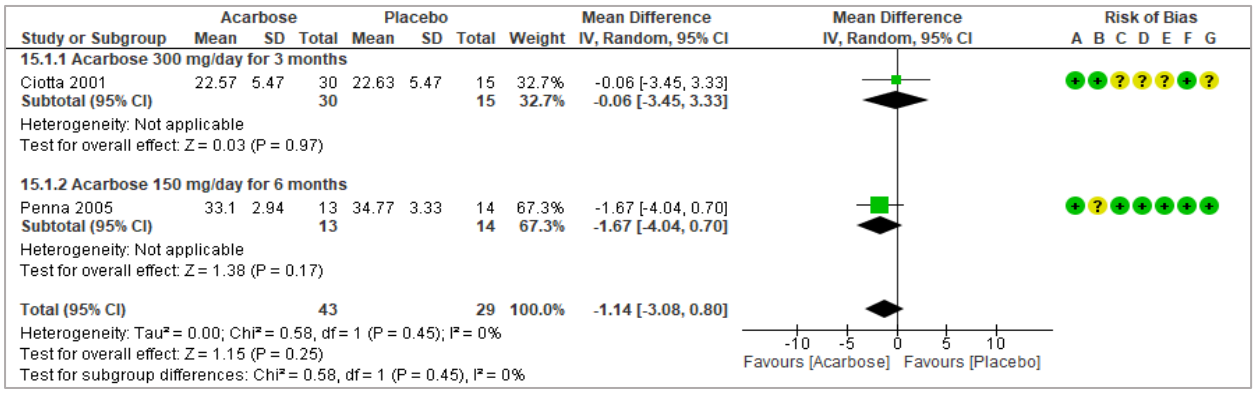


5.3.5.11 Acarbose versus placebo

In one RCT, acarbose 300 mg QD for three months compared with placebo has no effect on the mean BMI (MD: -0.06 kg/m²; 95% CI: -3.45, 3.33). In one RCT, acarbose 150 mg QD for six months compared with placebo has no effect on the mean BMI (MD: -1.67 kg/m²; 95%CI: -

4.04, 0.70). Overall, acarbose at various dosage has no effect on the mean BMI compared with placebo (MD: -1.14 kg/m²; 95% CI: -3.08, 0.80, I^2= 0%) (Figure 5-19) (low grade evidence, table 8).

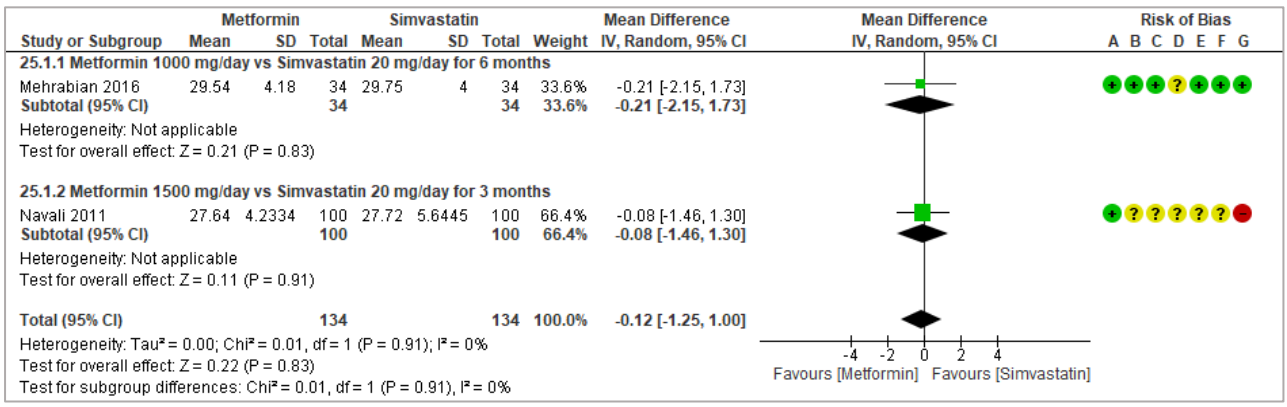
Figure 5-19: Forest plot of Acarbose versus placebo on BMI (kg/m^2)



5.3.5.12 Metformin versus Simvastatin

In one RCT, compared metformin 1000 mg QD with simvastatin 20 mg QD for six months showed no effect on the mean BMI (MD:-0.21 kg/m²; 95%CI: -2.15, 1.73). One RCT compared metformin 1500 mg QD with simvastatin 20 mg for three months showed no effect on the mean BMI (MD:-0.08 kg/m²;95%CI: -1.46,1.30). Overall, metformin at various dosage compared with simvastatin 20 mg QD showed no effect on the mean BMI (MD: -0.12 kg/m²; 95% CI: -1.25, 1.00, I^2= 0%) (Figure 5-20) (very low-grade evidence, table 8).

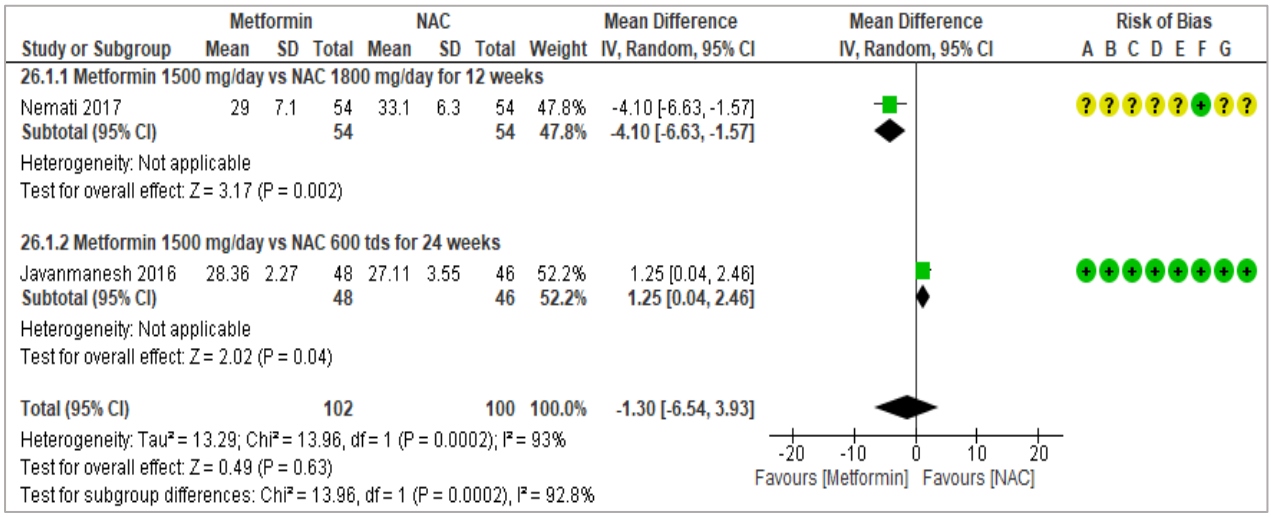
Figure 5-20: Forest plot of Metformin versus Simvastatin on BMI (kg/m^2)



5.3.5.13 Metformin versus NAC

One RCT compared metformin 1500 mg QD with NAC 1800 mg QD for 12 weeks showed a significant reduction in the mean BMI (MD: -4.10 kg/m²; 95% CI: -6.63,-1.57). Another RCT compared metformin 1500 mg QD with NAC 600 mg TDS for 24 weeks showed a significant increase in the mean BMI (MD: 1.25 kg/m²; 95% CI: 0.04, 2.46). Overall, metformin 1500 mg QD compared with various dosage of NAC for various duration has no effect on the mean BMI (MD: -1.30 kg/m²; 95%CI: -6.54, 3.93, I^2= 92.8%) (Figure 5-21) (very low-grade evidence, table 8).

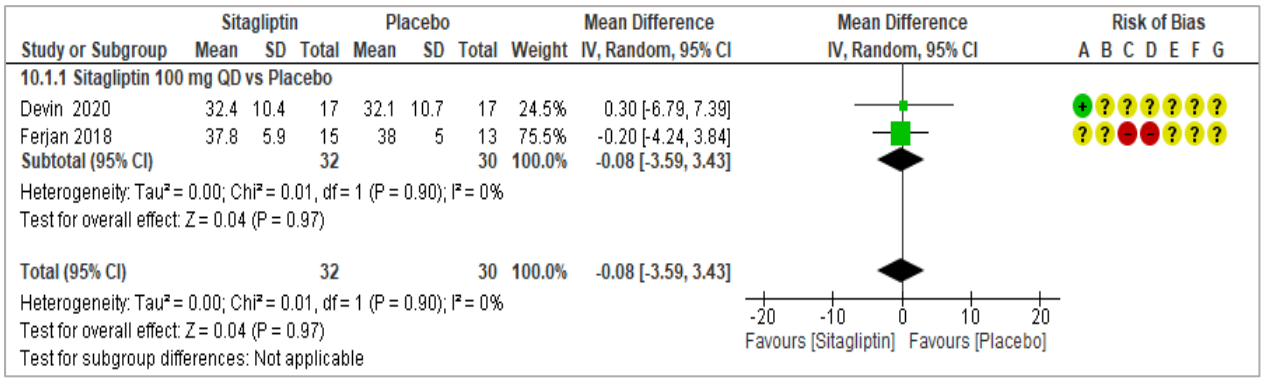
Figure 5-21: Forest plot of Metformin versus NAC on BMI (kg/m²)



5.3.5.14 Sitagliptin versus placebo

In two RCTs compared sitagliptin 100 mg QD with placebo showed no effect on the mean BMI (MD: -0.08 kg/m²; 95%CI: -3.59, 3.43, I^2= 0%) (Figure 5-22) (very low-grade evidence, table 8).

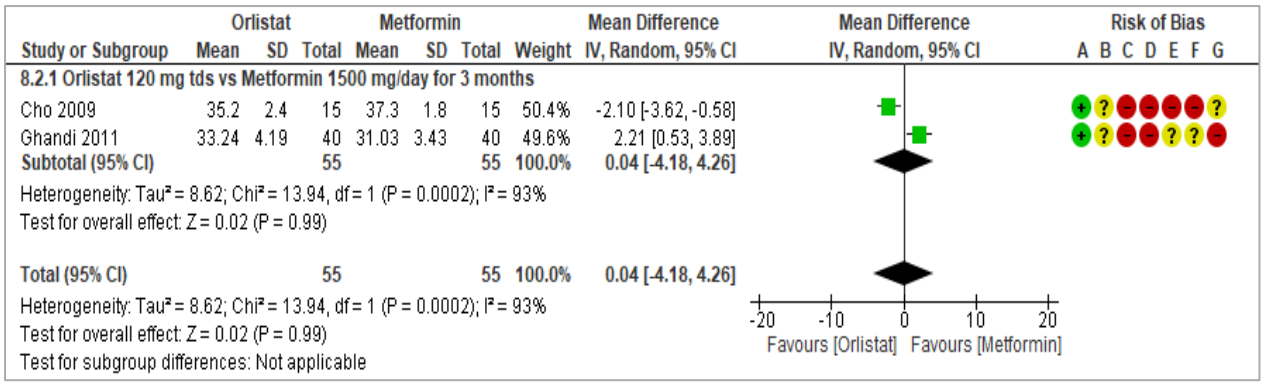
Figure 5-22: Forest plot of Sitagliptin versus placebo on BMI (kg/m^2)



5.3.5.15 Orlistat versus Metformin

In two RCTs compared orlistat 120 mg TDS with metformin 1500 mg QD for three months, showed no effect on the mean BMI (MD: 0.04 kg/m²; 95%CI: -4.18, 4.26, I^2= 93%) (Figure 5-23) (very low-grade evidence, table 8).

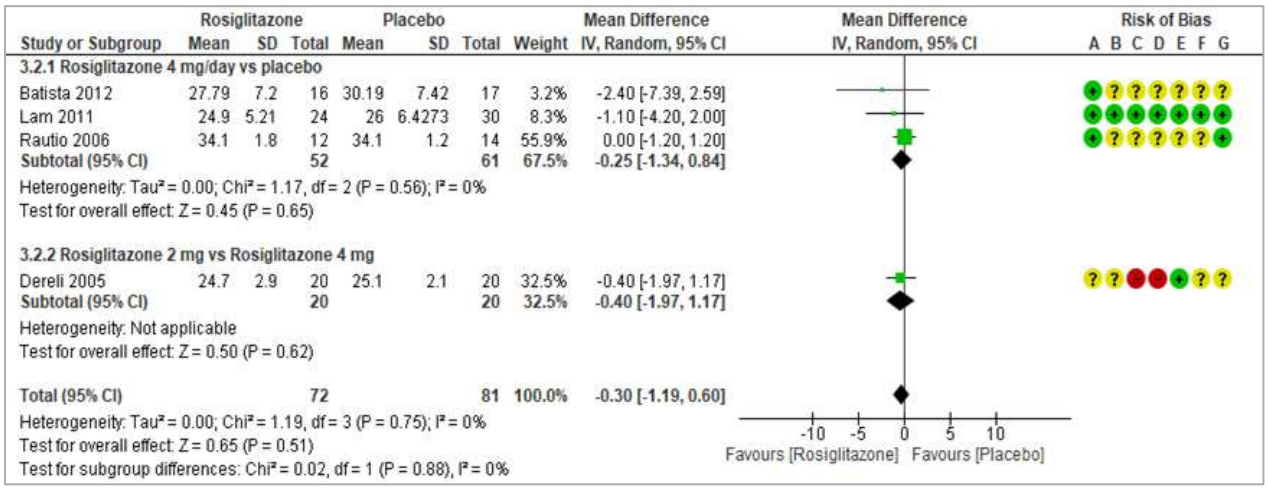
Figure 5-23: Forest plot of Orlistat versus Metformin on BMI (kg/m^2)



5.3.5.16 Rosiglitazone versus placebo

In four RCTs compared rosiglitazone 4 mg QD with either rosiglitazone 2 mg QD or placebo showed no effect on the mean BMI (MD: -0.30 kg/m²; 95% CI: -1.19, 0.60, I^2= 0%) (Figure 5-24) (very low-grade evidence, table 8).

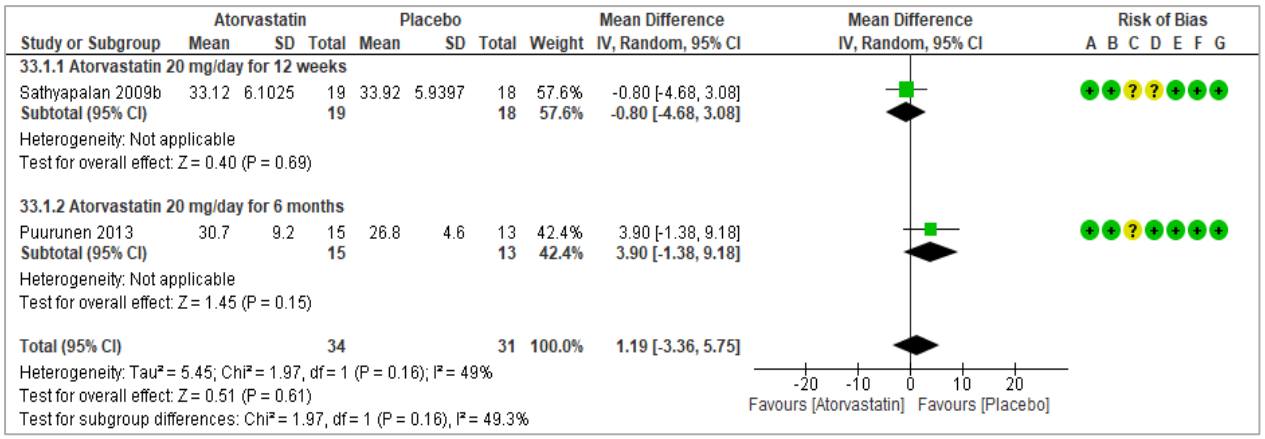
Figure 5-24: Forest plot of Rosiglitazone versus placebo on BMI (kg/m^2)



5.3.5.17 Atorvastatin versus placebo

Atorvastatin 20 mg QD for various duration compared with placebo has no effect on the mean BMI (MD: 1.19 kg/m²; 95%CI: -3.36, 5.75, I^2= 49.3%) (Figure 5-25) (very low-grade evidence, table 8).

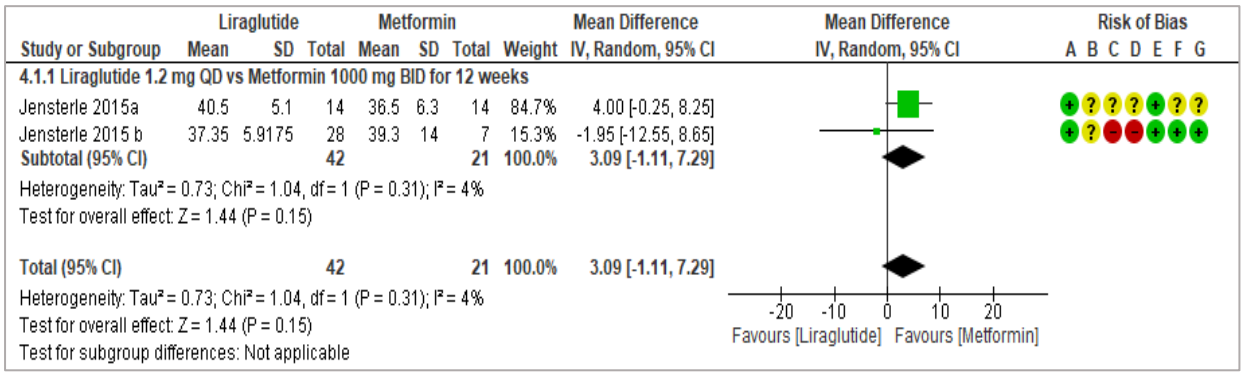
Figure 5-25: Forest plot of Atorvastatin versus placebo on BMI (kg/m^2)



5.3.5.18 Liraglutide versus Metformin

In two RCTs liraglutide 1.2 mg QD compared with metformin 1000 mg BID for 12 weeks has no effect on the mean BMI (MD: 3.09 kg/m²; 95%CI: -1.11, 7.29, I^2= 4%) (Figure 5-26) (low grade evidence, table 8).

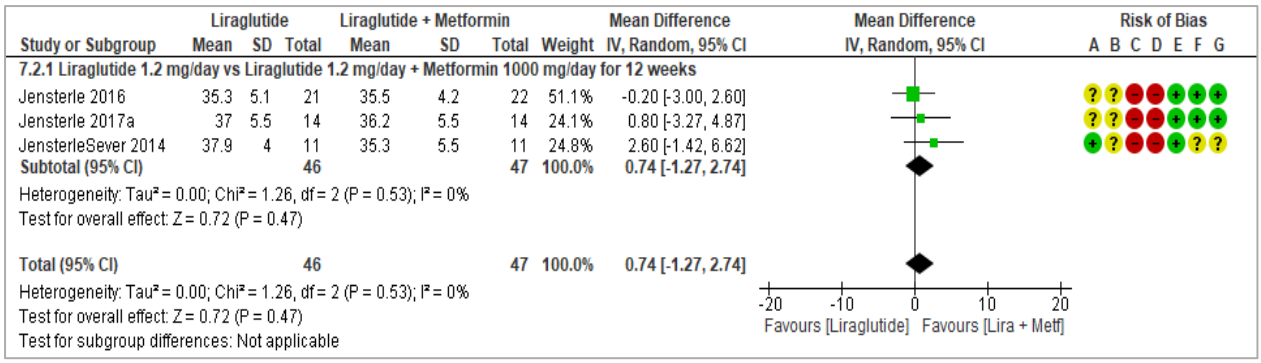
Figure 5-26: Forest plot of Liraglutide versus Metformin on BMI (kg/m^2)



5.3.5.19 Liraglutide versus Liraglutide + Metformin

Three RCTs compared liraglutide 1.2 mg QD with liraglutide 1.2 mg QD added to metformin 1000 mg QD for 12 weeks showed no effect on the mean BMI (MD: 0.74 kg/m^2; 95%CI: -1.27, 2.74, I^2= 0%) (Figure 5-27) (low grade evidence, table 8).

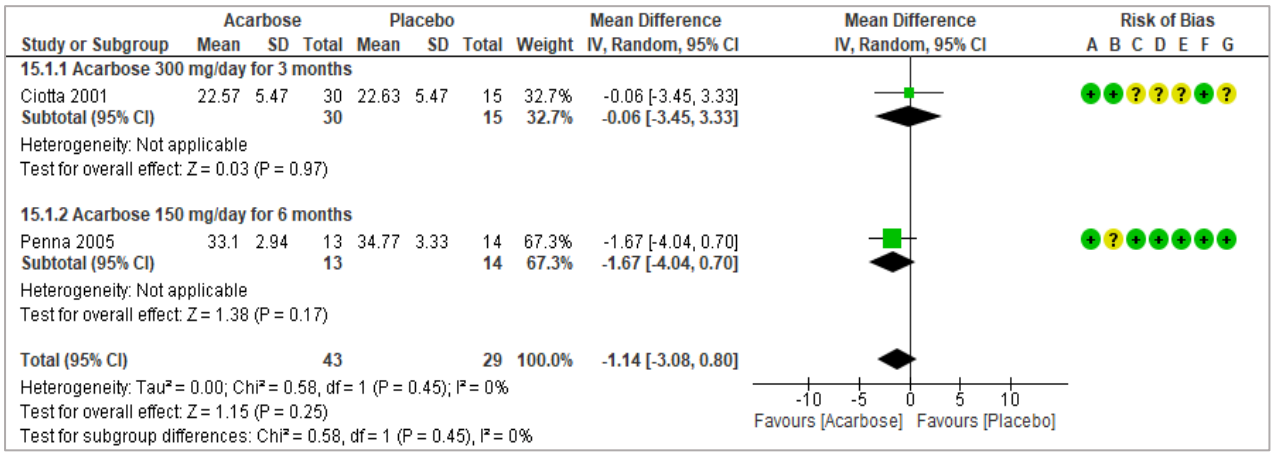
Figure 5-27: Forest plot of Liraglutide versus Liraglutide + Metformin on BMI (kg/m^2)



5.3.5.20 Acarbose versus placebo

One RCT compared acarbose 300 mg QD for three months with placebo showed no effect on the mean BMI (MD: -0.06 kg/m^2; 95% CI: -3.45, 3.33). One RCT compared acarbose 150 mg QD for six months with placebo has no effect on the mean BMI (MD: -1.67 kg/m^2; -4.04, 0.70). Overall, acarbose at various dosage has no effect on the mean BMI compared with placebo (MD: -1.14 kg/m^2; 95%CI: -3.08, 0.80, I^2= 0%) (Figure 5-28) (low-grade evidence, table 8).

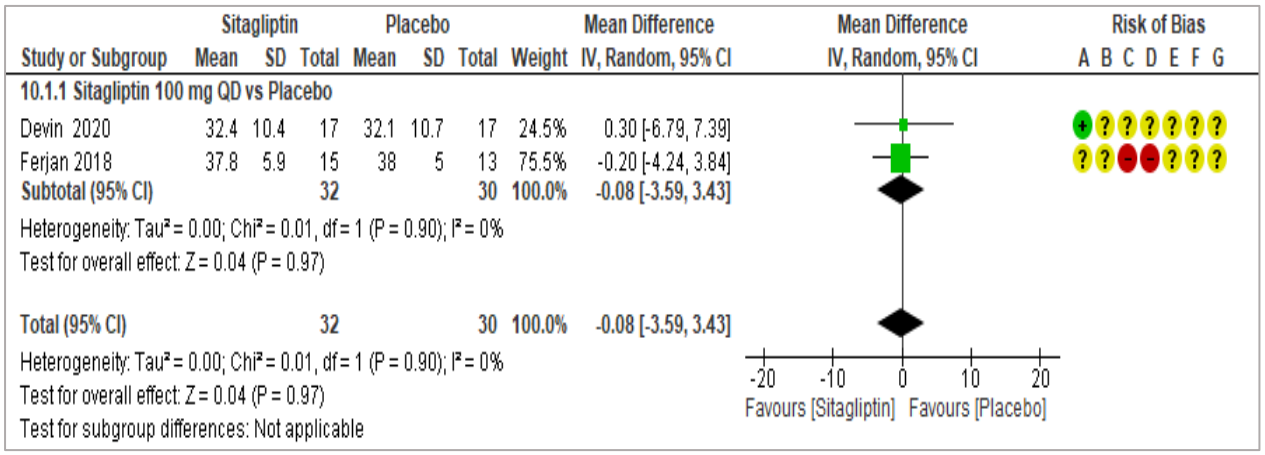
Figure 5-28: Forest plot of Acarbose versus placebo on BMI (kg/m^2)



5.3.5.21 Sitagliptin versus placebo

Two RCTs compared sitagliptin 100 mg QD with placebo showed no effect on the mean BMI (MD: - 0.08 kg/m²; 95%CI: -3.59,3.43, I^2= 0%) (Figure 5-29) (very low-grade evidence, table 8).

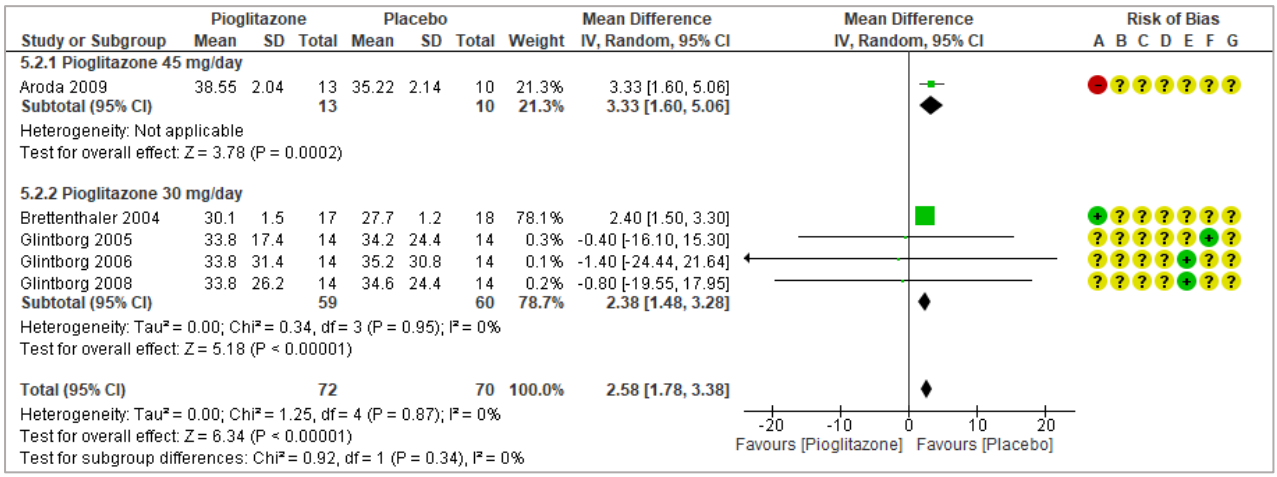
Figure 5-29: Forest plot of Sitagliptin versus placebo on BMI (kg/m^2)



5.3.5.22 Pioglitazone versus placebo

One RCT compared pioglitazone 45 mg QD, and four RCTs compared pioglitazone 30 mg QD with placebo. Showed a significant increase in the mean BMI (MD: 2.58 kg/m²; 95%CI: 1.78, 3.38, I^2= 0%) (Figure 5-30) (low-grade evidence, table 8).

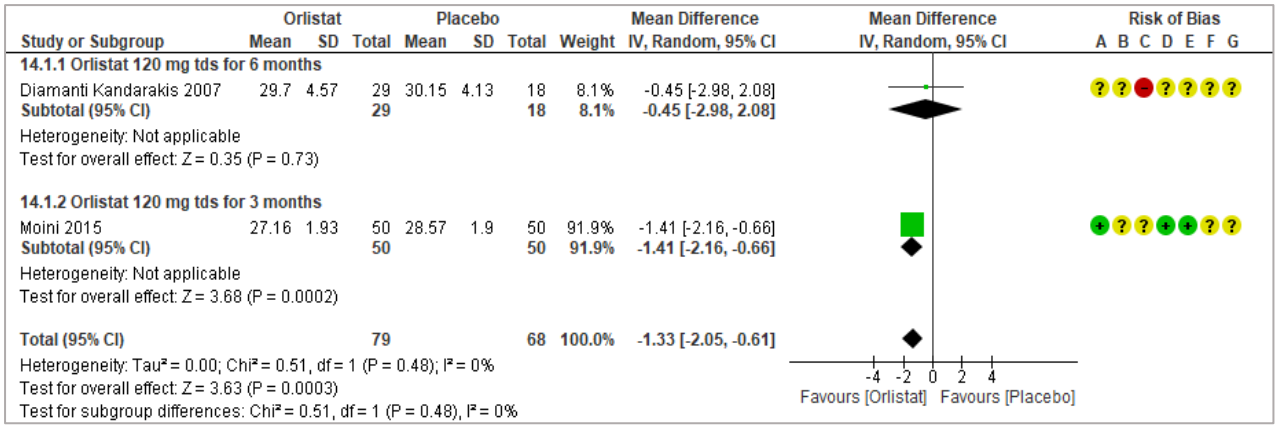
Figure 5-30: Forest plot of Pioglitazone versus placebo on BMI (kg/m^2)



5.3.5.23 Orlistat versus placebo

In two RCT compared orlistat 120 mg TDS with placebo for various duration showed a significant reduction in the mean BMI (MD: -1.33 kg/m²; 95%CI: -2.05, -0.61, I^2= 0%) (Figure 5-31) (very low-grade evidence, table 8).

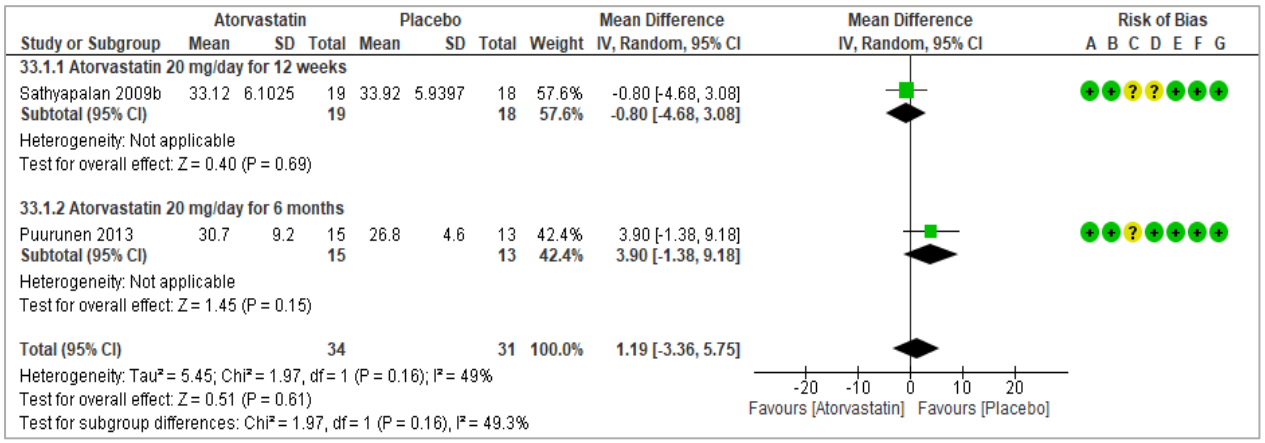
Figure 5-31: Forest plot of Orlistat versus placebo on BMI (kg/m^2)



5.3.5.24 Atorvastatin versus placebo

In two RCTs compared atorvastatin 20 mg QD for various duration with placebo showed no effect on the mean BMI (MD: 1.19 kg/m²; 95%CI: -3.36, 5.75, I^2= 49.3%) (Figure 5-32) (very low-grade evidence, table 8).

Figure 5-32: Forest plot of Atorvastatin versus placebo on BMI (kg/m^2)

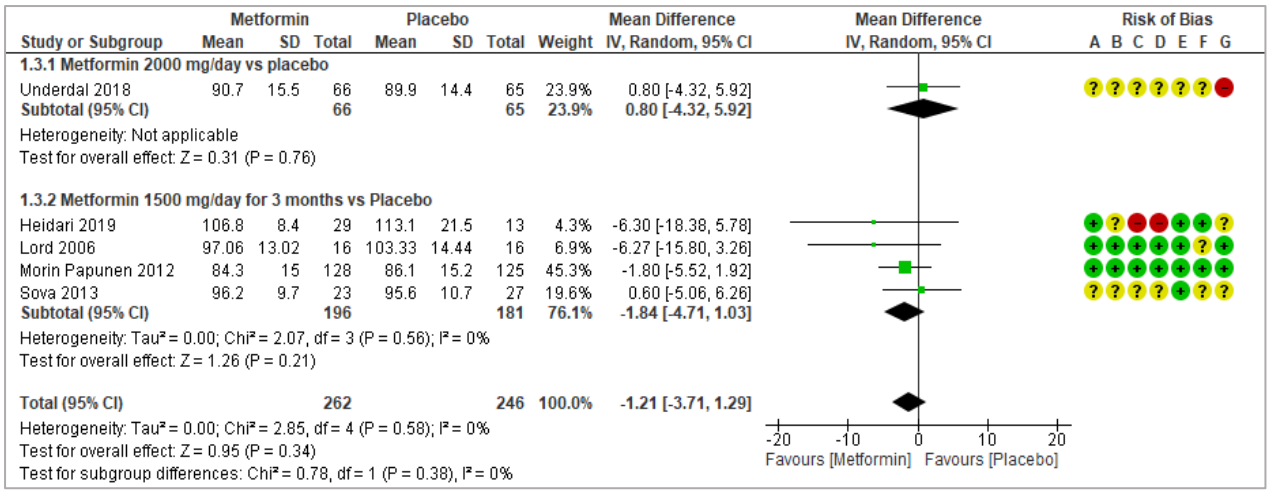


5.3.6 Waist Circumference (WC)

5.3.6.1 Metformin versus placebo

In one RCT, metformin 2000 mg QD was associated with no significant change in waist circumference (WC) (MD: 0.80 cm; 95%CI:-4.32, 5.92), while in four RCTs, metformin 1500 mg QD for three months significantly reduced WC by 1.84 cm (95% CI: -4.71, 1.03) when compared with placebo. However, regardless of the dosage, metformin insignificantly reduced the mean WC by 1.21 cm (95% CI: -3.71, 1.29, I^2= 0%) (Figure 5-33) (moderate grade evidence, Table 8).

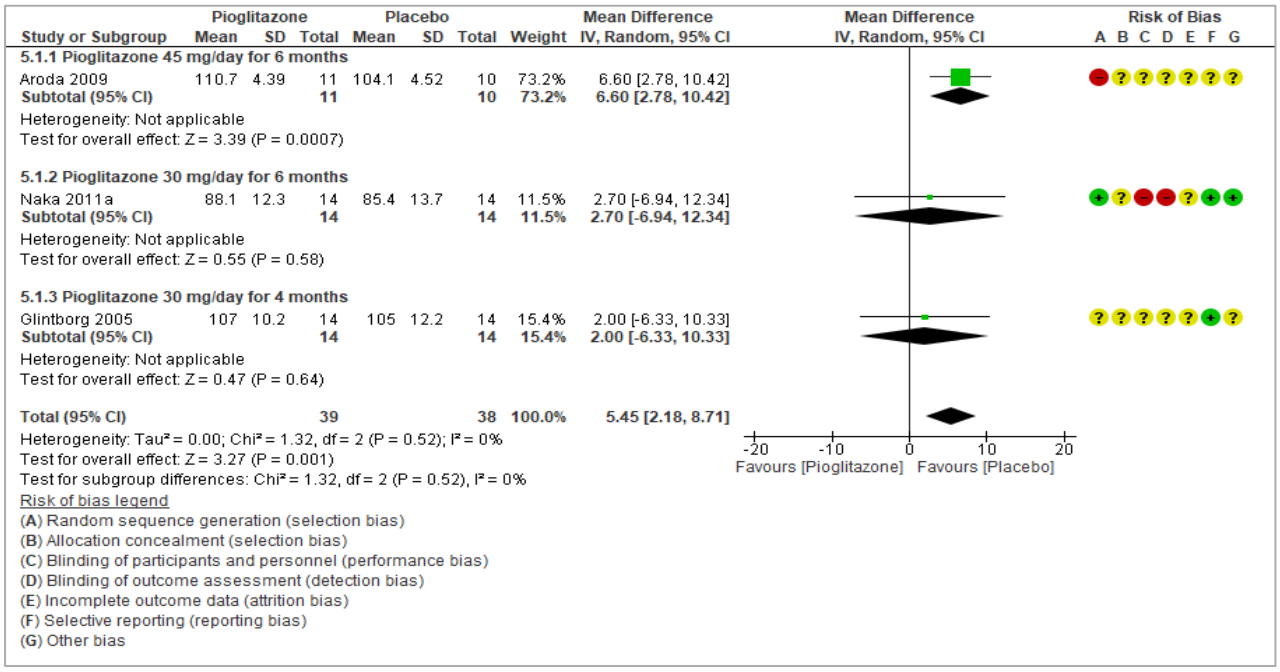
Figure 5-33: Forest plot of Metformin versus placebo on WC (cm)



5.3.6.2 Pioglitazone versus placebo

The pooled estimate showed that there was a significant increase in the mean WC when women received pioglitazone 45 mg QD for six months (MD: 6.60 cm; 95% CI: 2.78, 10.42), pioglitazone 30 mg QD for six months (MD: 2.70 cm; 95% CI: -6.94, 12.34) and pioglitazone 30 mg QD for four months (MD: 2.0 cm; 95% CI: -6.33,10.33). However, regardless of the dosage, frequency, and duration, the mean WC was significantly increased by 5.45 cm (95% CI: 2.18, 8.71, I^2= 0%) in 39 women who received pioglitazone compared to 38 women who received placebo (Figure 5-34) (very low-grade evidence, Table 8).

Figure 5-34: Forest plot of Pioglitazone versus placebo on WC (cm)

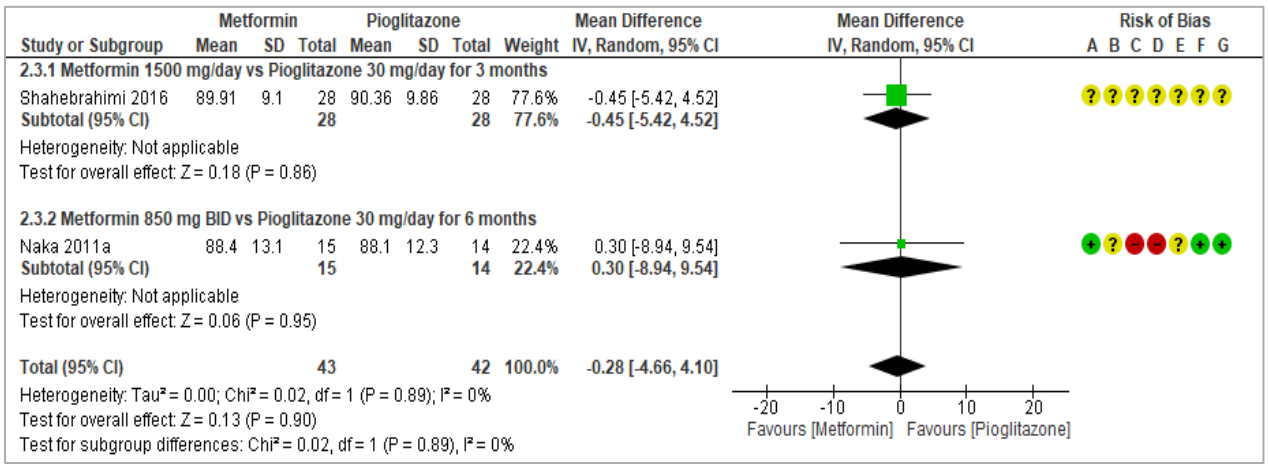


5.3.6.3 Metformin versus Pioglitazone

In one RCT, metformin 1500 mg QD compared with pioglitazone 30 mg QD for three months has no effect on the mean WC (MD: -0.45 cm; 95%CI: -5.42, 4.52). Another RCT, compared metformin 850 mg BID with pioglitazone 30 mg for six months, has no effect on the mean WC (MD: 0.30 cm; 95%CI: -8.94, 9.54). Overall, metformin at various dosages compared with

pioglitazone 30 mg QD for various duration has no effect on the mean WC (MD: -0.28 cm; 95% CI: -4.66, 4.10, I^2= 0%) (Figure 5-35) (very low-grade evidence, table 8).

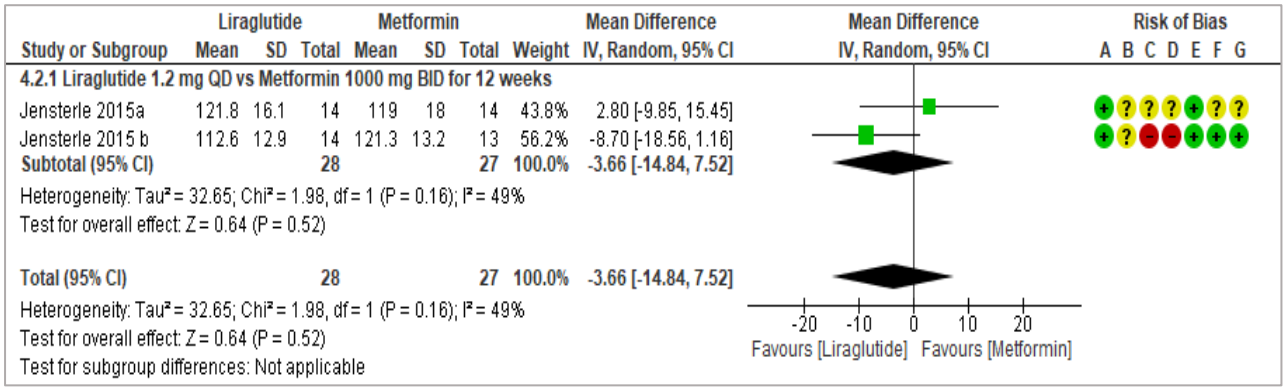
Figure 5-35: Forest plot of Metformin versus Pioglitazone on WC (cm)



5.3.6.4 Liraglutide versus Metformin

In two RCTs compared liraglutide 1.2 mg QD with metformin 1000 mg BID for 12 weeks showed no effect on the mean WC (MD: -3.66 cm; 95%CI: -14.84, 7.52, I^2= 49%) (Figure 5-36) (very low-grade evidence, table 8).

Figure 5-36: Forest plot of Liraglutide versus Metformin on WC (cm)

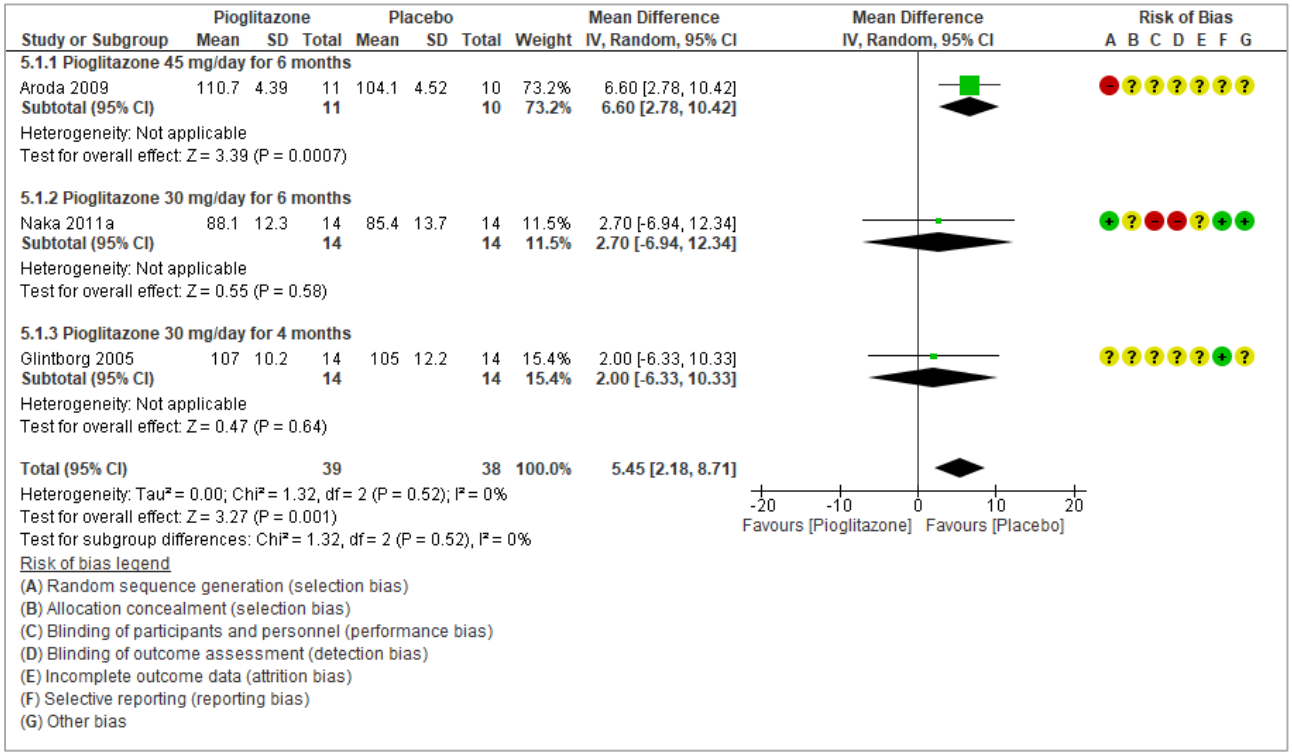


5.3.6.5 Pioglitazone versus placebo

One RCT compared pioglitazone 45 mg QD with placebo for six months showed a significant increase in the mean WC (MD: 6.60 cm; 95%CI: 2.78, 10.42). Another RCT compared

pioglitazone 30 mg QD with placebo for six months showed no effect on the mean WC (MD: 2.70 cm; 95% CI: -6.94,12.34). Finally, one RCT compared pioglitazone 30 mg QD with placebo for four months showed no effect on the mean WC (MD: 2.00 cm; 95% CI: -6.33,10.33). Overall, regardless of the dosage and the duration, pioglitazone significantly increased the mean WC by 5.45 cm (95% CI: 2.18, 8.71, I^2=0%) (Figure 5-37) (very low-grade evidence, table 8).

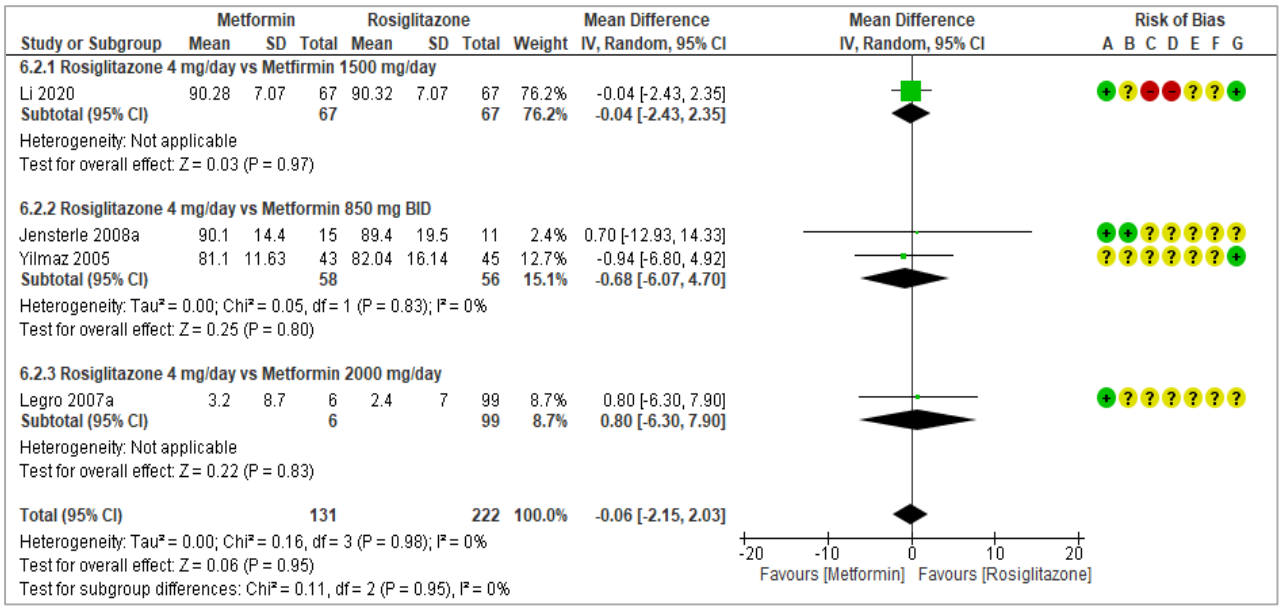
Figure 5-37: Forest plot of Pioglitazone versus placebo on WC (cm)



5.3.6.6 Metformin versus Rosiglitazone

In four RCTs compared rosiglitazone 4 mg QD with various metformin dosage, showed no effect on the mean WC (MD: -0.06 cm; 95%CI: -2.15, 2.03, I^2=0%) (Figure 5-38)(low-grade evidence, table 8).

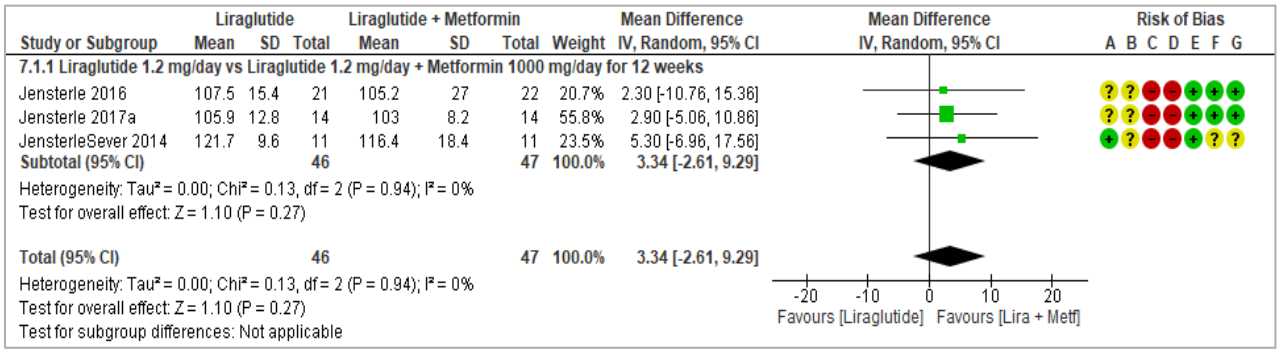
Figure 5-38: Forest plot of Metformin versus Rosiglitazone on WC (cm)



5.3.6.7 Liraglutide versus Liraglutide + Metformin

Three RCTs compared liraglutide 1.2 mg QD with liraglutide 1.2 mg QD added to metformin 1000 mg QD for 12 weeks, showed no effect on the mean WC (MD: 3.34 cm; 95% CI: -2.61, 9.29, I^2=0%) (Figure 5-39) (low-grade evidence, table 8).

Figure 5-39: Forest plot of Liraglutide versus Liraglutide + Metformin on WC (cm)

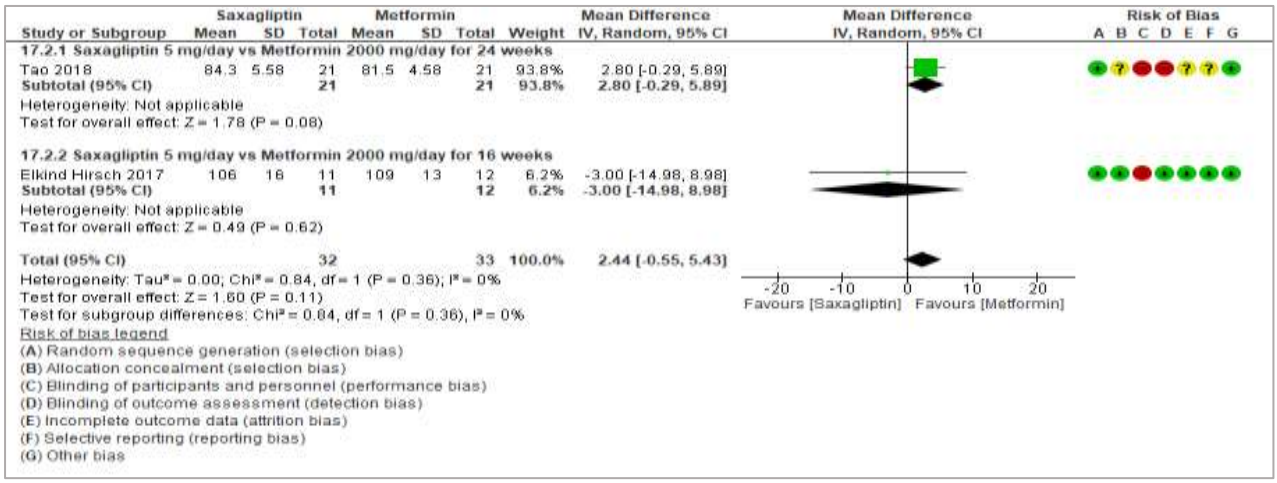


5.3.6.8 Saxagliptin versus Metformin

One RCT compared saxagliptin 5 mg QD with metformin 2000 mg QD for 24 weeks showed an increase in the mean WC by 2.80 cm (95%CI: -0.29, 5.89). However, another RCT compared saxagliptin 5 mg QD with metformin 2000 mg DQ for 16 weeks showed no effect on the mean

WC (MD: -3.00 cm; 95%CI: -14.98, 8.98). Overall, saxagliptin 5 mg QD compared with metformin 2000 mg QD for various duration has no effect on the mean WC (MD: 2.44 cm; 95% CI:-0.55, 5.43, I^2=0%) (Figure 5-40) (very low-grade evidence, table 8).

Figure 5-40: Forest plot of Saxagliptin versus Metformin on WC (cm)

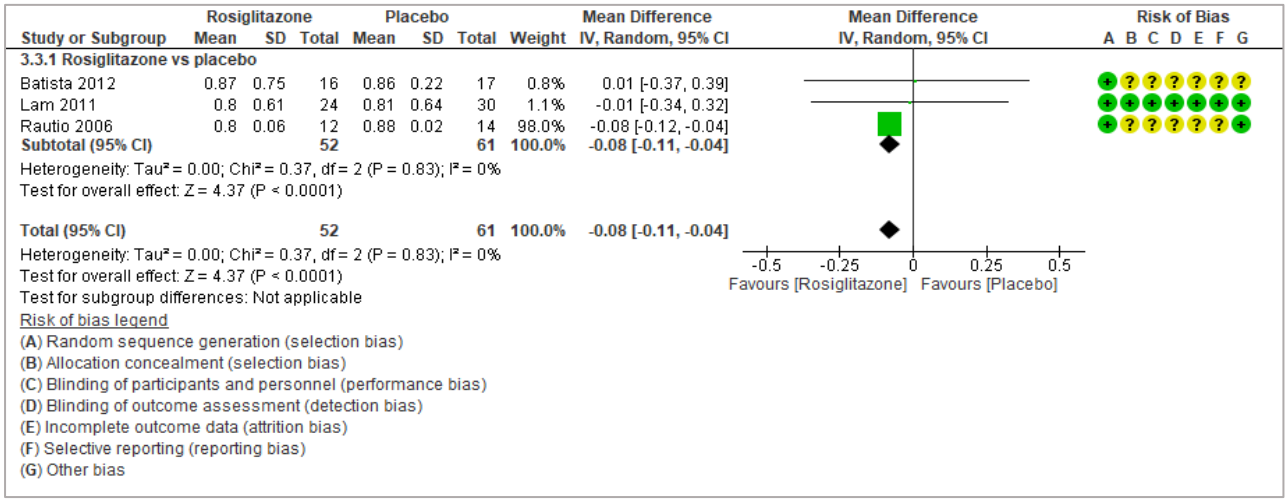


5.3.7 Waist to Hip Ratio (WHR)

5.3.7.1 Rosiglitazone versus placebo

The meta-analysis showed that there was a significant reduction in the waist to hip ratio (WHR) when rosiglitazone was compared with placebo (MD: -0.08; 95%CI: -0.11, 0.04, I^2= 0%) (Figure 5-41) (very low-grade evidence, Table 8).

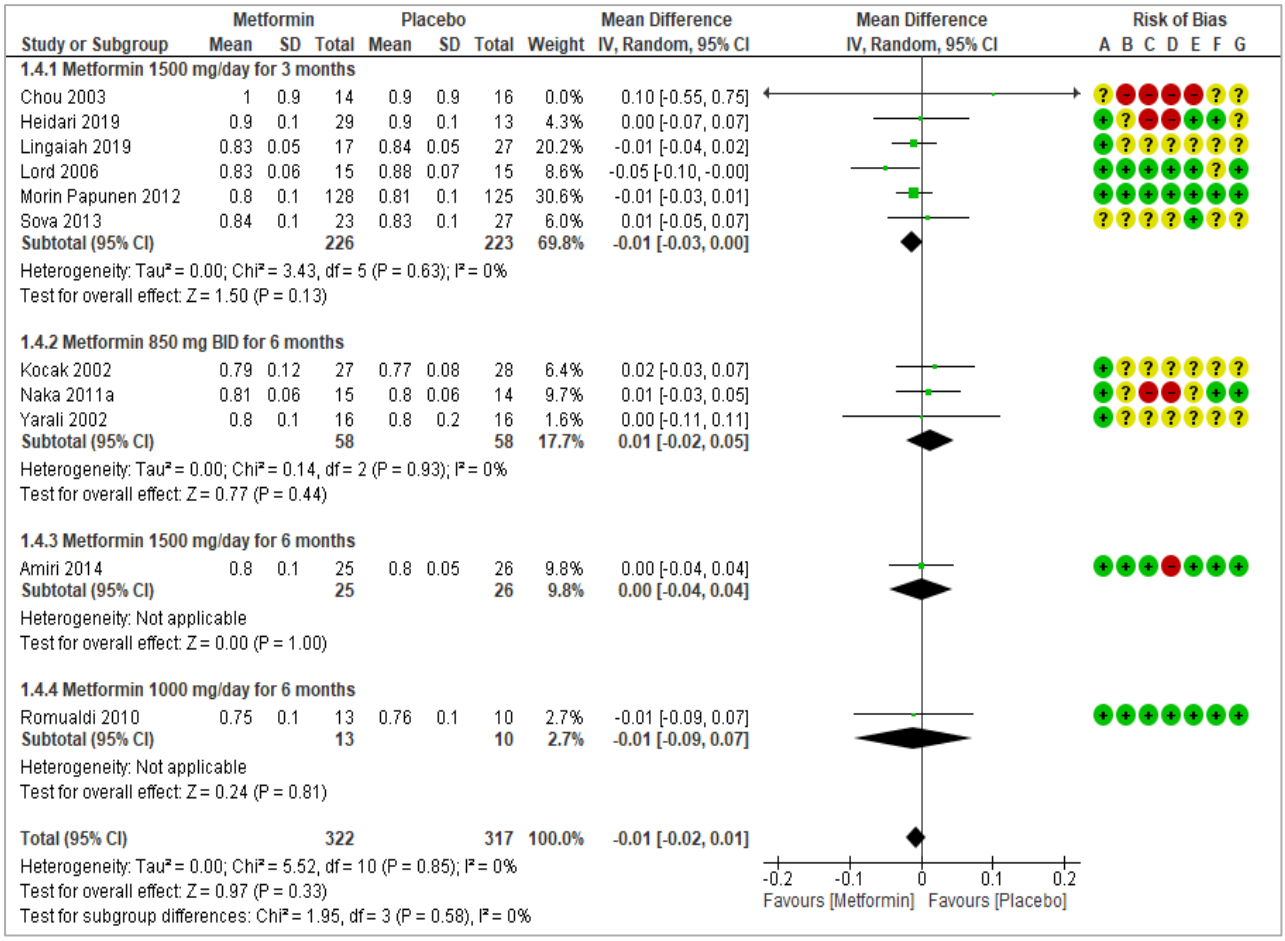
Figure 5-41: Forest plot of Rosiglitazone versus placebo on WHR



5.3.7.2 Metformin versus placebo

In 11 RCTs, regardless of the dosage and the duration, metformin has no effect on the mean WHR compared with placebo (MD: -0.01; 95%CI: -0.02, 0.01, I^2= 0%) (Figure 5-42) (moderate grade evidence, table 8).

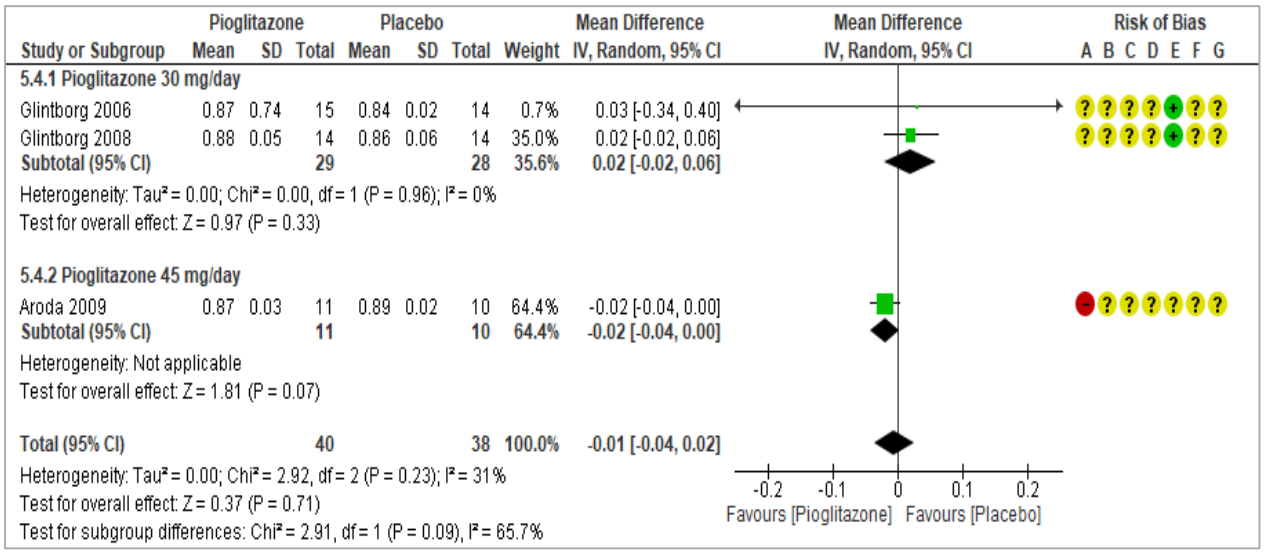
Figure 5-42: Forest plot of Metformin versus placebo on WHR



5.3.7.3 Pioglitazone versus placebo

In two RCTs, pioglitazone 30 mg QD compared with placebo has no effect on the mean WHR (MD: 0.02; 95%CI: -0.02, 0.06). One RCT compared pioglitazone 45 mg QD with placebo significantly reduced the mean WHR (MD: -0.02; 95%CI: -0.04, 0.00). Overall, pioglitazone of various dosage has no effect on the mean WHR compared with placebo (MD: -0.01; 95%CI: -0.04, 0.02, I^2= 0%) (Figure 5-43) (low grade evidence, table 8).

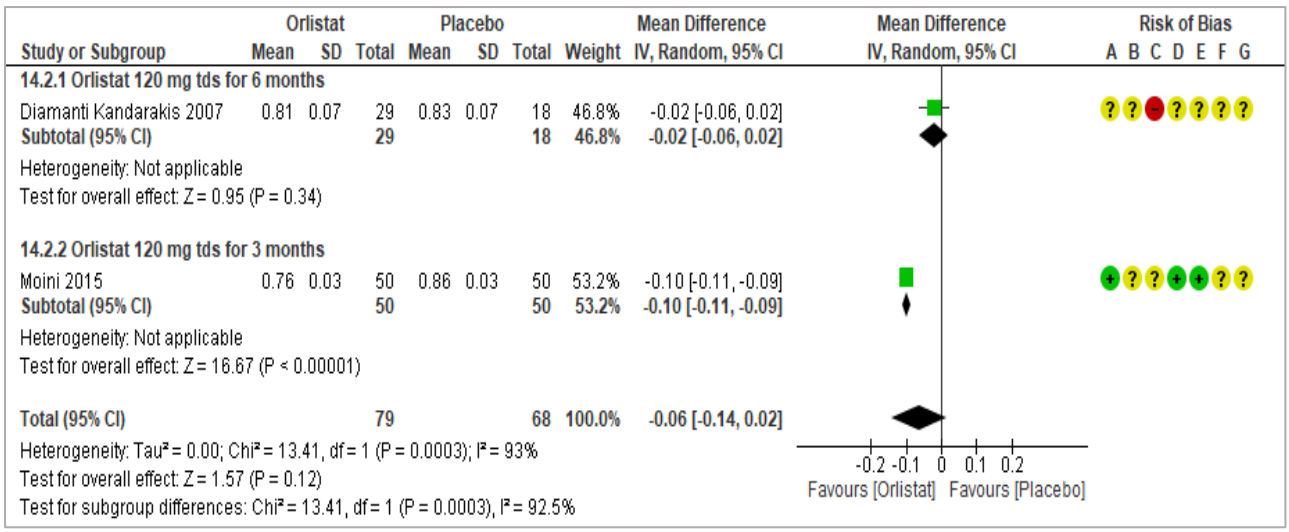
Figure 5-43: Forest plot of Pioglitazone versus placebo on WHR



5.3.7.4 Orlistat versus placebo

In one RCT compared orlistat 120 mg TDS for six months with placebo showed no effect on the mean WHR (MD: -0.02; 95%CI: -0.06, 0.02). Another RCT compared orlistat 120 mg TDS for three months with placebo showed a significant reduction in the mean WHR (MD: -0.10; 95%CI: -0.11, -0.09). Overall, regardless of the duration, orlistat has no effect on the mean WHR compared with placebo (MD: -0.06; 95%CI: -0.14, 0.02, I^2= 92.5%) (Figure 5-44) (very low-grade evidence, table 8).

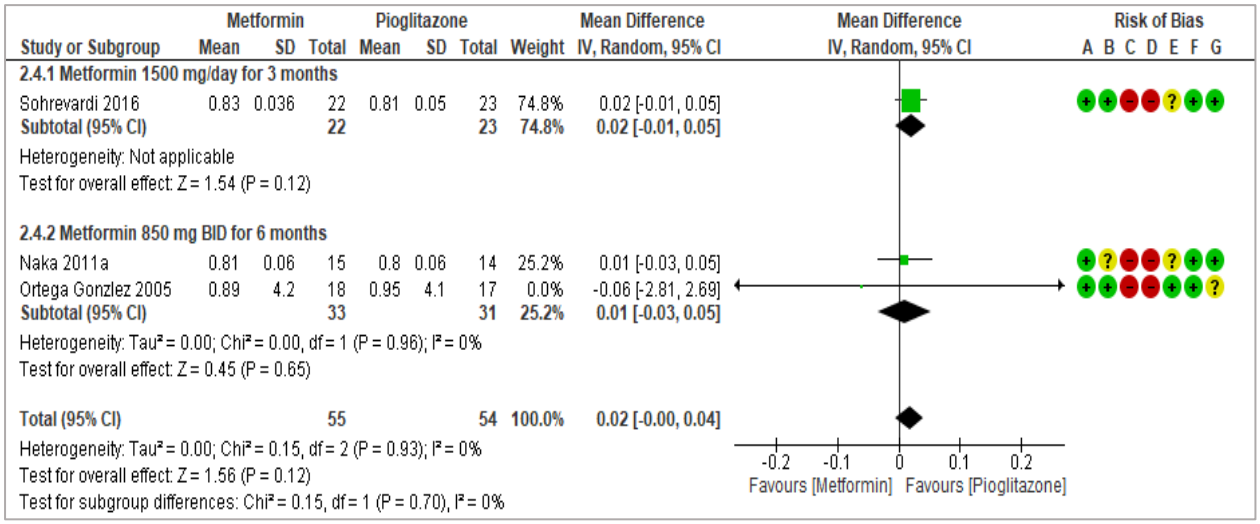
Figure 5-44: Forest plot of Orlistat versus placebo on WHR



5.3.7.5 Metformin versus Pioglitazone

Three RCTs compared metformin of various dosages for different durations with pioglitazone showed no effect on the mean WHR (MD: 0.02; 95%CI: -0.00, 0.04, I^2= 92.5%) (Figure 5-45) (very low-grade evidence, table 8).

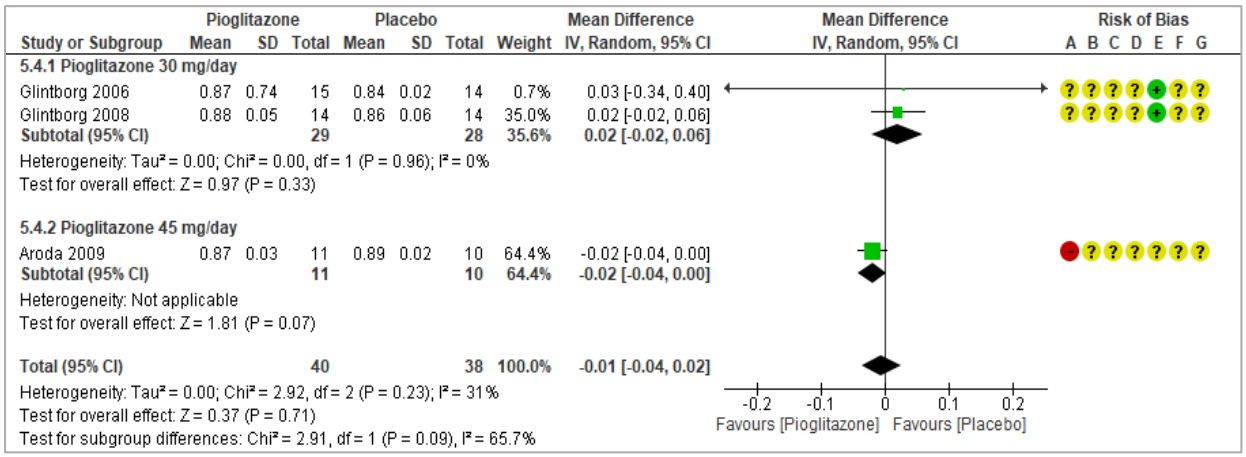
Figure 5-45: Forest plot of Metformin versus Pioglitazone on WHR



5.3.7.6 Pioglitazone versus placebo

Two RCTs compared pioglitazone 30 mg QD with placebo showed no effect on the mean WHR (MD: 0.02; 95%CI: -0.02, 0.06). One RCT compared pioglitazone 45 mg QD with placebo showed a significant reduction in the mean WHR (MD: -0.02; 95%CI: -0.04, 0.00). Overall, regardless of the administered dosage, pioglitazone showed no effect on the mean WHR compared with placebo (MD:-0.01;95%CI: -0.04, 0.02, I^2=65.7%) (Figure 5-46) (low-grade evidence, table 8).

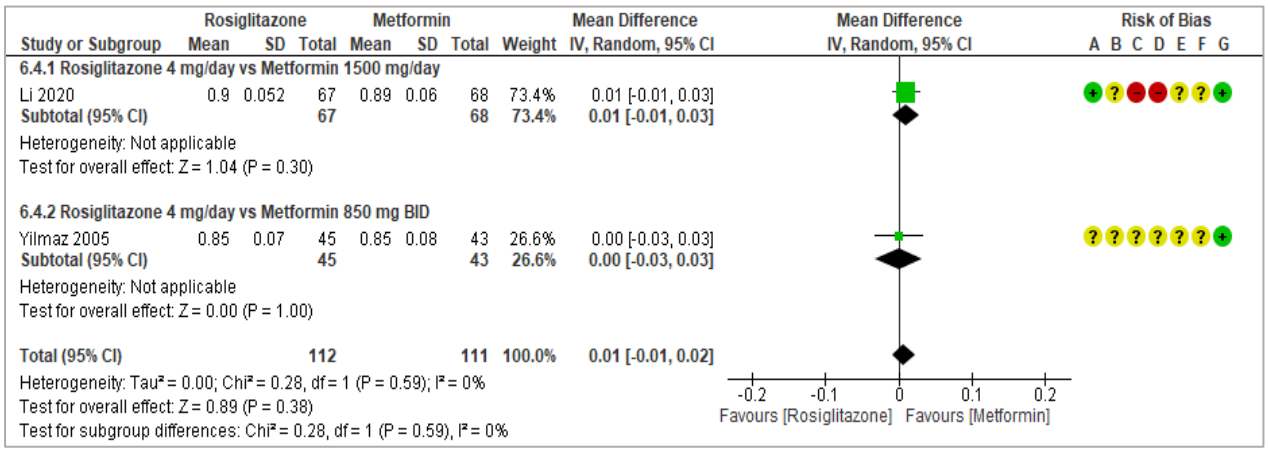
Figure 5-46: Forest plot of Pioglitazone versus placebo on WHR



5.3.7.7 Rosiglitazone versus Metformin

Two RCTs compared rosiglitazone 4 mg QD with various metformin dosages showed no effect on the mean WHR (MD: 0.01; 95% CI: -0.01, 0.02, I^2=0%) (Figure 5-47) (low-grade evidence, table 8).

Figure 5-47: Forest plot of Rosiglitazone versus Metformin on WHR

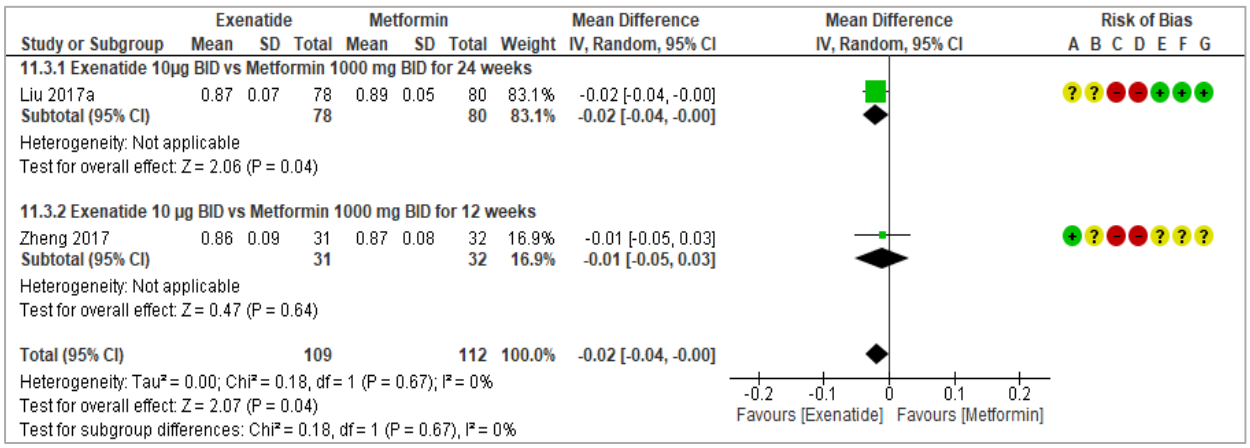


5.3.7.8 Exenatide versus Metformin

One RCT compared exenatide 10 µg BID with metformin 1000 mg BID for 24 weeks significantly reduced the mean WHR by 0.02 (95%CI: -0.04, -0.00). However, another RCT compared exenatide 10 µg BID with metformin 1000 mg BID for 12 weeks showed no effect on the mean WHR (MD: -0.01; 95% CI: -0.05, 0.03). Overall, exenatide 10 µg compared with

metformin 1000 mg BID for various duration significantly reduced the mean WHR by 0.02 (95%CI: -0.04, 0.00, I^2=0) (Figure 5-48) (low-grade evidence, table 8).

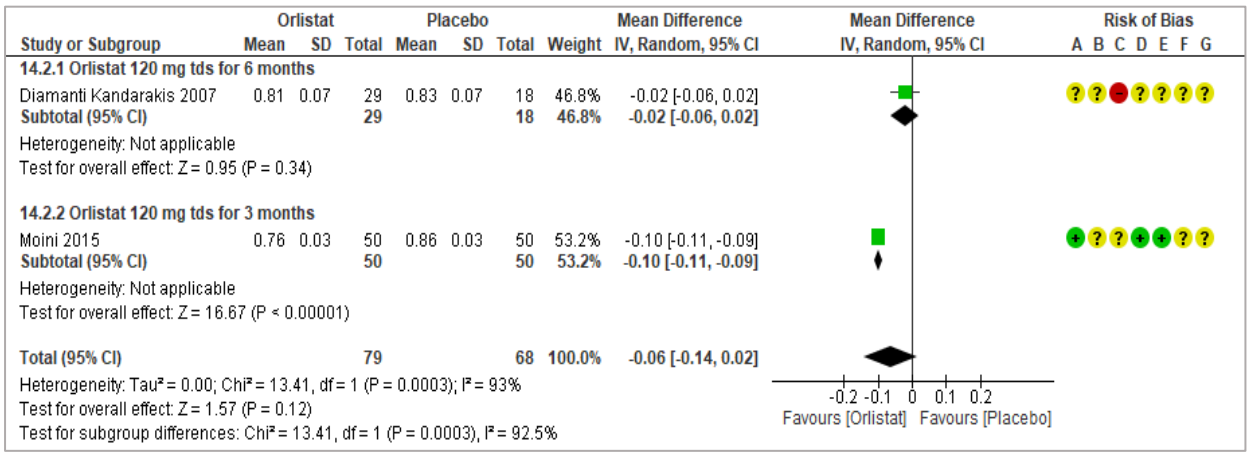
Figure 5-48: Forest plot of Exenatide versus Metformin on WHR



5.3.7.9 Orlistat versus placebo

One RCT compared orlistat 120 mg TDS with placebo for six months showed no effect on the mean WHR (MD: -0.02; 95%CI: -0.06, 0.02). Another RCT compared orlistat 120 mg TDS with placebo for three months showed a significant reduction in the mean WHR (MD: -0.10; 95% CI: -0.11, -0.09). Overall, regardless to the duration orlistat 120 mg TDS has no effect on the mean WHR compared with placebo (MD: -0.06; 95% CI: -014, 0.02, I^2=92.5%) (Figure 5-49) (very low-grade evidence, table 8).

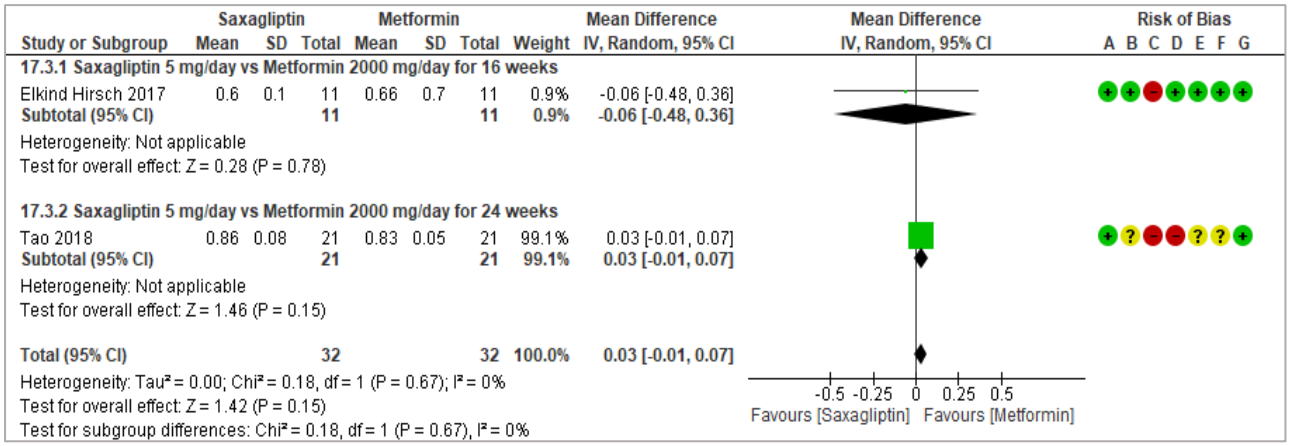
Figure 5-49: Forest plot of Orlistat versus placebo on WHR



5.3.7.10 Saxagliptin versus Metformin

In two RCTs compared saxagliptin 5 mg QD with metformin 2000 mg QD for various duration showed no effect on the mean WHR (MD: 0.03; 95%CI: -0.01, 0.07, I^2= 0%) (Figure 5-50) (very low-grade evidence, table 8).

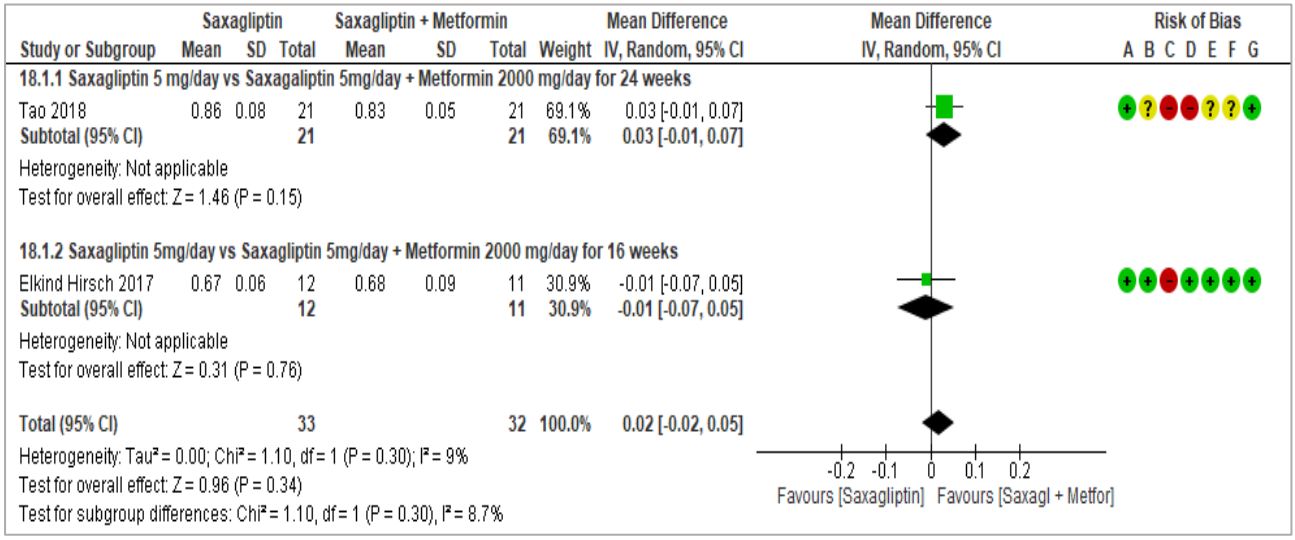
Figure 5-50: Forest plot of Saxagliptin versus Metformin on WHR



5.3.7.11 Saxagliptin versus Saxagliptin + Metformin

Two RCTs compared saxagliptin 5 mg QD with saxagliptin 5 mg QD added to metformin 2000 mg QD for the various durations of time showed no effect on the mean WHR (MD: 0.02; 95%CI: -0.02, 0.05, I^2= 8.7%) (Figure 5-51) (low-grade evidence, table 8).

Figure 5-51: Forest plot of Saxagliptin versus Saxagliptin + Metformin on WHR



5.3.7.12 Spironolactone versus Metformin

Two RCTs compared spironolactone 50 mg QD with metformin 1000 mg QD for six months significantly increased the mean WHR (MD: 0.03; 95%CI: 0.01, 0.05, I^2=0%) (Figure 5-52) (very low-grade evidence, table 8).

Figure 5-52: Forest plot of Spironolactone versus Metformin on WHR

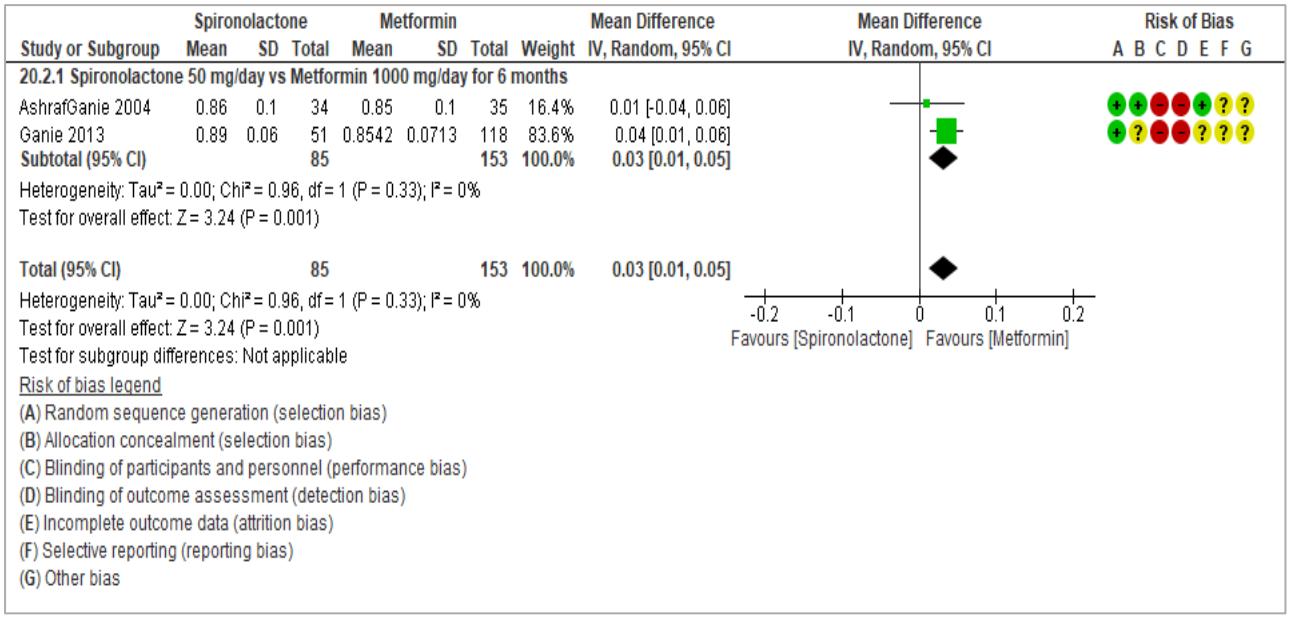


Table 8: Summary of findings for the outcomes

Patient or population: PCOS **Setting:** **Intervention: First treatment (T1)** **Comparison: Second treatment (T2)**					
Outcome	**№ of participants (studies)**	**Certainty of the evidence (GRADE)**	**Relative effect (95% CI)**	**Anticipated absolute effects**	
				Assumed risk	
				Risk difference with intervention	**Risk difference with comparison**
<u>Meformin versus placebo</u>					**(T1 minus T2)**
Body weight	739 (10 RCTs)	⊕⊕⊕◯ MODERATE a	-	The mean body weight ranged from 62.3-100.9 KG	MD 3.13 KG lower (5.33 lower to 0.93 lower)
BMI	1314 (22 RCTs)	⊕⊕⊕◯ MODERATE a	-	The mean BMI ranged from 22.6- 38.4 Kg/m2	MD 0.67 Kg/m2 lower (1.08 lower to 0.27 lower)
WC	508 (5 RCTs)	⊕⊕⊕◯ MODERATE a	-	The mean WC ranged from 86.1- 113.1 cm	MD 1.21 cm lower (3.71 lower to 1.29 higher)
WHR	639 (11 RCTs)	⊕⊕⊕◯ MODERATE b	-	The mean WHR ranged from 0.76- 0.90 cm	MD 0.01 cm lower (0.02 lower to 0.01 higher)
<u>Metformin versus Pioglitazone</u>			-		**(T1 minus T2)**
Body weight	185 (5 RCTs)	⊕⊕◯◯ LOW a,b	-	The mean body weight ranged from 72.1-97.1	MD 1.17 lower (3.05 lower to 0.71 higher)
BMI	236 (6 RCTs)	⊕◯◯◯ VERY LOW a,c,d	-	The mean BMI ranged from 25.83-to 37.3	MD 1.17 lower (1.8 lower to 0.54 lower)
WC	85 (2 RCTs)	⊕◯◯◯ VERY LOW b,e	-	The mean WC ranged from 88.1 to 90.36	MD 0.28 lower (4.66 lower to 4.1 higher)
WHR	109 (3 RCTs)	⊕◯◯◯ VERY LOW b,f	-	The mean WHR was 0.80-0.95	MD 0.02 higher (0 to 0.04 higher)
<u>Liraglutide versus Metformin</u>					**(T1 minus T2)**
Body weight	69 (2 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean body weight was 101.3- 108.1	MD 2.17 higher (10.66 lower to 14.99 higher)
BMI	55 (2 RCTs)	⊕⊕◯◯ LOW a,b,c	-	The mean BMI was 36.5-39.3	MD 0.25 higher (7.3 lower to 7.79 higher)
WC	55 (2 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean WC was 119-121.3	MD 3.66 lower (14.84 lower to 7.52 higher)
<u>Orlistat versus Metformin</u>					**(T1 minus T2)**
Body weight	121 (3 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean body weight was 75.85- 98.4	MD 3.28 higher (0.74 lower to 7.29 higher)
BMI	140 (3 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean BMI was 31.03-37.3	MD 0.22 lower (2.74 lower to 2.31 higher)
<u>Sitagliptin + Metformin versus Metformin</u>					**(T1 minus T2)**
BMI	34 (2 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean BMI was 32-39.5	MD 3.94 lower (7.81 lower to 0.08 lower)
<u>Sitagliptin versus placebo</u>					**(T1 minus T2)**
BMI	62 (2 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean BMI was 32.1-38	MD 0.08 lower (3.59 lower to 3.43 higher)
<u>Exenatide versus Metformin</u>					**(T1 minus T2)**
Body weight	221 (2 RCTs)	⊕⊕◯◯ LOW a,b	-	The mean body weight was 68.17- 68.49	MD 0.22 higher (2.01 lower to 2.44 higher)
BMI	249 (3 RCTs)	⊕⊕◯◯ LOW a,c	-	The mean BMI was 27.2-27.27	MD 0.85 lower (1.61 lower to 0.08 lower)
WHR	221 (2 RCTs)	⊕⊕◯◯ LOW a,c	-	The mean WHR was 0.87-0.89	MD 0.02 lower (0.04 lower to 0)

Orlistat versus placebo					**(T1 minus T2)**
BMI	147 (2 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean BMI was 28.57- 30.15	MD 1.33 lower (2.05 lower to 0.61 lower)
WHR	147 (2 RCTs)	⊕◯◯◯ VERY LOW a,b,c,d	-	The mean WHR was 0.83-0.86	MD 0.06 lower (0.14 lower to 0.02 higher)
Acarbose versus placebo					**(T1 minus T2)**
BMI	72 (2 RCTs)	⊕⊕◯◯ LOW a,b	-	The mean BMI was 22.63- 34.77	MD 1.14 lower (3.08 lower to 0.8 higher)
Acarbose versus Metformin					**(T1 minus T2)**
BMI	146 (3 RCTs)	⊕⊕◯◯ LOW a,b	-	The mean BMI was 27-30.6	MD 1.26 lower (2.13 lower to 0.38 lower)
Spironolactone versus Metformin					**(T1 minus T2)**
BMI	274 (3RCTs)	⊕◯◯◯ VERY LOW a,c,d	-	The mean BMI was 24.38-31.9	MD 0.16 higher (-0.62 lower to 0.94 higher)
WHR	238 (3 RCTs)	⊕◯◯◯ VERY LOW a,c,d	-	The mean WHR was 0.07-0.1	MD 0.03 higher (0.01 lower to 0.05 higher)
Saxagliptin versus Saxagliptin + Metformin					**(T1 minus T2)**
WHR	65 (2 RCTs)	⊕⊕◯◯ LOW a,b	-	The mean WHR was 0.68-0.83	MD 0.02 higher (0.02 lower to 0.05 higher)
Metformin versus Simvastatin					**(T1 minus T2)**
BMI	268 (2 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean BMI was 27.72- 29.75	MD 0.12 lower (1.25 lower to 1 higher)
Metformin versus NAC					**(T1 minus T2)**
BMI	202 (2 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean BMI was 27.11-33.1	MD 1.3 lower (6.54 lower to 3.93 higher)
Atorvastatin versus Placebo					**(T1 minus T2)**
BMI	65 (2 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean BMI was 26.8-33.92	MD 1.19 higher (3.36 lower to 5.75 higher)
Rosiglitazone versus Placebo					**(T1 minus T2)**
Body weight	87 (2 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean BMI was 26-30.19	MD 1.12 lower (1.73 lower to 0.51 lower)
BMI	87 (2 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean body weight was 64.2- 72.45	MD 2.94 lower (9.42 lower to 3.54 higher)
WHR	87 (2 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean WHR was 0.81-0.86	MD 0 (0.25 lower to 0.25 higher)

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **MD:** Mean difference; **RCT:** randomised clinical trials; **BMI:** body mass index; **WHR**: waist to hip ratio; **WC:** waist circumference; **T1:** first treatment; **T2:** second traeatment

GRADE Working Group grades of evidence.

High certainty: We are very confident that the true effect lies close to that of the estimate of the effect.

Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.

Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

Explanations

a. Two studies have an unclear risk of bias across five or more domains. One study has a high risk of performance bias. Thus, we downgraded one level.

b. A small number of participants with a wide confidence interval. So, we downgraded one level.

c. There is no overlapping of confidence interval between the studies, which could mean there are small studies with negative results been unreported. Thus, we downgraded one level.

d. Six studies showed an unclear risk of bias across more than five domains. One study has a high-performance bias. Thus, we downgraded one level.

e. A small number of participants with a wide confidence interval. Thus, we downgraded one level.

f. One study has a high risk of performance bias. Thus, downgraded one level.

g. A considerable level of heterogeneity. Therefore, we downgraded one level.
h. Only two studies, and there is no overlapping of confidence intervals. So, we downgraded one level.
i. There is an unclear risk of bias across many domains in all the studies. One study has a high risk of performance bias. We downgraded one level.
j. unclear risk of bias across many domains of the studies. Therefore, we downgraded one level.
k. There is a wide range of confidence intervals across the studies with a significant effect of CL and MD. Thus, we downgraded one level.

5.3.8 Sensitivity analysis

The impact of each study on heterogeneity and the strength of the summary was assessed using sensitivity analysis. Small sample-sized trials and those with overall high RoB were removed from the analysis while observing their effects on the cumulative results. Thus, no significant effect was found, and hence no trial was removed from the meta-analysis.

5.4 Discussion

This systematic review summarises the up-to-date evidence supporting the pharmacological interventions used in PCOS management. When metformin was administered at various therapeutic doses, there was a statistically significant reduction in the mean body weight, BMI and WC compared with placebo. Such effects were also observed when metformin was compared with sitagliptin and acarbose. On the other hand, pioglitazone and rosiglitazone were associated with a significant increase in the mean body weight, WC and BMI.

Significant beneficial changes were found with metformin versus placebo on body weight and BMI. Subgroup analyses also indicated significant body weight and BMI reductions were noted with differing doses of metformin administered for short or long durations. These findings are in-line with previous systematic reviews in which metformin was compared with lifestyle modification or placebo (638, 731). The most recent meta-analysis (638) reported a large reduction in BMI (WMD: -1.25 kg/m², 95%CI: -1.60, -0.91, p<0.00001) following treatment with metformin. Another meta-analysis (731) also reported a significant reduction in BMI (MD: -0.73 kg/m²,95% CI: -1.14, -0.32, P = 0.0005) with metformin compared with lifestyle or placebo. Therefore, these results agree that metformin as monotherapy can significantly reduce weight and BMI in women with PCOS. In the present review, it was shown that metformin could also reduce BMI when compared with pioglitazone. This observation is

consistent with a previous meta-analysis in which BMI was increased with pioglitazone treatment to a large extent compared with metformin (393).

Similarly, another systematic review compared the effect of pioglitazone versus metformin in PCOS and showed a decreased effect with pioglitazone than with metformin in reducing BMI (656). We also found a significant reduction in body weight with metformin administered at various doses compared with rosiglitazone; however, there was no difference between metformin and either liraglutide or exenatide. Another observation was a significant reduction in BMI with orlistat compared with placebo, which is in line with the findings of other groups (470, 732). Nevertheless, no reduction in BMI was seen for orlistat compared with metformin. Finally, a significant reduction of BMI was found with sitagliptin added to metformin versus metformin alone.

Results in the current study are in accord with many clinical trials of varying designs that have evaluated the effects of different pharmacological interventions on the body composition in patients with T2DM. In an observational study of 51 newly diagnosed patients with T2DM, metformin 1g/day for six months was associated with a significant improvement in body composition when compared with placebo (733). A recent systematic review and meta-analysis evaluating the efficacy of different pharmacological interventions on adults with T2DM showed that body weight was either significantly reduced or maintained in treatment with metformin and DPP-4 inhibitors (734). Another meta-analysis of 15 RCTs evaluating the effects of pioglitazone on the glycaemic indices, lipid profiles, BMI and body weight in T2DM reported a significant increase in body weight and BMI (WMD: 1.755, 95% CI 0.674 to 2.837 and 1.145, 95% CI 0.389 to 1.901, respectively) (735). In an RCT of 676 newly diagnosed patients with T2DM (343 in the acarbose group and 333 in the metformin group), examined

the effect of metformin and acarbose on WHR, it was reported that a significant reduction in WHR in both groups occurred after 25 weeks (acarbose: -0.015, 95% CI: -0.018 to -0.012, $P < 0.001$; metformin: -0.013, 95%CI: -0.016 to -0.010, $P < 0.001$) (736).

This study followed a comprehensive and systematic search of relevant databases and grey sources that only included RCTs. Furthermore, to minimise the risk of bias, all observational studies and non-randomised clinical trials were excluded. However, there are some limitations that must be considered for this systematic review. We applied a language filter, and only RCTs reported in the English language were included; hence several trials in foreign languages may not have been retrieved. Assessing such trials requires sophisticated translation, which is challenging and might also affect the methodology of this review.

Furthermore, only fully published trials were eligible to be included in the review. The majority of the trials were of a small sample size. Therefore, the statistical power used to calculate sample size and detect the significant differences between the groups was not fully reported. In addition, all the trials were of short duration and reported baseline and immediate post-intervention data. Thus, the long-term effects of different pharmacological interventions in women with PCOS are not clear.

5.5 Conclusion

Metformin, alone or in combination with other medications and irrespective of the dosage and duration of therapy, can significantly reduce mean body weight, BMI and WC in women with PCOS. Orlistat also significantly reduced mean BMI compared with placebo. On the other hand, both rosiglitazone and pioglitazone, alone or combined with other medications, were associated with significant increases in mean body weight, BMI and WC.

6 Chapter 6: Impact of pharmacological interventions on biochemical hyperandrogenaemia in women with polycystic ovary syndrome: a systematic review and meta-analysis of randomised controlled trials

6.1 Introduction

Polycystic ovary syndrome (PCOS) is a challenging endocrine condition that affects women of reproductive age and is associated with androgen excess (737). Hyperandrogenism is one of three diagnostic criteria for its diagnosis, and pathognomonic features for PCOS include anovulation, menstrual irregularity, acne and hirsutism (66,738). In women, the adrenal gland produces androgen precursors which activated to testosterone in the ovaries and the peripheral tissue (60). Testosterone and dihydrotestosterone (DHT) are the most bioactive androgens; however, over 60% of testosterone is bound to sex hormone-binding globulin (SHBG) and approximately 30% bound to albumin, resulting in 1% being biologically active testosterone that exerts its effects (61). Testosterone can be transformed into oestradiol by the action of the aromatase enzyme and synergise their action to control female reproductive functions (62). An excessive amount of oestradiol leads to disturbance in the hypothalamic-pituitary-gonadal adrenal axis, which affects the secretion of gonadotrophin-releasing hormone (GnRH) (63). This, in turn, will affect the release of luteinising hormone (LH) and the follicular stimulating hormone (FSH) and alter the ratio of LH to FSH (64). Excessive LH increases ovarian androgen production, and the low FSH drives anovulation (65). Hyperandrogenism is also an independent risk factor for long-term health consequences associated with PCOS, including obesity and type 2 diabetes (66).

Management strategies for PCOS are primarily based on managing the androgen-related symptoms, menstrual disturbances and infertility (436). Oral contraceptives pills are the treatment of choice for managing excessive hair growth by inhibiting ovarian androgen production. However, this option alone might not lead to desirable results (739). Anti-androgen therapies such as spironolactone, finasteride and flutamide suppress the androgen effect by competing with its receptors and inhibiting testosterone conversion to its most active form, DHT (506). As a 5-α reductase inhibitor, finasteride prevents the conversion of testosterone to DHT. There was a modest increase in testosterone levels in men taking finasteride for benign prostatic hyperplasia (740). Metformin and thiazolidinediones (TZDs) reduce androgen levels by improving insulin sensitivity, menstrual cyclicity and ovulation and reducing the cardiometabolic risks related to hyperinsulinemia in PCOS (72,163). However, most of these therapeutic options were studied in smaller clinical trials, which sparingly reported their results in the literature. This review aimed to retrieve, assess and appraise the existing literature. A meta-analysis was used to combine the results of individual studies to provide greater reliability of the estimates of effects of pharmacological interventions on hyperandrogenaemia in women with PCOS.

6.2 Methods and materials

6.2.1 Protocol and registration

The protocol and the registration of this systematic review and meta-analysis are explained in chapter 2, section 2.1.1.1.

6.2.2 Eligibility criteria for the included studies

The eligibility criteria, including inclusion/exclusion criteria, is explained in chapter 2, section 2.1.1.2.

6.2.3 Literature search

The search strategy, including the search in the medical databases, is explained in chapter 2, section 2.1.1.3.

6.2.4 Study selection

The study selection, including screening titles and abstracts of the retrieved studies, is explained in chapter 2, section 2.1.1.4.

6.2.5 Data extraction

The method used to extract information from the included studies is explained in chapter 2, section 2.1.1.5.

6.2.6 Risk of bias assessment in the included studies

The RoB assessment for the included studies is explained in chapter 2, section 2.1.1.6.

6.2.7 GRADE scoring

The robustness of evidence for each chosen outcome is assessed using the GRADE scoring system and is explained in chapter 2, section 2.1.1.7.

6.2.8 Statistical analysis

The statistical method used to estimate the pooled effects of the various interventions is explained in chapter 2, section 2.1.1.12.

6.2.9 Assessment of heterogeneity

Heterogeneity for the outcomes across each RCT was evaluated and explained in chapter 2, section 2.1.1.8.

6.2.10 Subgroup analysis

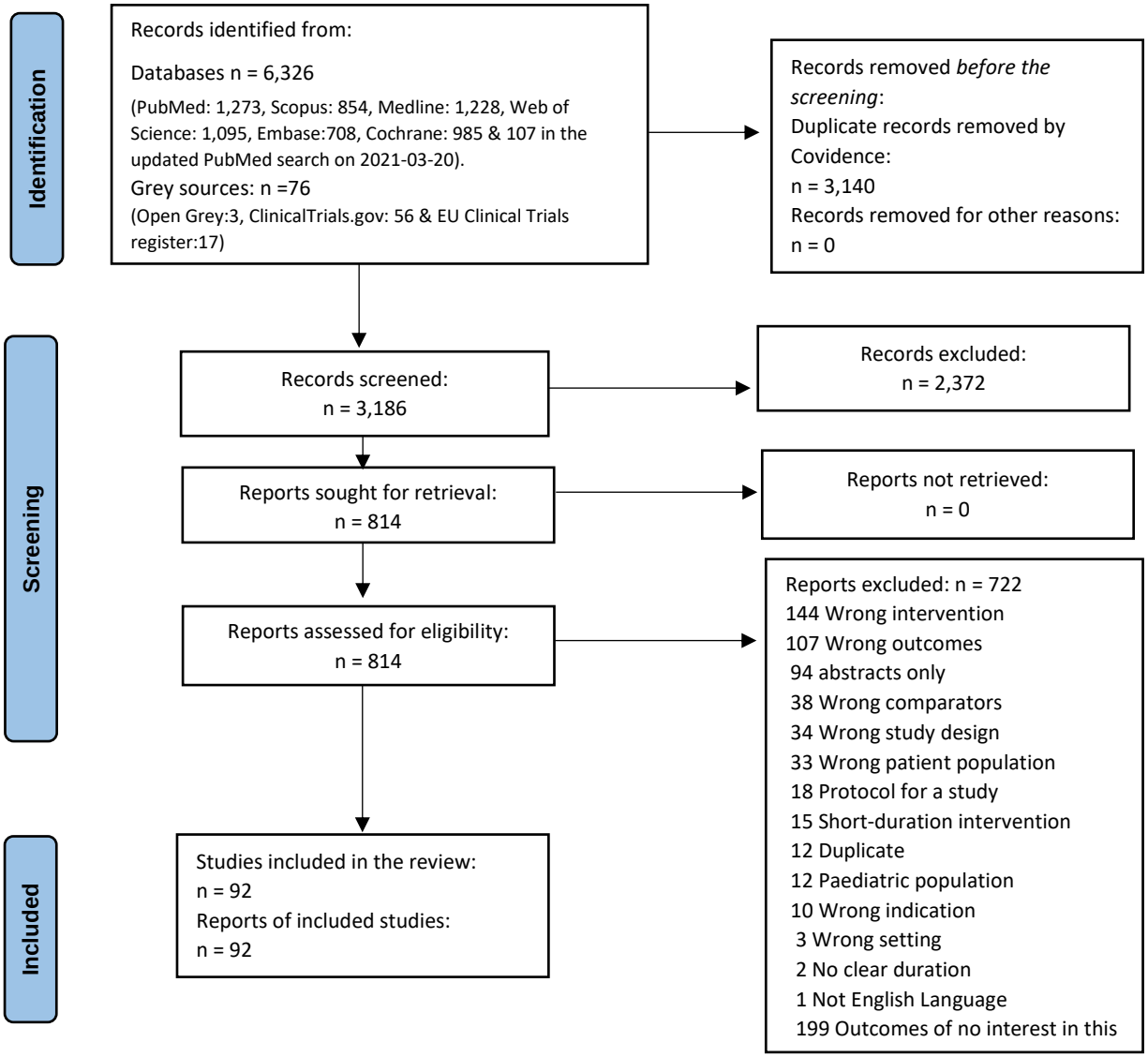
Subgroup analysis was conducted for the included RCTs and explained in chapter 2, section 2.1.1.9.

6.3 Results

6.3.1 Search results

A total of 3,326 studies were identified from the searched databases; after removing duplicates, 3,186 were initially screened for eligibility based on both titles and abstract. A total of 814 articles were then retrieved for more detailed screening, of which 722 articles were excluded due to reasons presented in the PRISMA flow diagram. Figure 6-1. After exclusion, a total of 92 RCTs met the eligibility criteria and were included in the meta-analysis.

Figure 6-1: PRISMA flow diagram



6.3.2 Characteristics of the included RCTs

The 92 RCTs were published until 2020, of which 40 RCTs (607, 610, 612, 613, 615, 616, 619, 636, 659, 661, 663, 666, 670, 672, 677, 678, 680, 686, 696, 710, 714, 715, 741-758) (43.47%) diagnosed PCOS using the Rotterdam diagnostic criteria 2003 (30). Three RCTs (3.24%)(720, 759, 760) diagnosed PCOS based on Androgen Excess Society criteria (32). Eleven RCTs (11.95%) (609, 622, 623, 679, 681, 684, 694, 724, 761-763) used the National Institute of Health (NIH/NICHD) criteria (626) to diagnose PCOS. No diagnostic criteria were specified for the remaining RCTs. The characteristics of the included RCTs are presented in Table 9.

6.3.3 Interventions and comparisons details of the included RCTs

Sixteen (17.39%) RCTs (607, 610, 616, 628-630, 663, 670, 671, 673, 686-688, 714, 715, 722) evaluated metformin versus placebo. Two RCTs (693,694) (2.17%) assessed acarbose versus placebo. Two RCTs (764,765) (2.17%) examined bromocriptine versus placebo. Two RCTs (745,756) (2.17%) tested dexamethasone versus placebo. Four RCTs (608, 633, 637, 679) (4.34%) examined pioglitazone versus placebo. Two RCTs (661, 666) (2.17%) tested sitagliptin versus placebo. Four RCTs (607, 627, 741, 747) (4.34%) compared flutamide versus placebo. Two RCTs (613,636) (2.17%) compared atorvastatin versus placebo. Two RCTs (720,766) (2.17%) used rosiglitazone versus placebo. Five RCTs (615,632,677,686,690) (5.43%) evaluated metformin versus pioglitazone. Two RCTs (767) (2.17%) examined liraglutide versus metformin. Eight RCTs (8.69%) (612, 622, 659, 672, 678, 681, 726, 761) examined metformin versus rosiglitazone. Three RCTs (623, 668, 669) (3.26%) examined liraglutide+ metformin versus metformin. Three RCTs (611, 619, 696) (3.26%) compared exenatide versus metformin. Three RCTs (607, 627,747) (3.26%) compared flutamide added to metformin versus flutamide alone. Two RCTs (743, 754) (2.17%) compared two different combined oral contraceptive pills

(OCPs) (30 µg Ethinyl Estradiol (EE)+ Drospirenone (DRSP) versus 20 µg Ethinyl Estradiol (EE) + Drospirenone (DRSP)). Two RCTs (609, 617) (2.17%) compared saxagliptin versus metformin. Two RCTs (746, 760) (2.17%) examined cabergoline added to metformin versus metformin alone. Eleven RCTs (748, 755, 757, 762, 763, 768-773) (11.95%) examined an OCP versus metformin. Ten RCTs (9.2%) (744, 748, 749, 751, 757, 768, 769, 771, 774, 775) examined OCP added to metformin versus OCP alone. Two RCTs (758, 776) (2.17%) tested clomiphene citrate added to metformin versus clomiphene citrate alone. Three RCTs (759, 762, 777) (3.26%) compared OCP (Ethinyl Estradiol (EE)/cyproterone acetate (CPA) versus OCP (EE/DRSP). Two RCTs (742,778) (2.17%) examined simvastatin added to OCP versus OCP alone. Two RCTs (779, 780)(2.17%) compared finasteride versus flutamide. Two RCTs (751, 753)(2.17%) compared OCP added to spironolactone versus OCP alone.

Table 9: Characteristics of the studies included in the systematic review and meta-analysis

Author	Country	PCOS diagnostic criteria	PCOS patient's age Mean±SD	Patient, n (PCOS)	Patient n, (control)	Interventions	Duration	Biomarkers
Ahmed et al. (761)	India	NIH	22.81±4.52	31	30	Metformin, Rosiglitazone	12months	DHEAS, TT, A4
Ajossa et al. (741)	Italy	Rotterdam	24.3 ± 2.8	11	11	Flutamide, placebo	Three months	TT, FT,DHEAS, 17-OHP,A4
Amiri et al. (607)	Iran	Rotterdam	25.6±4.02	52	53	Flutamide, Metformin Flutamide+ Metformin placebo	Six months	TT, FT, DHEAS, SHBG
Amiri et al. (759)	Iran	AES	-	100	100	EE+LNG, EE+CPA, EE+DRSP, EE+DSG	Three months	SHBG, DHEAS, TT, FAI
Aroda et al. (679)	USA	NIH	27.87 ± 0.87	23	6	Pioglitazone , placebo	Six months	TT, FT, SHBG, DHEAS, 17-OHP, A4
Banaszewska et al. (742)	USA	Rotterdam	24 ±3.5	24	24	Simvastatin, OCP	12 weeks	SHBG,TT,FT, DHEAS
Batista et al. (720)	Brazil	AES	24.56±4.33	16	17	Rosiglitazone, placebo	12 weeks	A4,TT,DHEAS, FT,SHBG, 17-OHP
Bhattacharya et al. (743)	India	Rotterdam	21.47 ± 4.27	55	57	30 µg EE, 20 µg EE	12 months	TT, FAI, SHBG
Bilgir et al. (744)	Turkey	Rotterdam	24.3±5.7	20	20	EE/CPA, EE/CPA+ Metformin	12 weeks	DHEAS, FT
Brettenthaler et al. (608)	Switzerland	-	30.2 ±1.4	17	18	Pioglitazone, placebo	Three months	DHEAS, TT, SHBG, FAI
Buvat et al. (764)	France	-	-	27	28	Bromocriptine, placebo	Six months	E, TT, A4, DHEAS,17-OHP
Carlsen et al. (745)	UK	Rotterdam	26.4+3.8	18	20	Dexamethasone, placebo	26 weeks	DHEAS, A4, TT
Cetinkalp et al. (659)	Turkey	Rotterdam	-	94	-	Metformin, Rosiglitazone, ECA	Four months	DHEAS,17-OHP,FT,TT
Cho et al. (686)	UK	Rotterdam	26· 4 ± 1·5	15	15	Orlistat, Metformin, Pioglitazone	12 weeks	SHBG, FAI
Chou et al. (689)	Brazil	-	24±5	14	16	Metformin, placebo	Three months	TT, SHBG
Cibula et al. (774)	Czech Republic	-	23.2 ±4.6	14	14	COC, Metformin + COC	Six months	SHBG, FAI, TT,A4, DHEAS
Ciotta et al. (693)	Italy	-	20.5±0.6	15	15	Acarbose, placebo	Three months	TT, A4, DHEAS, SHBG, 17-OHP
Devin et al. (661)	USA	Rotterdam	30.3 ± 3.3	18	-	Sitagliptin, placebo	Two months	TT,FT,E,SHBG
Duleba et al. (778)	Poland	-	24.0 ± 0.7	24	24	Simvastatin+ OCP, OCP	12 weeks	DHEAS, TT,SHBG
Eisenhardt et al. (663)	Germany	Rotterdam	27.0±2	22	23	Metformin, placebo	12 weeks	DHEAS,SHBG, A4, E,TT
Elkind Hirsch et al. (696)	USA	Rotterdam	28.2 ± 1.1	60	-	Exenatide, Metformin, Exenatide+ Metformin	24 weeks	FAI, SHBG, TT, DHEAS,
Elkind Hirsch et al (609)	USA	NIH	29.9 ±7	38	-	Saxagliptin, Metformin, Saxagliptin+ Metformin	16 weeks	TT,SHBG,FAI,DHEAS
ElsersyMAM et al. (746)	Egypt	Rotterdam	25.4 ± 4.7	127	123	Metformin , Cabergoline, placebo	Three months	DHEAS, TT
Elter et al. (775)	Turkey	-	24.9±6.6	20	20	OC, OC+ Metformin	Four months	TT, FT, A4, 17-OHP, SHBG, DHEAS

Falsetti et al. (779)	Italy	-	22.9 ±4.9	44	-	Finasteride, Flutamide	Six months	17-OHP, A4, TT, FT, DHEAS, SHBG
Falsetti et al. (780)	Italy	-	22.9 ±6.4	32	32	Finasteride, Flutamide	12 months	SHBG, 17-OHP, A4, TT, FT
Ferjan et al. (666)	Slovenia	Rotterdam	35.0 ± 7.2	15	15	Sitagliptin, placebo	12 weeks	TT, FT, A4, DHEAS, SHBG
Gambineri et al. (627)	Italy	-	26·1 ± 4·5	40	-	Metformin, Flutamide, Metformin+ Flutamide, placebo	Six months	TT, FT, SHBG, DHEAS, A4
Gambineri et al. (747)	Italy	Rotterdam	28 ± 8	20	17	Metformin, Flutamide, Metformin+ Flutamide, placebo	12 months	TT, FT, FAI, A4, DHEAS, SHBG
Ghaneei et al. (760)	Iran	AES	25.20 ± 4.8	54	51	Metformin+ Cabergoline, Metformin +placebo	Four months	DHEAS, TT
Glintborg et al. (637)	Denmark	-	32±6	14	14	Pioglitazone, placebo	16 weeks	SHBG, TT, FT,E
Glintborg et al (633)	Denmark	-	32±5	14	14	Pioglitazone, placebo	16 weeks	E,TT, FT, SHBG
Glintborg et al (725)	Denmark	-	-	14	14	Pioglitazone, placebo	16 weeks	FT
Glintborg et al (768)	Denmark	-	29±7	65	-	Metformin, OCP, Metformin +OCP	12 months	TT, SHBG
Glintborg et al. (748)	Denmark	Rotterdam	-	65	-	Metformin , OCP, Metformin + OCP	12 months	SHBG, TT
Glintborg et al. (769)	Denmark	-	27.9 ± 4.7	90	35	Metformin, OCP ,Metformin +OCP	12 months	E, TT, SHBG
Glintborg et al. (749)	Denmark	Rotterdam	30±4.2	65	-	Metformin, OCP, Metformin +OCP	12 months	TT, SHBG,
Hanjalic Beck et al. (680)	Germany	Rotterdam	-	62	-	Metformin, Acarbose	12 weeks	DHEAS, SHBG, A4, 17-OHP, TT
Harborne et al. (770)	UK	-	-	16	18	Metformin, Dianette	12 months	SHBG, TT, FAI, DHEAS, A4, 17-OHP
Heidari et al. (610)	USA	Rotterdam	32.4±7.5	29	13	Metformin, placebo	Three months	E, TT, DHEAS
Jensterle et al. (681)	Slovenia	NICHD	23.5±0.7	35	12	Metformin, Rosiglitazone	Six months	DHEAS, FT, TT
Jensterle et al. (622)	Slovenia	NICHD	23.1±3.7	15	11	Metformin, Rosiglitazone	Six months	A4, DHEAS, TT, FT
Jensterle et al. (724)	Slovenia	NICHD	29.5 ± 7.7	17	15	Liraglutide, Metformin	12 weeks	DHEAS, TT, FT, SHBG, FAI
Jensterle et al. (719)	Slovenia	Rotterdam	30.7 ± 7.9	45	-	Metformin, Liraglutide, Rosiglitazone	12 weeks	TT, FT, FAI, SHBG, DHEAS, A4
Jensterle et al (750)	Slovenia	Rotterdam	30.3±.4.4	44	-	Liraglutide, Metformin	12 weeks	A4, TT, FT, SHBG
Jensterle et al. (621)	Slovenia	Rotterdam	33.1 ± 6.1	30	-	Metformin +Liraglutide, Liraglutide	12 weeks	A4, TT, FT, SHBG
JensterleSever et al. (623)	Slovenia	NICHD	31.3 ±9.4	40	-	Metformin, Liraglutide, Metformin +Liraglutide	12 weeks	TT, FT, SHBG, DHEAS, A4
Kahraman et al. (777)	Turkey	AES	-	39	-	EE/CPA, EE/DSG	12 months	E, TT, FT, SHBG, FAI, DHEAS
Kazerooni et al. (670)	Iran	Rotterdam	25.6 ±4.32	42	42	Metformin + Simvastatin, Metformin + placebo	12 weeks	TT, DHEAS

Kebapcilar et al. (752)	Turkey	Rotterdam	24.0± 5.4	48	-	EE/CA, EE/CA+ Metformin, Metformin, EE/CA +Spironolactone	Three months	DHEAS, FT
Kebapcilar et al. (751)	Turkey	Rotterdam	23.4 ± 4.8	60	-	EE/CPA, EE/CPA+ Spironolactone	12 weeks	DHEAS, FT
Kocak et al. (671)	Turkey	-	26.2± 3.7	27	28	Metformin, placebo	-	DHEAS, TT, E
Kumar et al. (771)	India	-	23.2 ± 4.4	96	-	OCP, Metformin+ Metformin	Six months	F-G score, TT, DHEAS
Lam et al. (710)	China	Rotterdam	-	24	30	Rosiglitazone, placebo	12 months	TT,FT,SHBG
Leelaphiwat et al. (753)	Thailand	Rotterdam	26.29± 4.04	36	-	EE/DSG + Spironolactone, EE/CPA	3 months	TT,FT,FAI,SHBG,DHEAS
Legro et al. (726)	USA	-	27.9±4.0	55	72	CC, Metformin, CC + Metformin	Six months	SHBG, TT, FT
Li et al. (672)	China	Rotterdam	25.95± 4.36	204	-	Metformin, Rosiglitazone, Metformin +Rosiglitazone	Six months	TT
Lingaiah et al. (673)	Finland	Rotterdam	27.6 ±4.0	57	61	Metformin, placebo	Three months	E, TT, SHBG, FAI, DHEAS, A4
Liu et al. (611)	China	Rotterdam	27.69 ± 3.80	158	-	Metformin, Exenatide	12 weeks	TT, SHBG, FAI
Lord et al. (628)	UK	-	27.76±4.89	16	16	Metformin, placebo	12 weeks	TT, SHBG, FAI, DHEAS
Malkawi et al. (776)	Jordan	-	29 ± 3.1	16	12	Metformin /CC, placebo/CC	-	TT, FT, SHBG, FAI, DHEAS
Mohiyiddeen et al (612)	UK	Rotterdam	30.0±0.9	35	-	Metformin, Rosiglitazone	Three months	TT, SHBG, FAI
Morin Papunen et al. (773)	Finland	-	-	-	-	Metformin, Diane	Six months	TT, SHBG, DHEAS, A4, 17-OHP
Morin Papunen et al. (772)	Finland	-	28.2 ±1.4	17	-	Metformin, EE/CPA	Three months	TT, SHBG, FAI, DHEAS, A4
Murdoch et al. (765)	UK	-	-	7	9	Bromocriptine, placebo	12 months	TT, A4, SHBG, E
Naka et al(690)	Greece	-	23.3 ±4.9	43	14	Pioglitazone, Metformin, placebo	Six months	TT, SHBG, FAI
Ng et al. (629)	China	-	30.5±6	10	10	Metformin, placebo	Three months	TT,SHBG, A4,DHEAS
Ortega Gonzlez et al. (632)	Mexico	-	28.8 ±0.9	35	-	Metformin, Pioglitazone	Six months	DHEAS, FT, A4, E
Palomba et al. (722)	Italy	-	24.3 ±3.1	14	13	Metformin, placebo	24 months	SHBG,FAI,FT,TT,DHEAS,A4
Panidis et al. (762)	Greece	NICHD	21.07 ± 3.21	45	-	EE/CPA, EE/DRSP, Metformin	Six months	TT, DHEAS, SHBG, 17-OHP, FAI
Penna(694)	Brazil	NIH	26.69± 1.46	15	14	Acarbose, placebo	Six months	TT, A4, FAI, SHBG
Puurunen et al. (636)	Finland	Rotterdam	40.5±5.9	15	13	Atorvastatin, placebo	Six months	TT, SHBG, FAI, A4, DHEAS, E
Rautio et al. (766)	Finland	Anonymous	29.1 ± 1.2	15	15	Rosiglitazone, placebo	Four months	TT, SHBG, DHEAS, A4, FAI
Romualdi et al. (714)	Italy	Rotterdam	24.7 ±4.4	13	10	Metformin, placebo	Six months	A4, TT,FAI,SHBG,DHEAS,17-OHP
Romualdi et al. (754)	Italy	Rotterdam	22.92±3.80	26	-	20ugEE/DRSP, 30ugEE/DRSP	12 months	A4, TT,FAI,SHBG,DHEAS,17-OHP
Sahu et al. (755)	India	Rotterdam	27.0 ±5.2	86	-	Metformin, OCP	Six months	TT, SHBG, DHEAS
Sathyapalan et al. (613)	UK	Rotterdam	27.7 ± 1.4	19	18	Atorvastatin, placebo	12 weeks	FAI, TT, SHBG
Shahebrahimi et al. (677)	Iran	Rotterdam	27.5 ± 3.68	56	-	Metformin, Pioglitazone	Three months	TT, DHEAS
Sohrevardi et al. (615)	Iran	Rotterdam	-	84	-	Metformin, Pioglitazone, Metformin + Pioglitazone	Three months	DHEAS,SHBG,FAI
Sonmez et al. (684)	Turkey	NICHD	26.13±5.08	30	-	Metformin, Acarbose	Three months	E, TT

Sova et al. (616)	Finland	Rotterdam	27.7± 4.0	110	-	Metformin, placebo	Three months	SHBG, TT, SHBG, FAI, DHEAS
Tao et al. (617)	China	-	29 ± 5	75	-	Metformin, Saxagliptin, Saxagliptin + Metformin	24 weeks	TT, SHBG, FAI
Teede et al. (763)	Australia	NIH	33.5 ±6.7	56	-	Metformin, OCP	Six months	TT, FAI, SHBG
Trolle et al. (630)	Denmark	-	31±4	52	-	Metformin, placebo	six months	TT, SHBG
Vandermolen et al. (688)	USA	-	29.6 ±1.2	11	14	Metformin, placebo	Seven weeks	FT, TT, SHBG, E, A4, DHEAS, 17-OHP
Vanky et al. (756)	Norway	Rotterdam	26.4 ±6 3.8	18	20	Dexamethasone, placebo	Eight weeks	TT, SHBG, FT, A4, DHEAS, 17-OHP
Vanky et al. (715)	Norway	Rotterdam	28.9±4.8	18	22	Metformin, placebo	36 weeks	DHEAS, SHBG, TT, A4
Wu et al. (757)	China	Rotterdam	26.1+4.6	60	-	Diane, Metformin, Diane +Metformin	Three months	TT
Yarali et al. (687)	Turkey	-	29.7±5.6	16	16	Metformin, placebo	Six weeks	E, TT, FT, A4, DHEAS, 17-OHP
Yilmaz et al. (678)	Turkey	Rotterdam	24.67+4.60	88	-	Metformin, Rosiglitazone	Six months	FAI, SHBG, DHEAS, TT, FT, A4
Zain et al. (758)	Australia	Rotterdam	27.8 ±3.6	115	-	Metformin, CC, Metformin +CC	Six months	TT
Zheng et al. (619)	China	Rotterdam	27.70 ± 3.41	82	-	Exenatide, Metformin	12 weeks	FAI, DHEAS, SHBG

PCOS: polycystic ovary syndrome, **TT**: total testosterone**, FT:** free testosterone, **SHBG:** sex hormone-binding globulin, **A4:** Androstenedione, **DHEAS:** Dehydroepiandrosterone sulphate, **17-OHP:** 17-hydroxyprogesterone, **FAI:** free androgen index, **E:** oestradiol, **OCP:** oral contraceptive pills**, NIH:** national institute of health, **CC:** clomiphene citrate, **NICHD:** national institute of child health. **SD:** standard deviation

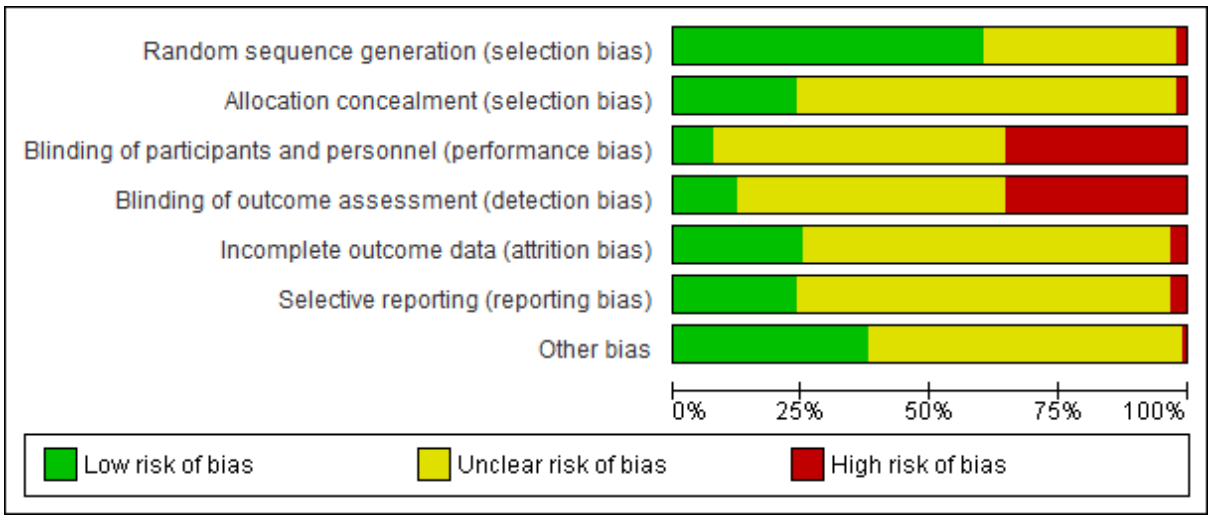
6.3.4 Outcomes in the included RCTs

The RCTs included in this review examined values of the desirable outcomes at baseline and post-intervention with few RCTs reporting differences from the baseline. Total testosterone was reported in 85 RCTs (92.39%, participants *n* = 3,459), free testosterone in 30 RCTs (32.6%, participants *n* = 1,080), FAI in 23 RCTs (25%, participants *n* = 851), DHEAS in 56 RCTs (60.86%, participants *n* = 2,542), SHBG in 62 RCTs (67.39%, participants *n* = 2,335) , oestradiol in 7 RCTs (7.6%, participants *n* = 255), androstenedione in 28 RCTs (30.43%, participants *n* = 1,016) and 17-OHP in 9 RCTs (9.78%, participants *n* = 467).

6.3.5 Risk of bias assessment in the included RCTs

Most of the RCTs were judged to have poor quality due to inadequate randomisation and blinding of assessors and participants. Moreover, the vast majority of the included RCTs were not sufficiently reported; therefore, they were judged to have an unclear RoB. The overall risk of bias for the included RCTs is shown in Figure 6-2.

Figure 6-2: Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included RCTs.

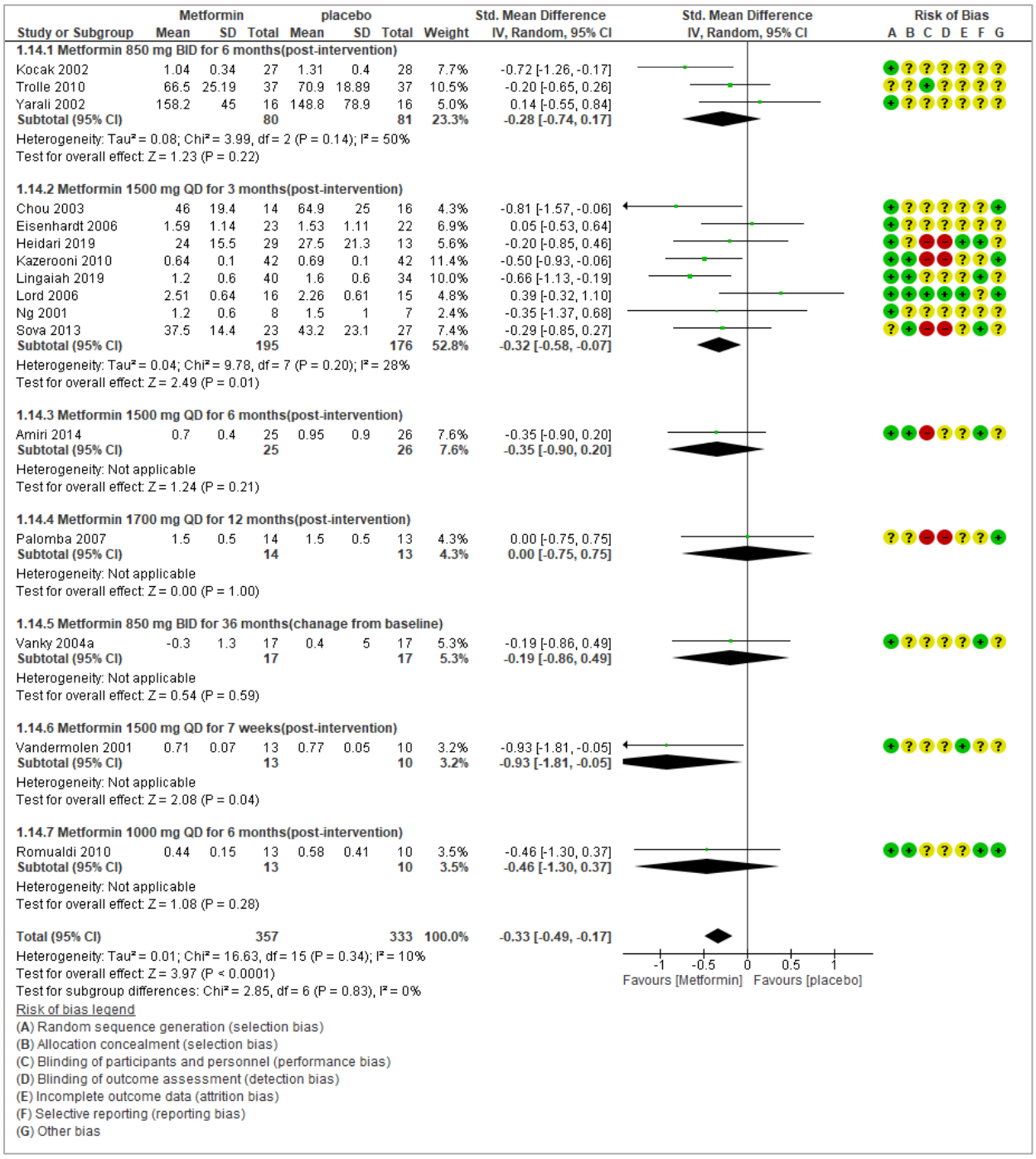


6.3.6 Total testosterone

6.3.6.1 Metformin versus placebo

Three RCTs compared metformin 850 mg BID with placebo for six months; the analysis of the post-intervention values showed no reduction in the total testosterone level (SMD: -0.28; 95% CI: -0.74 , 0.17). In eight RCTs comparing metformin 1500 mg QD for three months, there was a statistically significant reduction in total testosterone (SMD: -0.32; 95% CI:-0.58, -0.07). One RCT compared metformin 1500 mg for six months showed no reduction in the total testosterone (SMD: -0.35; 95% CI: -0.90, 0.20). One RCT compared metformin 1700 mg QD for 12 months with no reduction in the total testosterone (SMD: 0.00; 95% CI: -0.75, 0.75). In one RCT compared metformin 850 BID for 36 months, the changes from the baseline showed no reduction in the total testosterone (SMD: -0.19; 95% CI: -0.86, 0.49). Whereas in one RCT compared metformin 1500 mg QD for seven weeks showed a significant reduction in the total testosterone (SMD: -0.93; 95% CI: -1.81, -0.05). However, one RCT of metformin 1000 mg QD for six months showed no reduction in total testosterone (SMD: -0.46; 95% CI: -1.30, 0.37). Overall, regardless of the administered dosage or the duration, metformin was associated with a significant reduction in the total testosterone when compared with placebo (SMD: -0.33; 95% CI: -0.49, -0.17) (Figure 6-3) (moderate grade evidence, table 10).

Figure 6-3: Forest plot of Metformin versus placebo on total testosterone

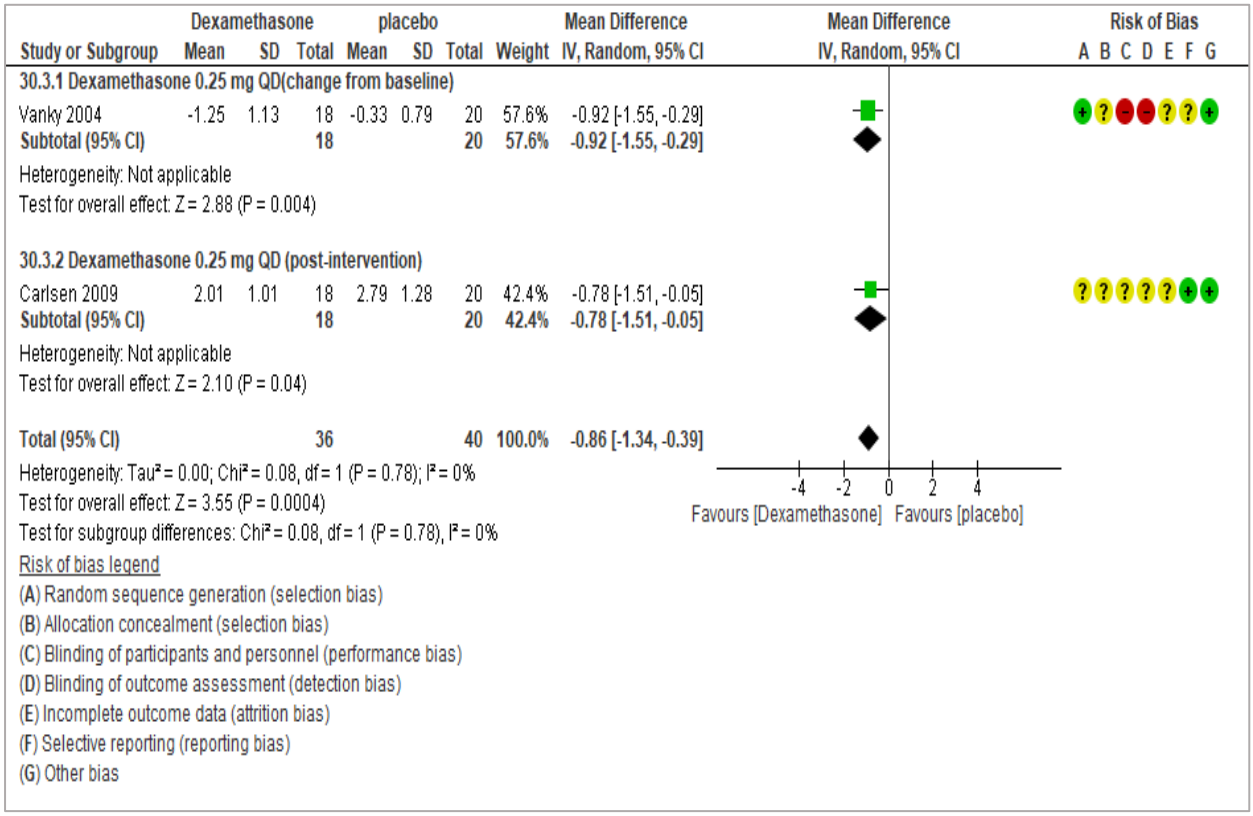


6.3.6.2 Dexamethasone versus placebo

In one RCT compared dexamethasone 0.25 mg QD, changes from baseline analysis showed a significant reduction in total testosterone (MD: -0.92 nmol/L; 95% CI: -1.55, -0.29). Similarly, in one RCT of dexamethasone 0.25 mg QD, the post-intervention result showed a significant

reduction in the total testosterone level (MD: -0.78 nmol/L; 95% CI: -1.51, -0.05). Overall, dexamethasone 0.25 mg QD was associated with a significant reduction in the total testosterone compared with placebo (MD: -0.86 nmol/L; 95% CI: -1.34, -0.39) (Figure 6-4) (very low-grade evidence, table 10).

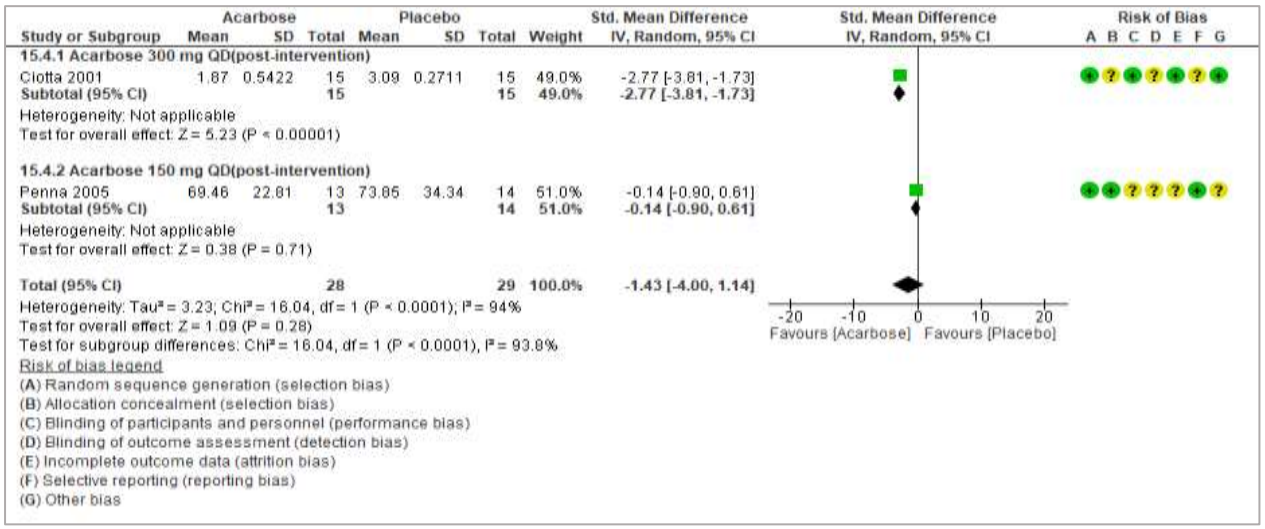
Figure 6-4: Forest plot of Dexamethasone versus placebo on total testosterone (nmol/L)



6.3.6.3 Acarbose versus placebo

In one RCT, acarbose 300 mg QD showed a significant reduction in the mean total testosterone compared with placebo (SMD: -2.77; 95% CI: -3.81, -1.73). In another RCT, acarbose 150 mg QD showed no effect on the mean total testosterone compared with placebo (SMD: -0.14; 95%CI: -0.90, 0.61). Overall, acarbose at various dosages showed no effect on the mean total testosterone compared with placebo (SMD: -1.43; 95% CI: -4.00, -1.14) (Figure 6-5) (very low-grade evidence, table 10).

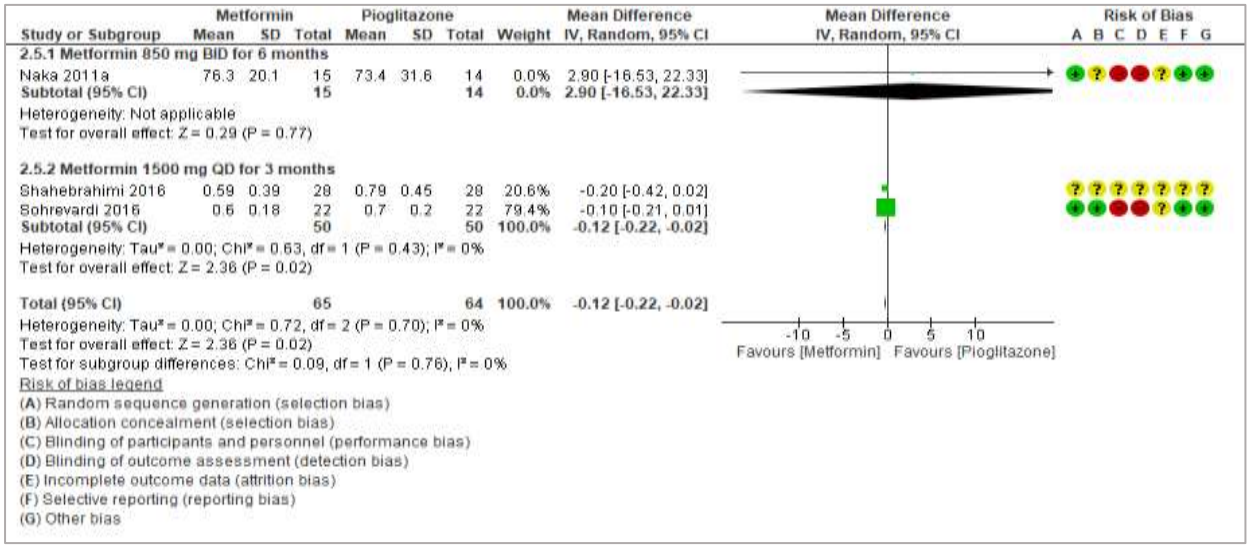
Figure 6-5: Forest plot of Acarbose versus placebo on total testosterone



6.3.6.4 Metformin versus Pioglitazone

In one RCT, metformin 850 mg BID compared with pioglitazone for six months had no effect on the total testosterone (MD: 2.90 nmol/L; 95% CI: -16.53, 22.33). However, in two RCTs, when metformin 1500 mg QD was compared with pioglitazone showed a significant reduction in the total testosterone (MD: -0.12 nmol/L; 95%CI: -0.22, -0.02). Overall, metformin significantly reduced the total testosterone when compared with pioglitazone (MD: -0.12 nmol/L; 95% CI:-0.22, -0.02) (Figure 6-6) (very low-grade evidence, table 10).

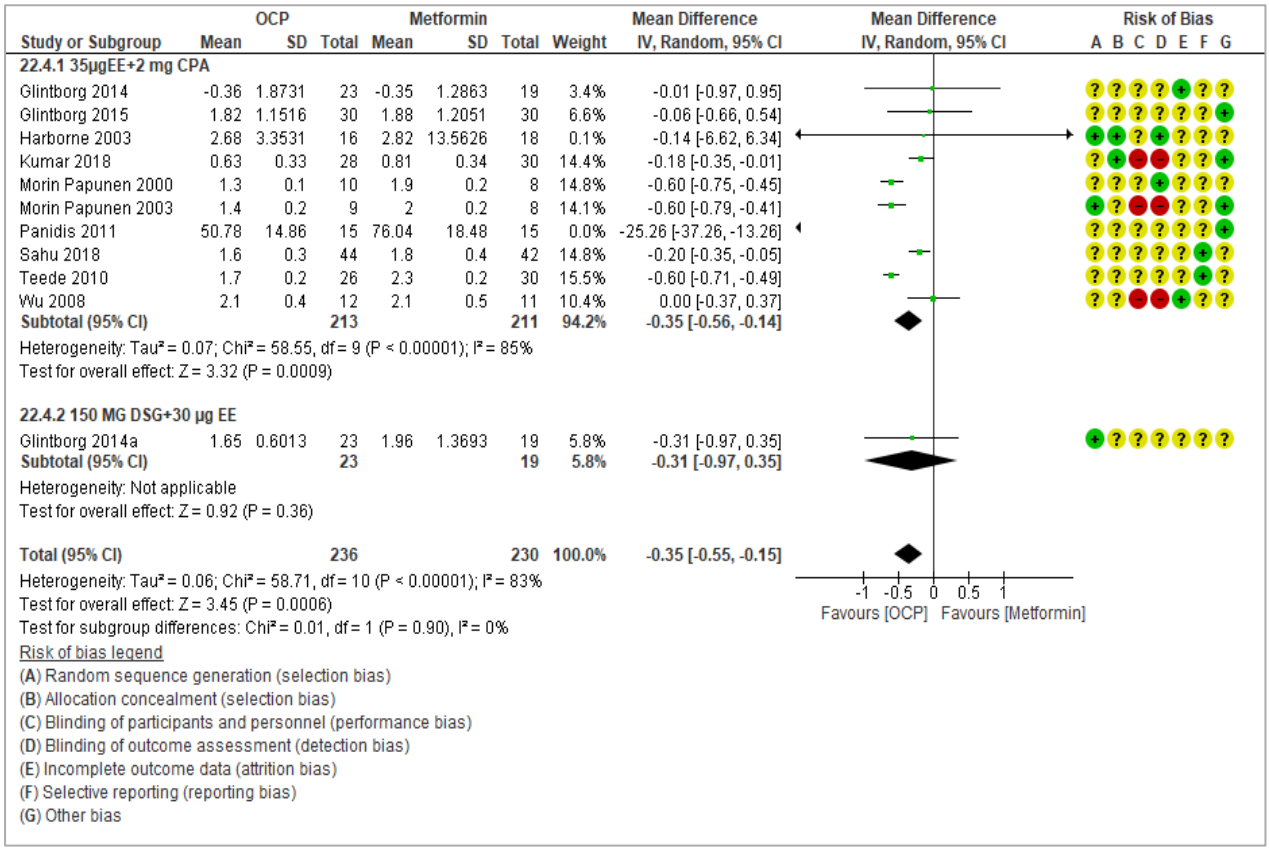
Figure 6-6: Forest plot of Metformin versus Pioglitazone on total testosterone (nmol/L)



6.3.6.5 OCP versus Metformin

In 10 RCTs compared OCP (35 µg EE/2 mg CPA) with metformin, there was a significant reduction in the total testosterone (MD: -0.35 nmol; 95% CI: -0.56, -0.14). Whereas one RCT that compared OCP (150 mg DSG/30 µg EE) with metformin had no effect on the total testosterone (MD: -0.31 nmol; 95%CI: -0.97, 0.35). Overall, OCP therapy significantly reduced the total testosterone when compared with metformin (MD: -0.35 nmol/L; 95% CI: -0.55, -0.15) (Figure 6-7) (very low-grade evidence, table 10).

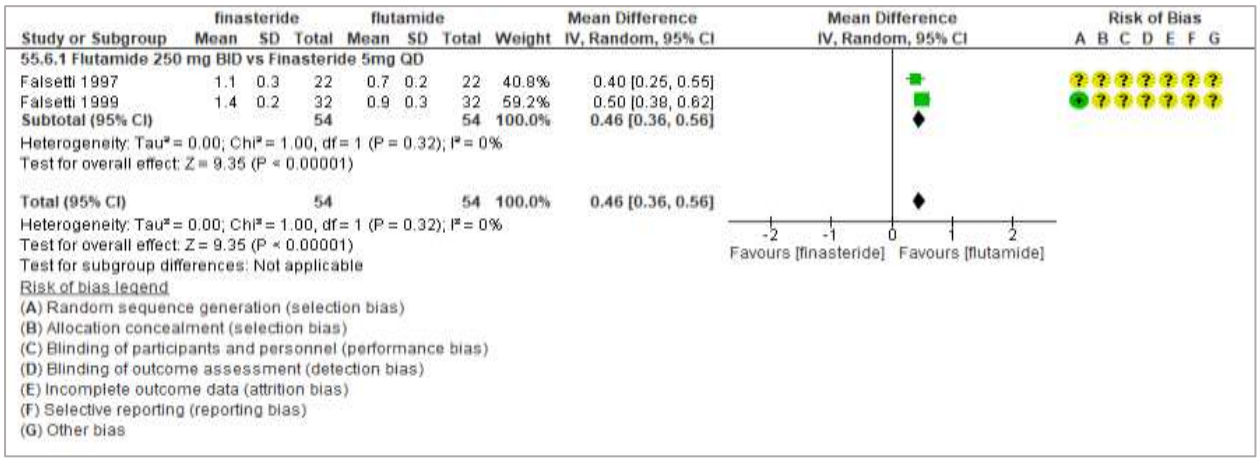
Figure 6-7: Forest plot of OCP versus Metformin on total testosterone (nmol/L)



6.3.6.6 Flutamide versus Finasteride

Two RCTs compared flutamide 250 mg BID with finasteride 5 mg QD and showed a significant increase in the total testosterone (MD: 0.46 nmol/L; 95% CI: -0.36, -0.56) (Figure 6-8) (very low-grade evidence, table 10).

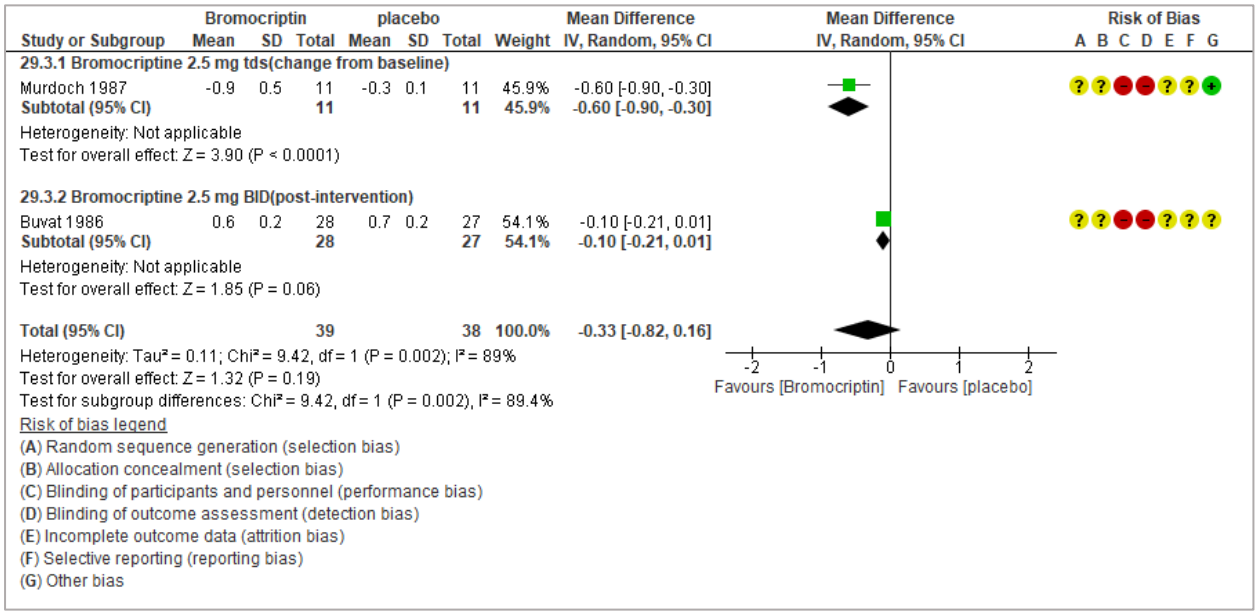
Figure 6-8: Forest plot of Flutamide versus Finasteride on total testosterone (nmol/L)



6.3.6.7 Bromocriptine versus placebo

One RCT compared bromocriptine 2.4 mg TDS with placebo showed a significant reduction in the mean total testosterone (MD: -0.60 nmol/L; 95%CI: -0.90, -0.30). Another RCT showed that bromocriptine 2.5 mg BID showed a significant reduction in the mean total testosterone compared with placebo (MD: -0.10 nmol/L; 95% CI: -0.21, 0.01). Overall, bromocriptine of various dosages has no effect on the mean total testosterone compared with placebo (MD: -0.33 nmol/L; 95%CI: 0.82, 0.16) (Figure 6-9) (very low-grade evidence, table 10).

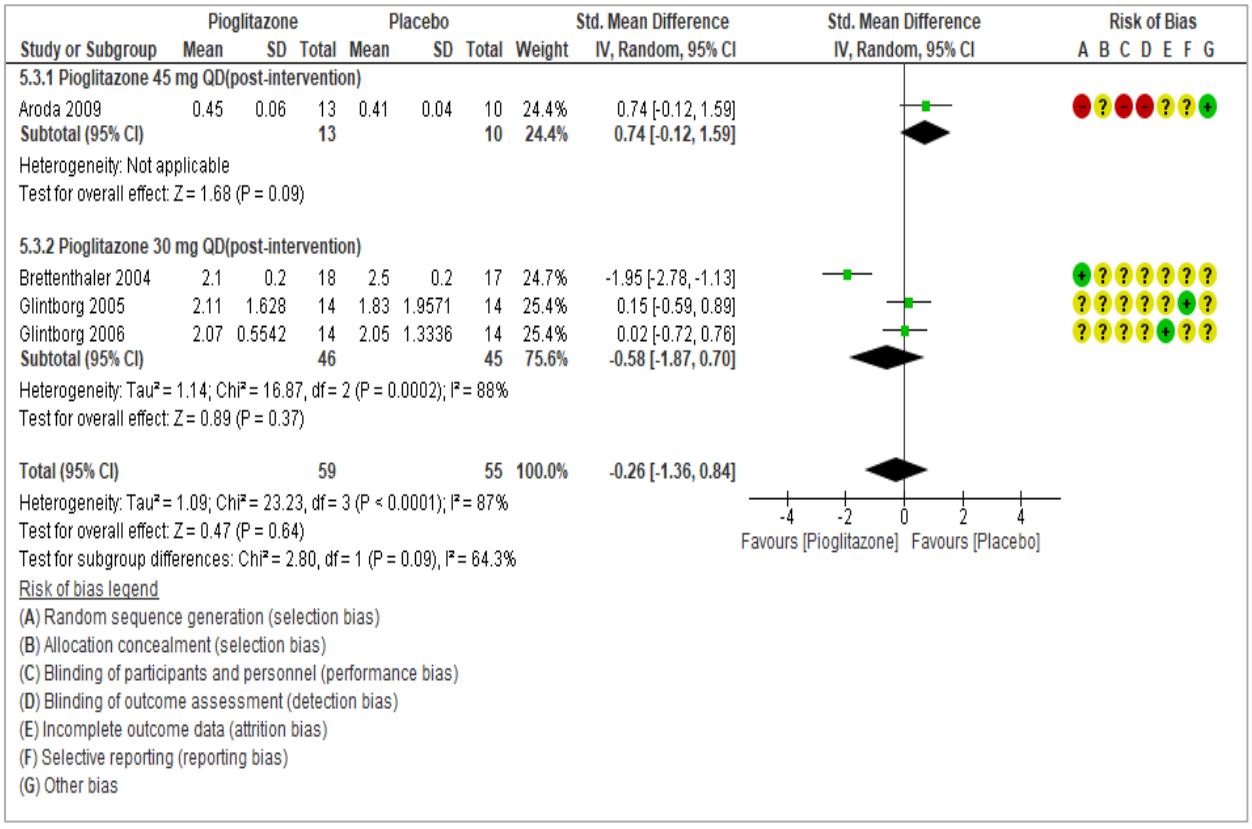
Figure 6-9: Forest plot of Bromocriptine versus placebo on total testosterone (nmol/L)



6.3.6.8 Pioglitazone versus placebo

One RCT compared pioglitazone 45 mg QD with placebo; the post-intervention result showed a significant increase in mean total testosterone. In three RCTs compared pioglitazone 30 mg QD with placebo, the post-intervention results showed no effect on the mean total testosterone. Overall, pioglitazone of various dosages has no effect on the mean total testosterone compared with placebo (SMD: -0.26; 95%CI: -1.36, 0.84) (Figure 6-10) (very low-grade evidence, table 10).

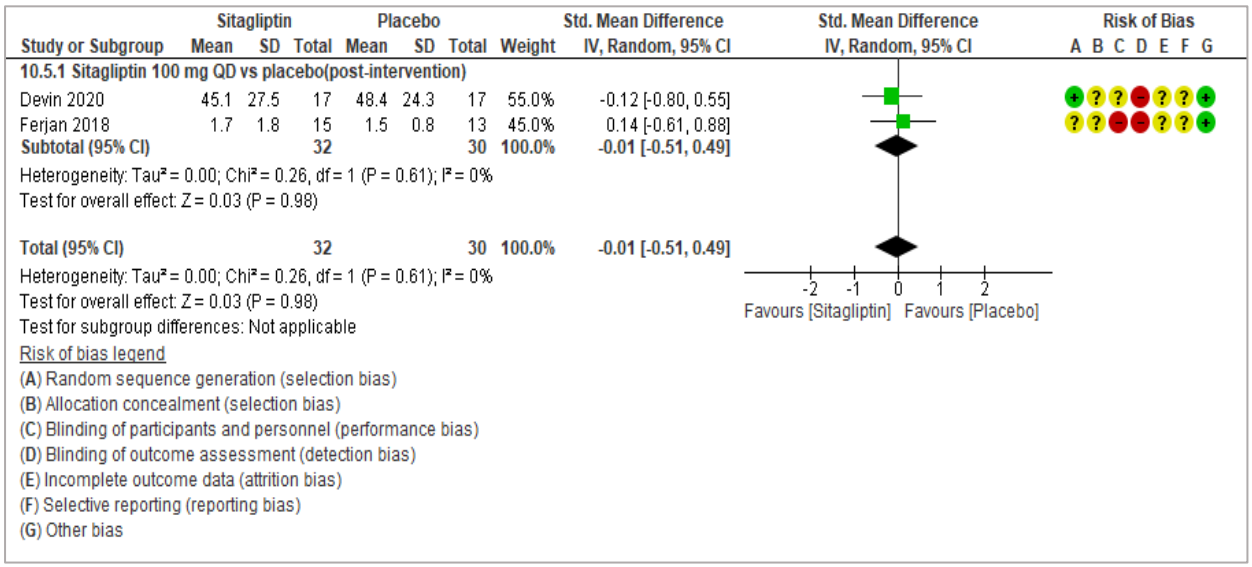
Figure 6-10: Forest plot of Pioglitazone versus placebo on total testosterone



6.3.6.9 Sitagliptin versus placebo

Two RCTs compared sitagliptin 100 mg QD with placebo showed no effect on the mean total testosterone (SMD: -0.01; 95%CI: -0.51, 0.49) (Figure 6-11) (very low-grade evidence, table 10).

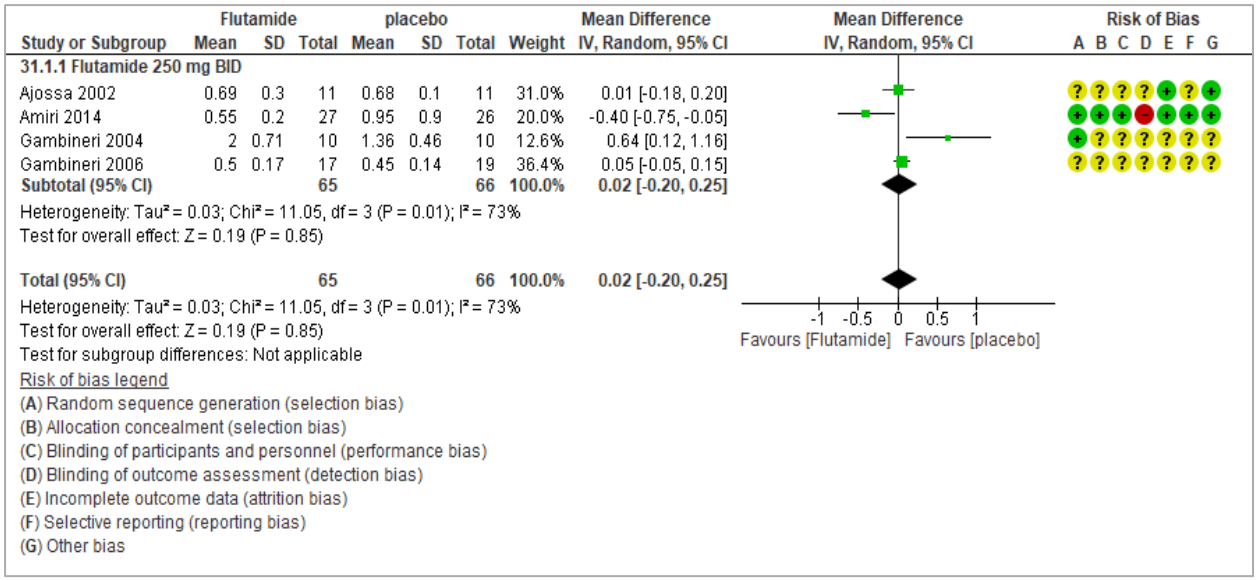
Figure 6-11: Forest plot of Sitagliptin versus placebo on total testosterone



6.3.6.10 Flutamide versus placebo

Four RCTs compared flutamide 250 mg BID with placebo showed no effect on the mean total testosterone (MD: 0.02 nmol/L; 95% CI: -0.20, 0.25) (Figure 6-12) (very low-grade evidence, table 10).

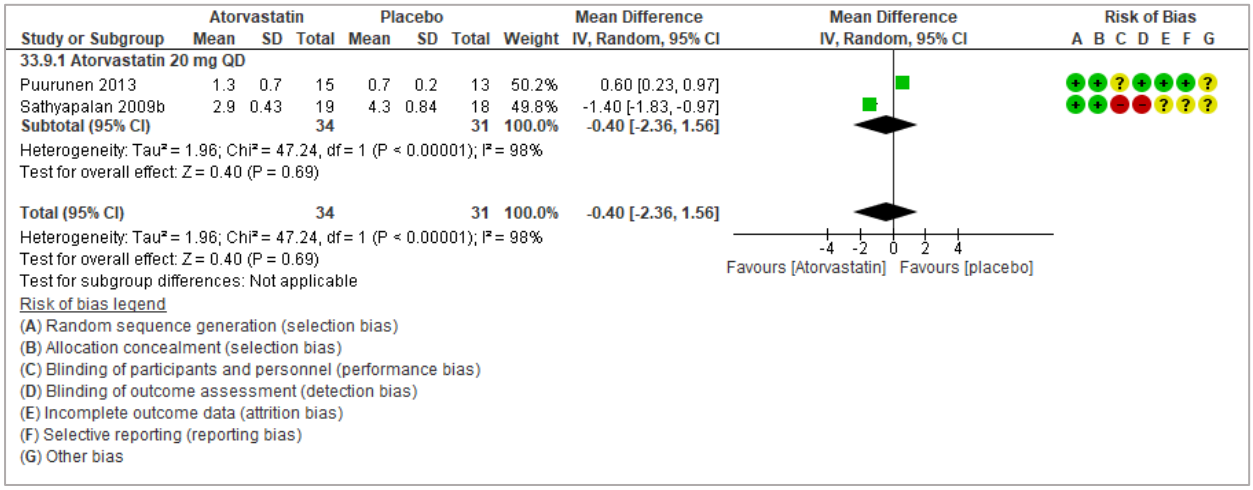
Figure 6-12: Forest plot of Flutamide versus placebo on total testosterone (nmol/L)



6.3.6.11 Atorvastatin versus placebo

Two RCTs compared atorvastatin 20 mg QD with placebo showed no effect on the mean total testosterone (MD: -0.40 nmol/L; 95%CI: -2.36, 1.56) (Figure 6-13) (very low-grade evidence, table 10).

Figure 6-13: Forest plot of Atorvastatin versus placebo on total testosterone (nmol/L)

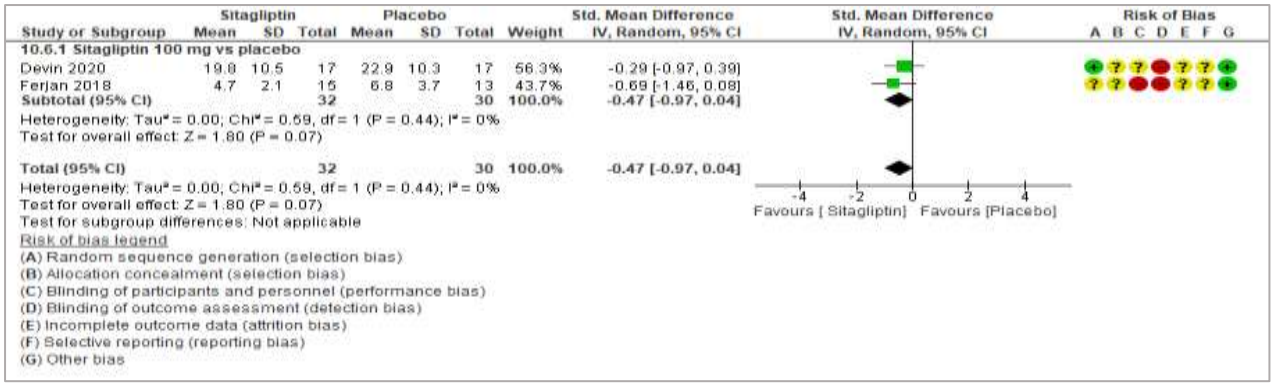


6.3.7 Calculated free testosterone

6.3.7.1 Sitagliptin versus placebo

In two RCTs, sitagliptin 100 mg QD significantly reduced the mean calculated free testosterone when compared with placebo (SMD: -0.47; 95% CI: -0.97, 0.04) (Figure 6-14) (very low-grade evidence, table 10).

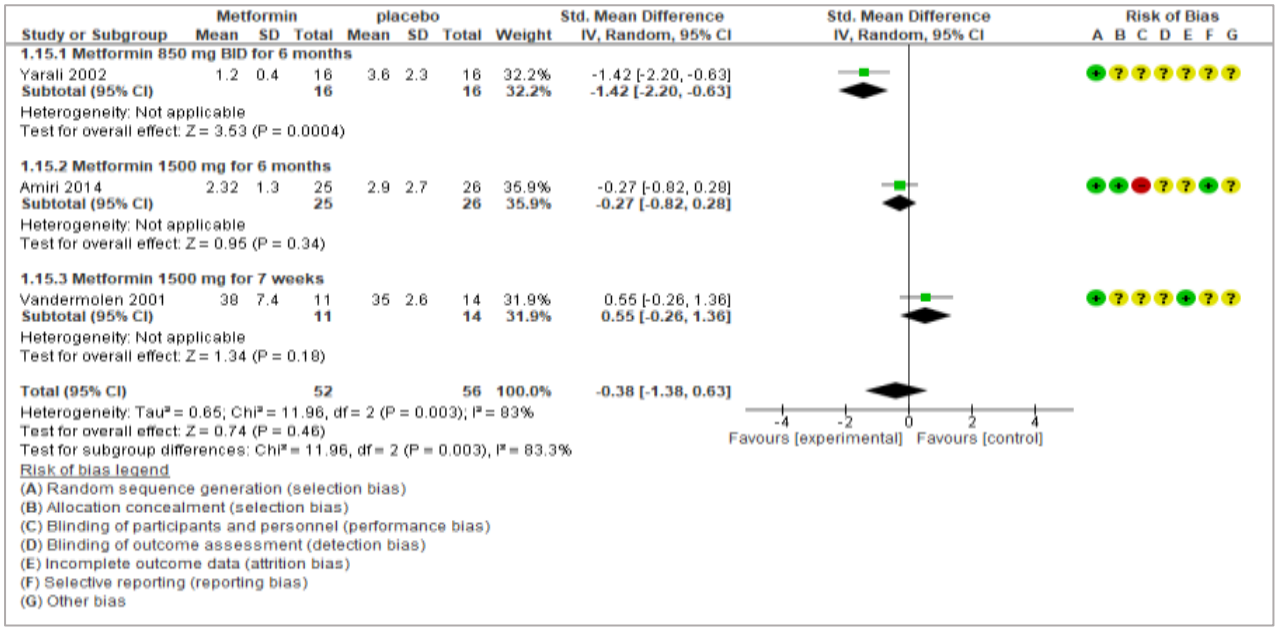
Figure 6-14: Forest plot of Sitagliptin versus placebo on the calculated free testosterone



6.3.7.2 Metformin versus placebo

One RCT compared metformin 850 mg BID for six months with placebo showed a significant reduction in the mean calculated free testosterone. However, two RCTs compared metformin 1500 mg QD for various durations did not affect the mean calculated free testosterone compared with placebo. Overall, metformin of various dosages for various duration showed no effect on the mean calculated free testosterone when compared with placebo (SMD: -0.38; 95%CI: -1.38, 0.63) (Figure 6-15) (very low-grade evidence, table 10).

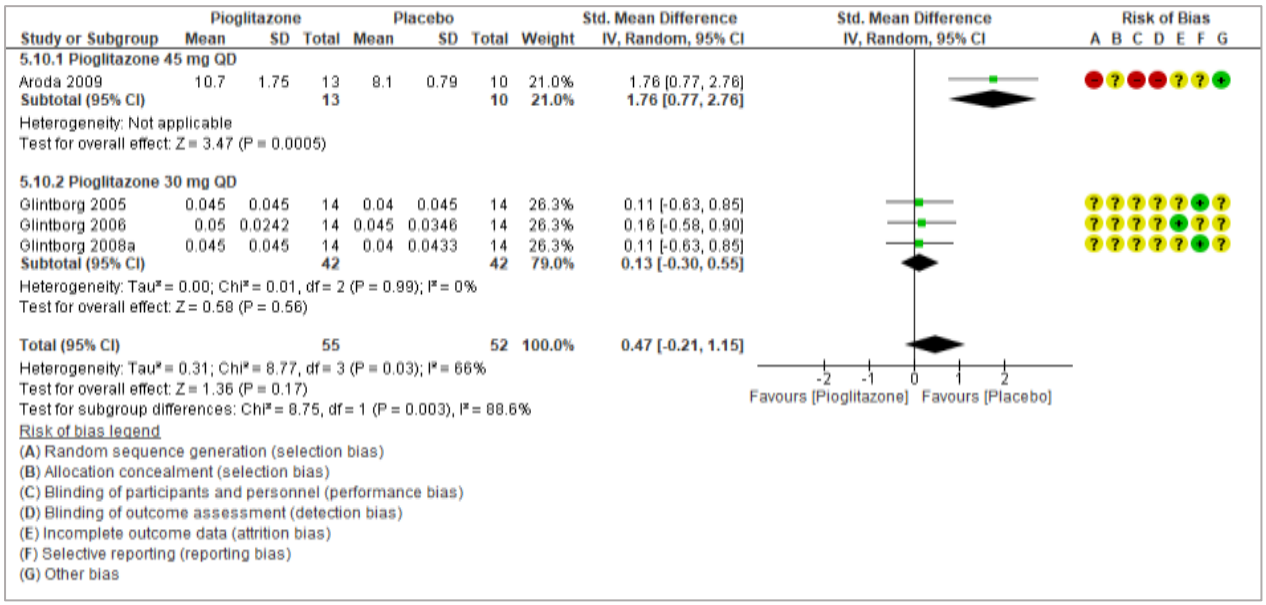
Figure 6-15: Forest plot of Metformin versus placebo on the calculated free testosterone



6.3.7.3 Pioglitazone versus placebo

One RCT compared pioglitazone 45 mg QD with placebo showed a significant increase in the mean calculated free testosterone (SMD: 1.76; 95%CI: 0.77, 2.76). On the other hand, in three RCTs, pioglitazone 30 mg QD with placebo showed no effect on the mean calculated free testosterone (SMD: 0.13; 95%CI: -0.30, 0.55). Overall, pioglitazone of various dosages has no effect on the mean calculated free testosterone compared with placebo (SMD: 0.47; 95% CI: -0.21, 1.15) (Figure 6-16) (very low-grade evidence, table 10).

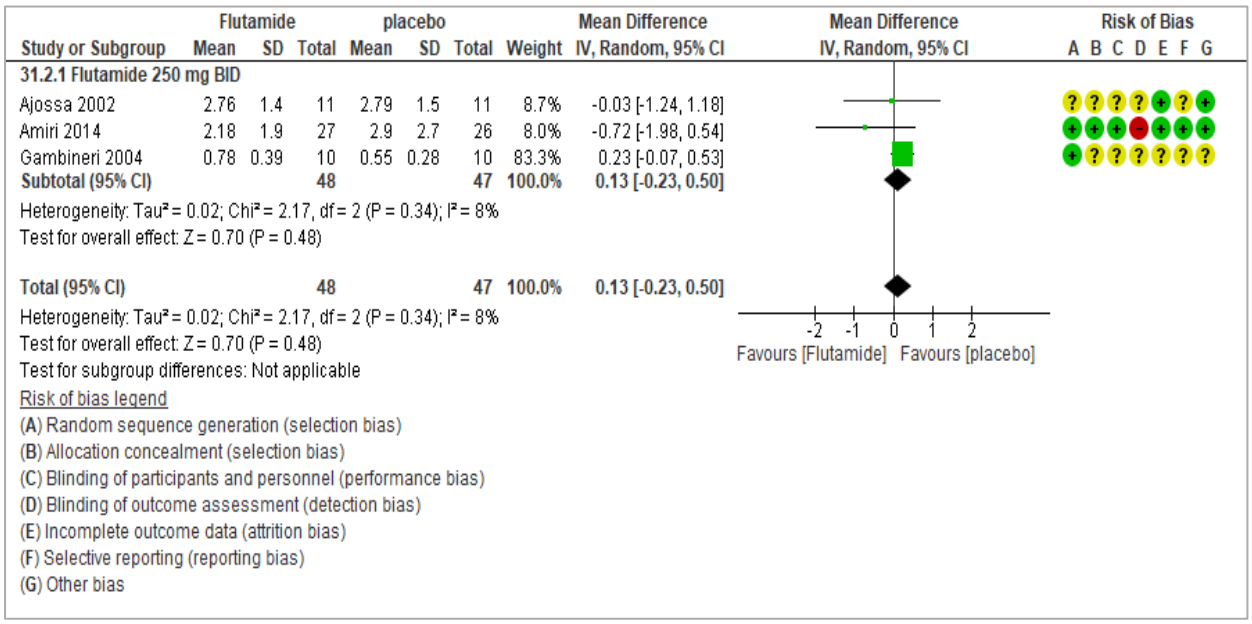
Figure 6-16: Forest plot of Pioglitazone versus placebo on the calculated free testosterone



6.3.7.4 Flutamide versus placebo

Three RCTs compared flutamide 250 mg BID with placebo showed no effect on the mean calculated free testosterone (MD: 0.13 pmol/L; 95% CI: -0.23, 0.50) (Figure 6-17) (very low-grade evidence, table 10).

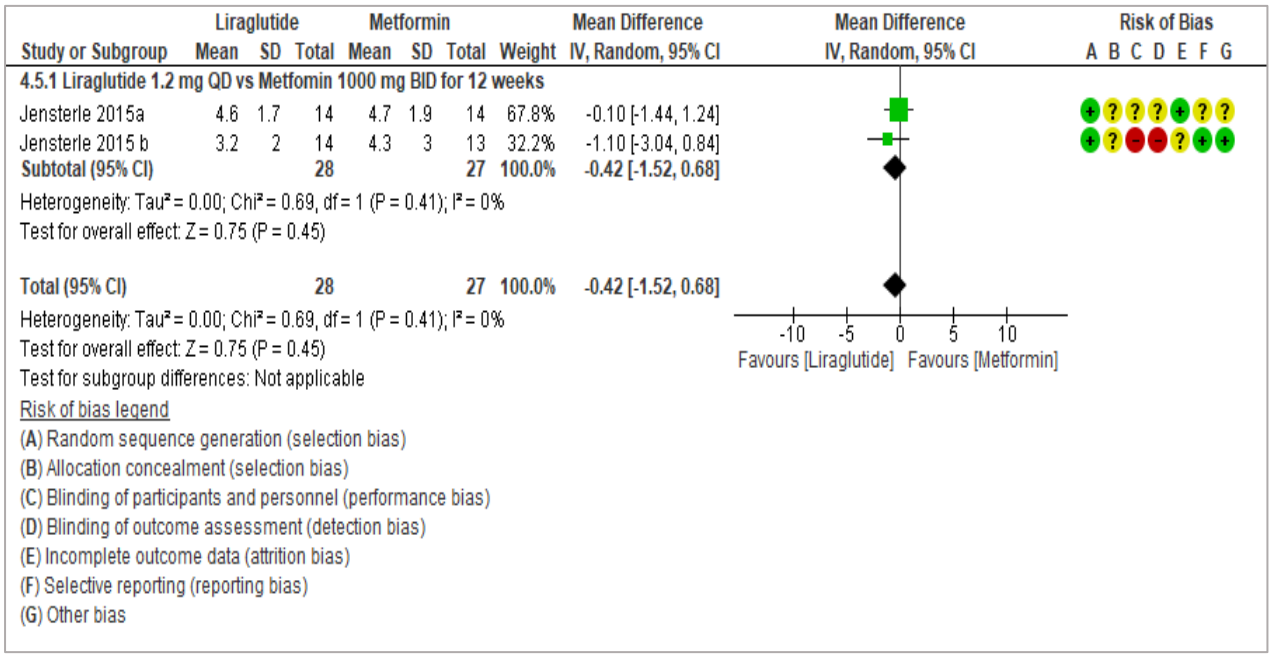
Figure 6-17: Forest plot of Flutamide versus placebo on the calculated free testosterone (pmol/L)



6.3.7.5 Liraglutide versus Metformin

Two RCTs compared liraglutide 1.2 mg QD with metformin 1000 mg BID for 12 weeks showed no effect on the mean calculated free testosterone (MD: -0.42 pmol/L; 95%CI: -1.52, 0.68) (Figure 6-18) (very low-grade evidence, table 10).

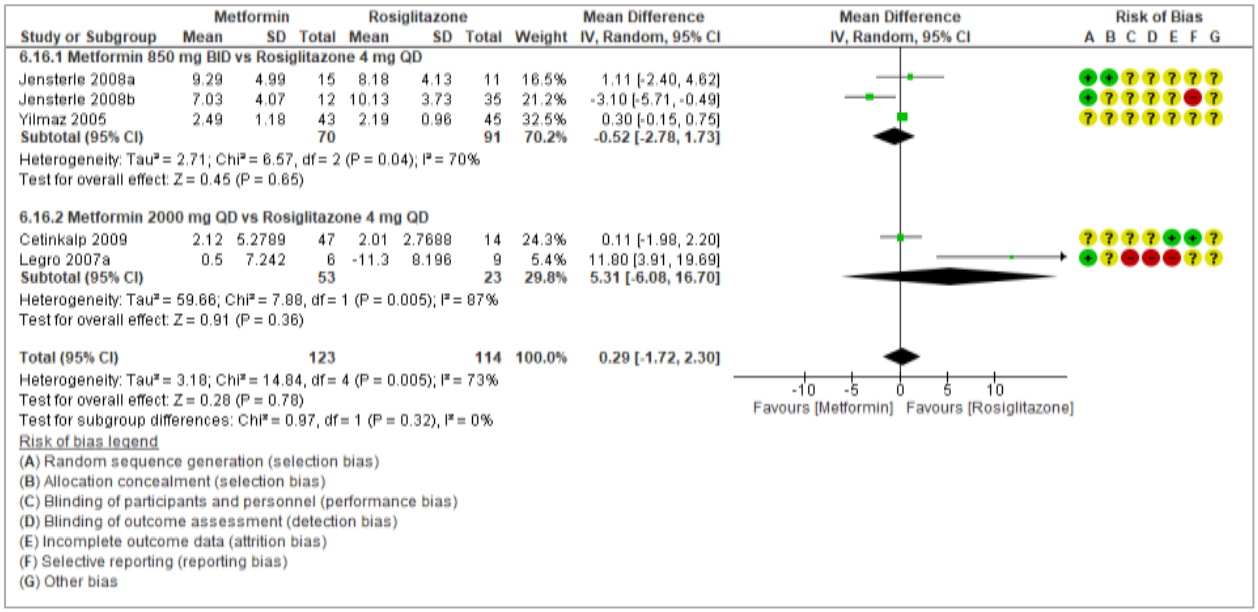
Figure 6-18: Forest plot of Liraglutide versus Metformin on the calculated free testosterone (pmol/L)



6.3.7.6 Metformin versus Rosiglitazone

Three RCTs compared metformin 850 mg BID with rosiglitazone 4 mg QD showed no effect on the mean calculated free testosterone (MD: -0.52 pmol/L; 95% CI: -2.78, 1.73). Likewise, two RCTs compared metformin 2000 mg with rosiglitazone 4 mg QD showed no effect on the mean calculated free testosterone (MD: 5.31 pmol/L; 95% CI: 6.08, 16.70). Overall, metformin of various dosages compared with rosiglitazone 4 mg has no effect on the mean calculated free testosterone (MD: 0.29 pmol/L; 95%CI: -1.72, 2.30) (Figure 6-19) (very low-grade evidence, table 10).

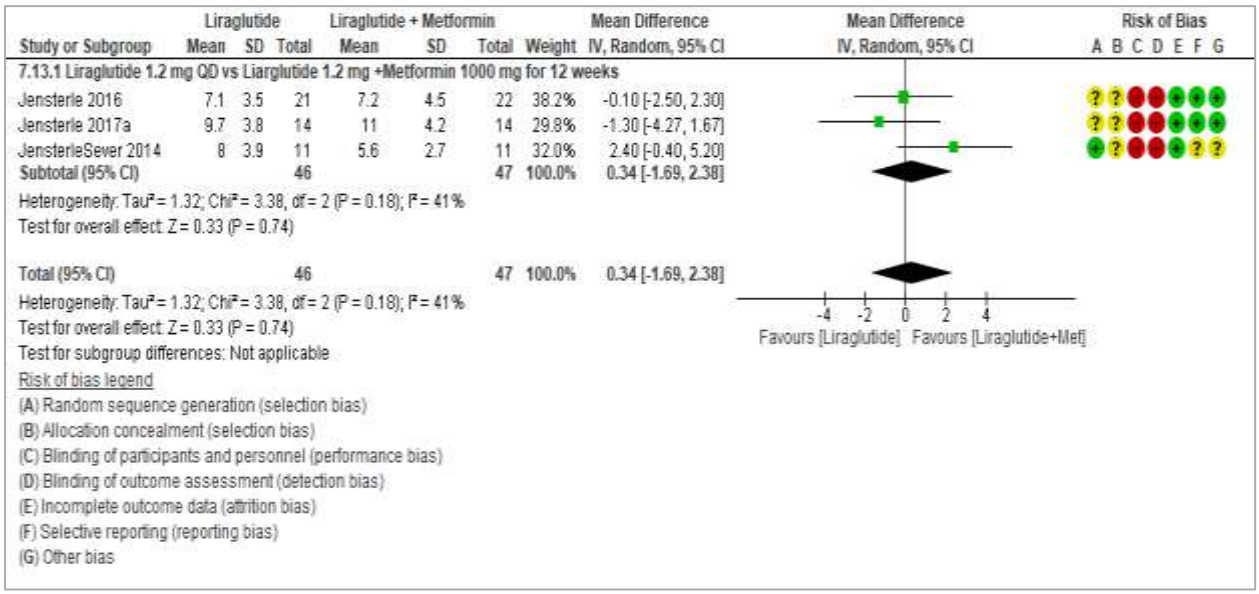
Figure 6-19: Forest plot of Metformin versus Rosiglitazone on the calculated free testosterone (pmol/L)



6.3.7.7 Liraglutide versus Liraglutide + Metformin

Three RCTs compared liraglutide 1.2 mg QD with liraglutide 1.2 mg QD added to metformin 100 mg QD for 12 weeks showed no effect on the mean calculated free testosterone (MD: 0.34 pmol/L; 95%CI: - 1.69, 2.38) (Figure 6-20) (very low-grade evidence, table 10).

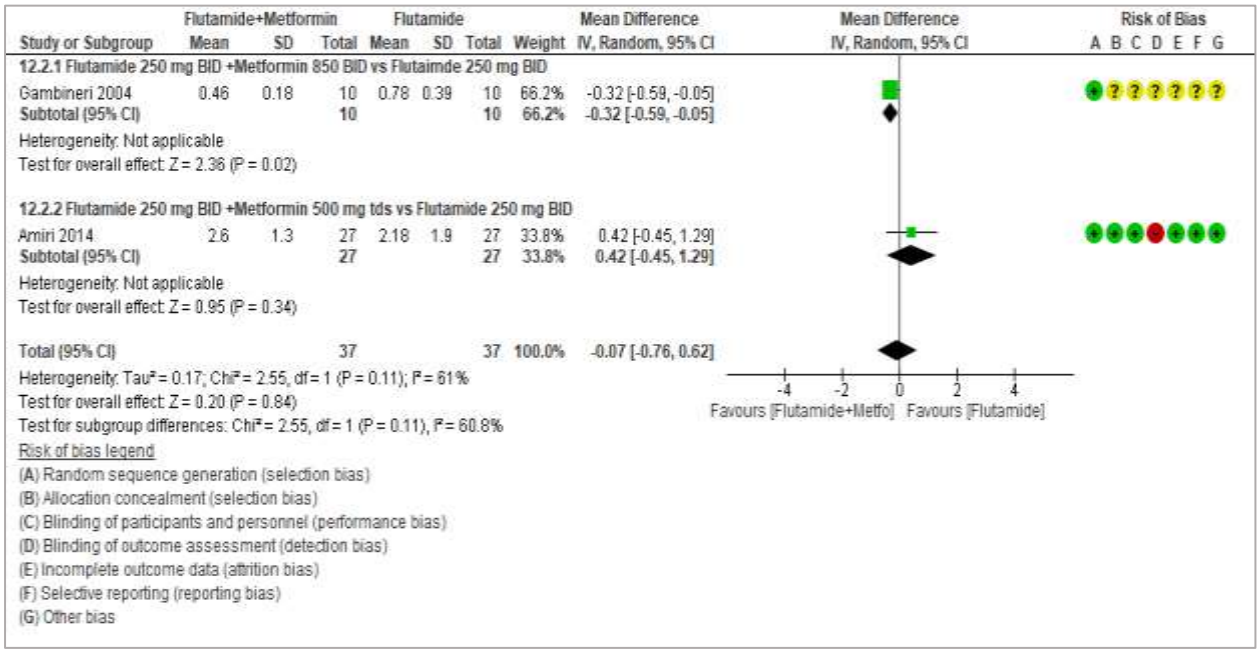
Figure 6-20: Forest plot of Liraglutide versus Liraglutide + Metformin on the calculated free testosterone (pmol/L)



6.3.7.8 Flutamide + Metformin versus Flutamide

One RCT compared flutamide 250 mg BID added to metformin 850 mg BID with flutamide 250 mg BID alone showed a significant effect on the mean calculated free testosterone (MD: -0.32 pmol/L; 95% CI: -0.59, -0.05). However, another RCT compared flutamide 250 mg BID added to metformin 500 mg TDS with flutamide 250 mg showed no effect on the mean calculated free testosterone (MD: 0.42 pmol/L;95% CI: -0.45, 1.29). Overall, flutamide 250 mg BID added to metformin of various dosages and compared with flutamide alone has no effect on the mean calculated free testosterone (MD: -0.07 pmol/L; 95% CI: -0.76, 0.62) (Figure 6-21) (very low-grade evidence, table 10).

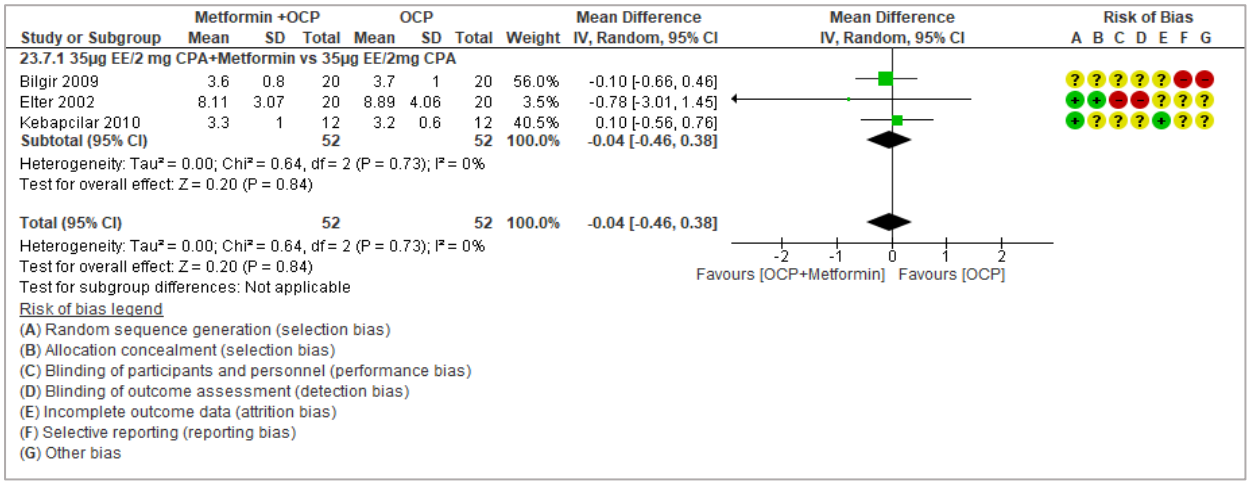
Figure 6-21: Forest plot of Flutamide+ Metformin versus Flutamide on the calculated free testosterone (pmol/L)



6.3.7.9 Metformin + OCP versus OCP

In three RCTs, metformin added to (EE 35µg/2 CPA) and compared with (EE 35µg/2 CPA) alone showed no effect on the mean calculated free testosterone (MD: -0.04 pmol/L; 95%CI: -0.46, 0.38) (Figure 6-22) (very low-grade evidence, table 10).

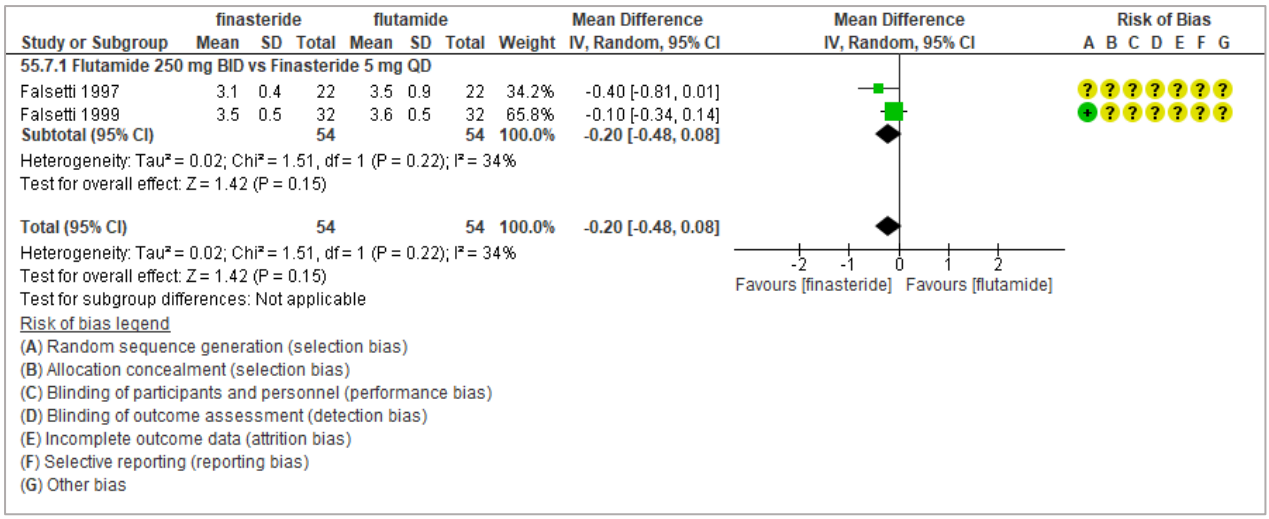
Figure 6-22: Forest plot of Metformin + OCP versus OCP on the calculated free testosterone (pmol/L)



6.3.7.10 Finasteride versus Flutamide

Two RCTs compared finasteride 5 mg QD with flutamide 250 mg BID showed no effect on the mean calculated free testosterone (MD: -0.20 pmol/L; 95%CI: -0.48, 0.08) (Figure 6-23) (very low-grade evidence, table 10).

Figure 6-23: Forest plot of Finasteride versus Flutamide on the calculated free testosterone (pmol/L)

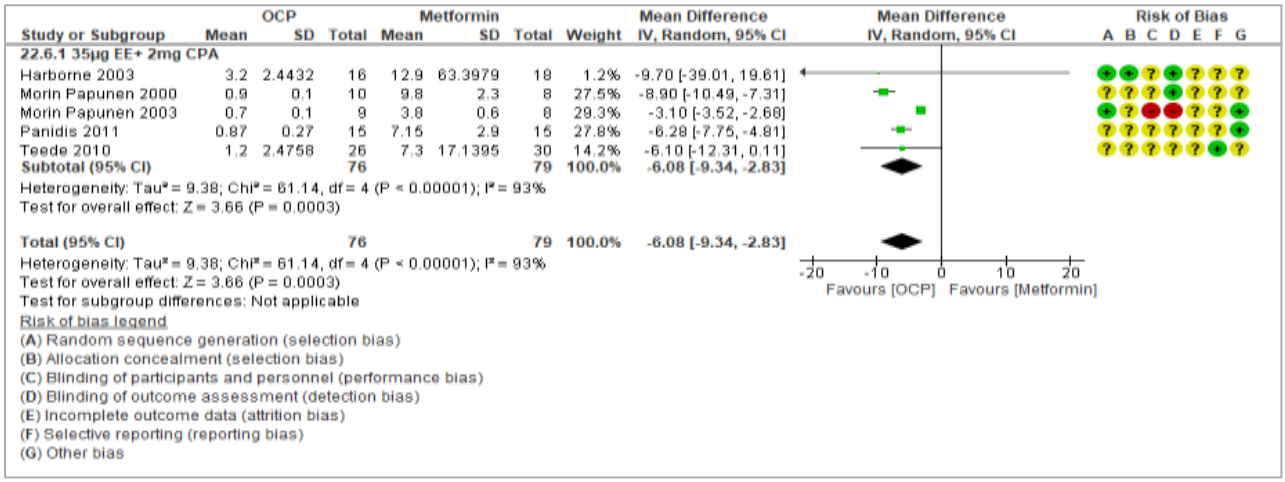


6.3.8 Free androgen index (FAI)

6.3.8.1 OCP versus Metformin

Five RCTs compared OCP (35 µg EE/2 mg CPA) with metformin showed a significant reduction in the mean FAI (MD: -6.68; 95% CI: -9.34, -2.83) (Figure 6-24) (very low-grade evidence, table 10).

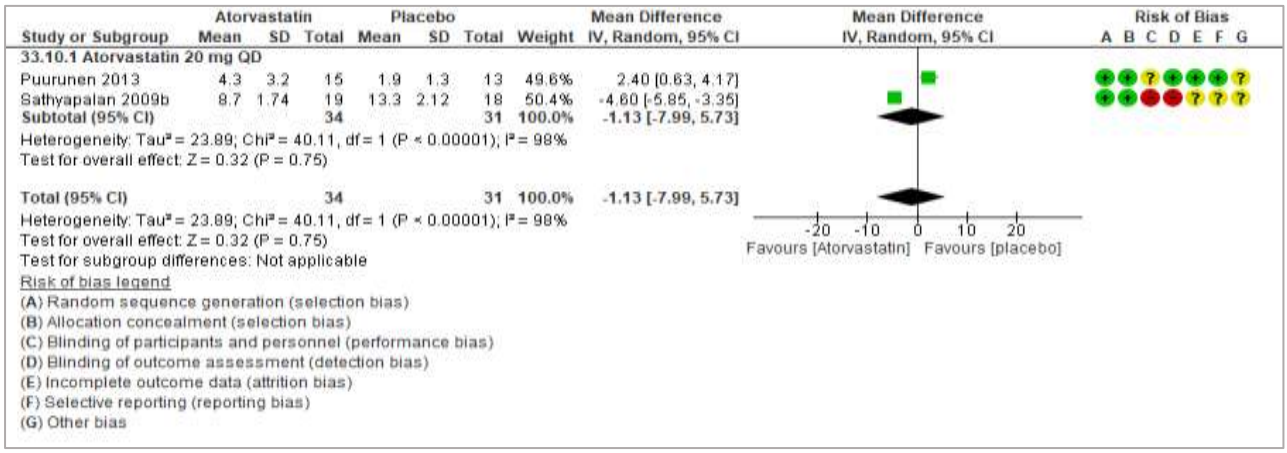
Figure 6-24: Forest plot of OCP versus Metformin on the FAI



6.3.8.2 Atorvastatin versus placebo

Two RCTs compared atorvastatin 20 mg QD with placebo showed no effect on the mean FAI (MD: -1.13; 95% CI: -7.99, 5.73) (Figure 6-25) (very low-grade evidence, table 10).

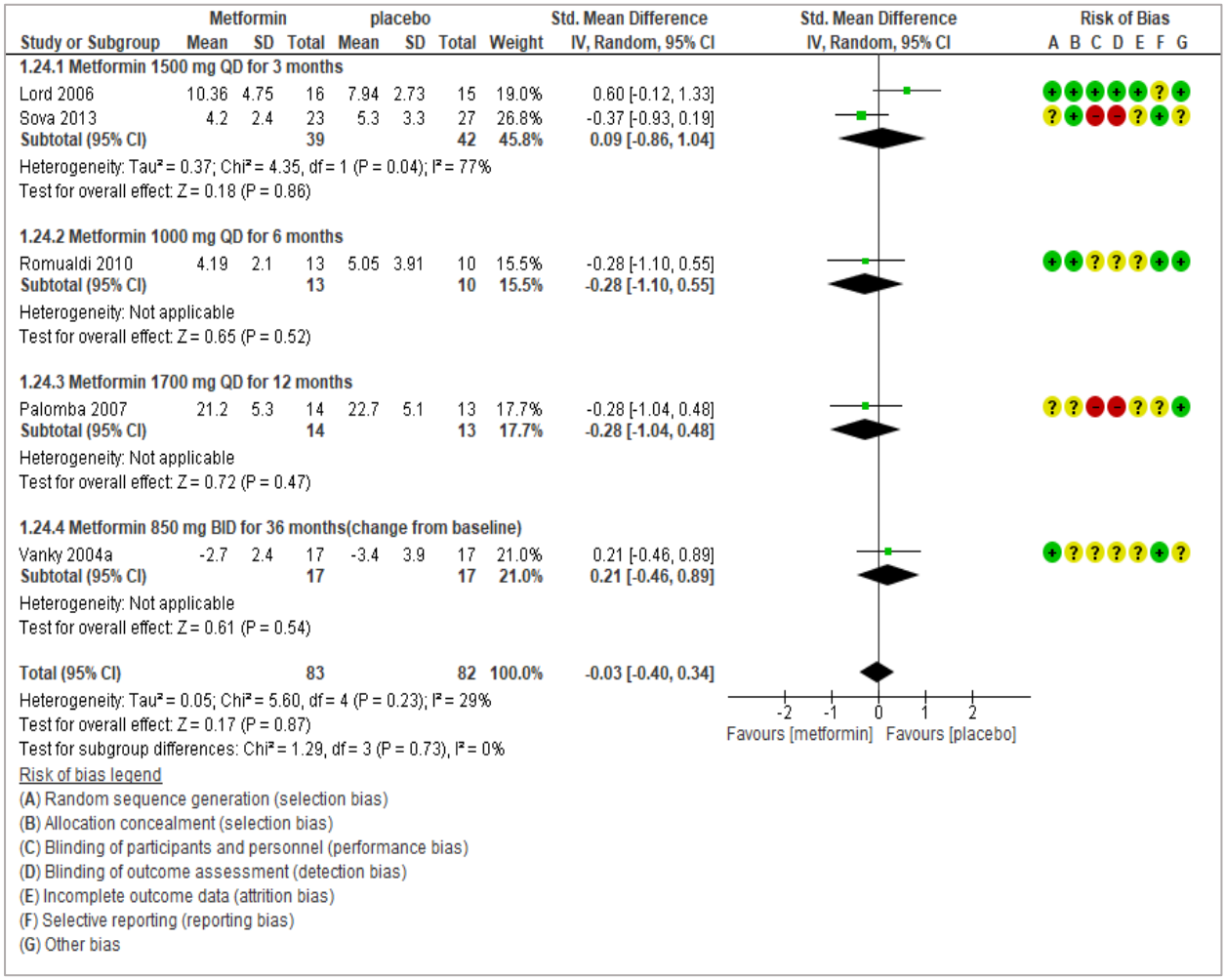
Figure 6-25: Forest plot of Atorvastatin versus placebo on the FAI



6.3.8.3 Metformin versus placebo

In five RCTs compared metformin of various dosages and for various durations with placebo showed no effect on the mean FAI (SMD: -0.03; 95% CI: -0.40, -0.34) (Figure 6-26) (very low-grade evidence, table 10).

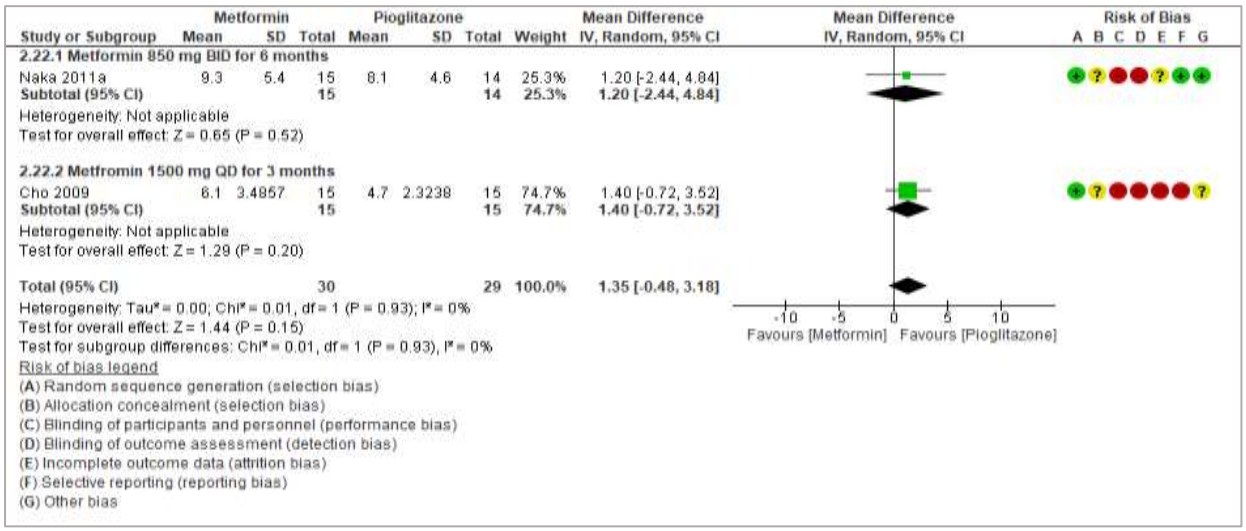
Figure 6-26: Forest plot Metformin versus placebo on the FAI



6.3.8.4 Metformin versus Pioglitazone

Two RCTs compared metformin of various dosages and for the various duration with pioglitazone showed no effect on the mean FAI (MD: 1.35; 95% CI: -0.48, 3.18) (Figure 6-27) (very low-grade evidence, table 10).

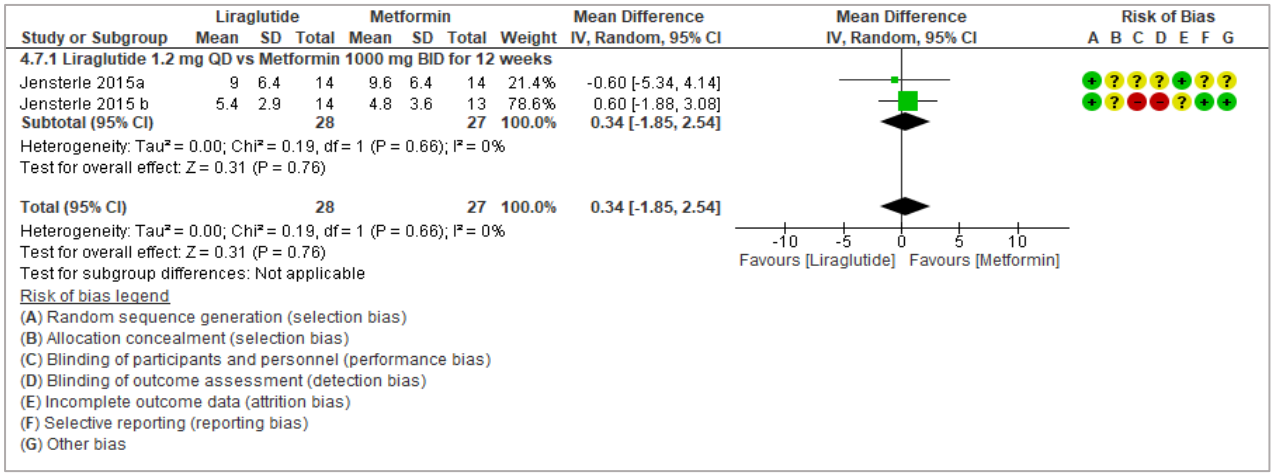
Figure 6-27: Forest plot Metformin versus Pioglitazone on the FAI



6.3.8.5 Liraglutide versus Metformin

Two RCTs compared liraglutide 1.2 mg QD with metformin 1000 mg BID for 12 weeks showed no effect on the mean FAI (MD: 0.34; 95%CI: -1.85, 2.54) (Figure 6-28) (very low-grade evidence, table 10).

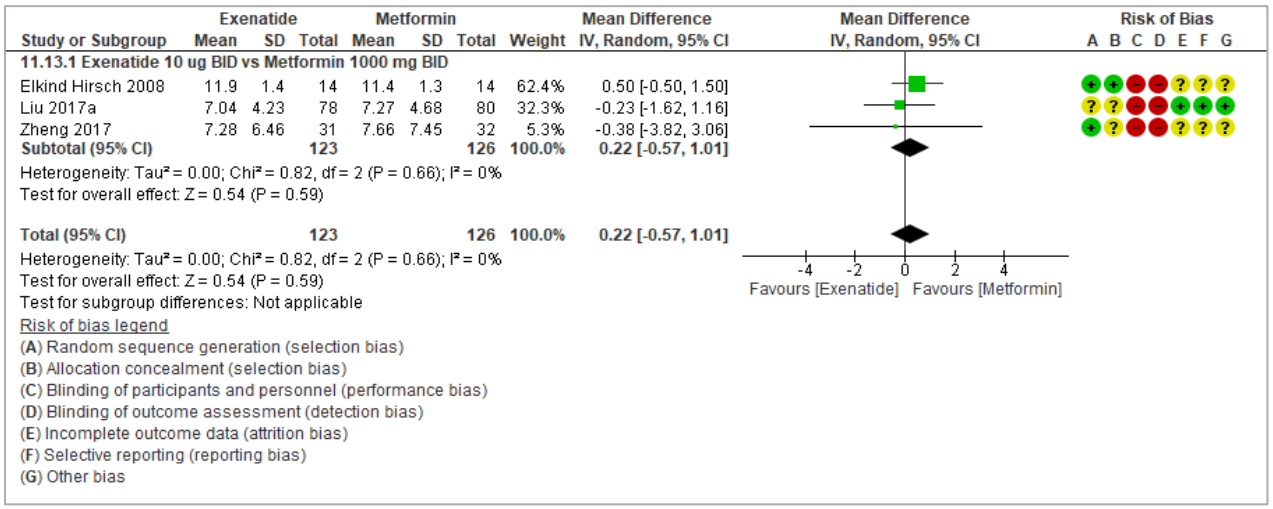
Figure 6-28: Forest plot Liraglutide versus Metformin on the FAI



6.3.8.6 Exenatide versus Metformin

Three RCTs compared exenatide 10 µg BID with metformin 1000 mg BID showed no effect on the mean FAI (MD: 0.22; 95% CI: -0.57,1.01) (Figure 6-29) (very low-grade evidence, table 10).

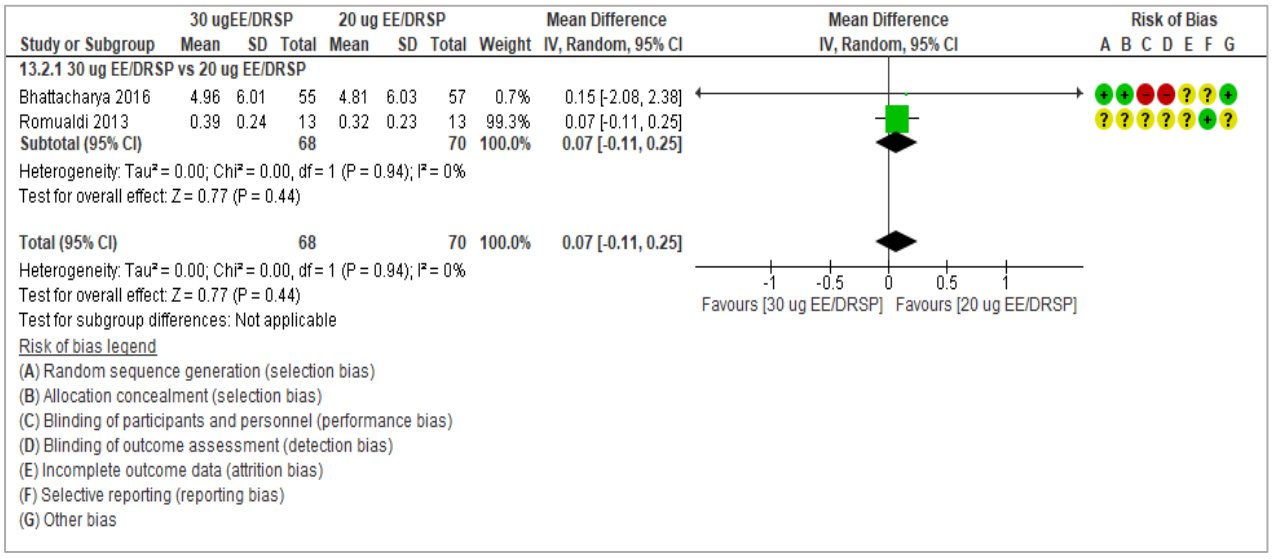
Figure 6-29: Forest plot Exenatide versus Metformin on the FAI



6.3.8.7 OCP (30µg EE/2 mg CPA) versus OCP (20µg EE/DRSP)

Two RCTs compared OCP (30µg EE/2 mg CPA) with OCP (20µg EE/DRSP) showed no effect on the mean FAI (MD: 0.07; 95% CI: -0.11, 0.25)(Figure 6-30) (very low-grade evidence, table 10).

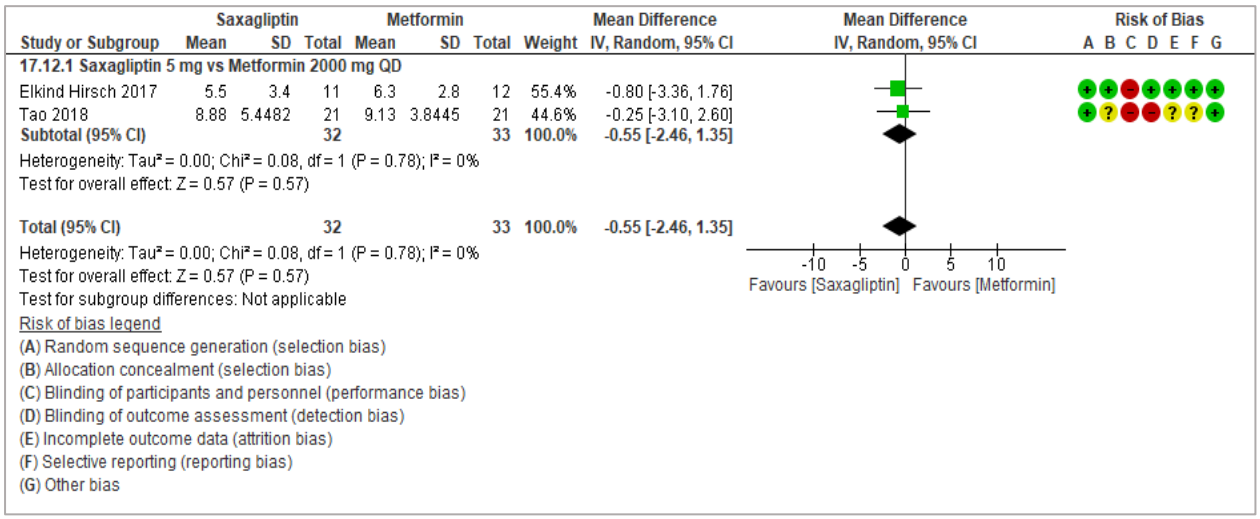
Figure 6-30: Forest plot of OCP (30µ9 EE/2 mg CPA) versus OCP (20µg EE/DRSP) on the FAI



6.3.8.8 Saxagliptin versus Metformin

Two RCTs compared saxagliptin 5 mg QD with metformin 2000 mg QD showed no effect on the mean FAI (MD:-0.55; 95% CI:-2.46, 1.35) (Figure 6-31) (very low-grade evidence, table 10).

Figure 6-31: Forest plot of Saxagliptin versus Metformin on the FAI

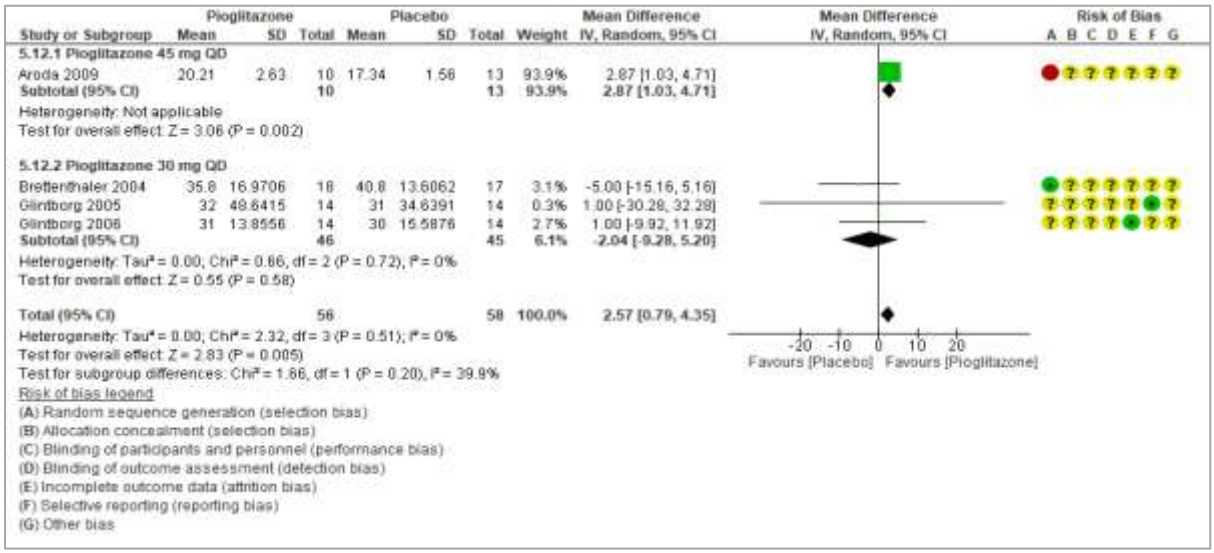


6.3.9 Sex hormone-binding globulin (SHBG)

6.3.9.1 Pioglitazone versus placebo

In one RCT, pioglitazone 45 mg QD significantly increased SHBG by 2.87 nmol/L (95% CI: 1.03, 4.71) compared with placebo, while in three RCTs, pioglitazone 30 mg QD had no effect on the SHBG compared with placebo (MD:-2.04 nmol/L; 95% CI: -9.28,5.20). Overall, pioglitazone at various dosages significantly increased SHBG compared with placebo (MD: 2.57 nmol/L; 95% CI: 0.79,4.35) (Figure 6-32) (very low-grade evidence, table 10).

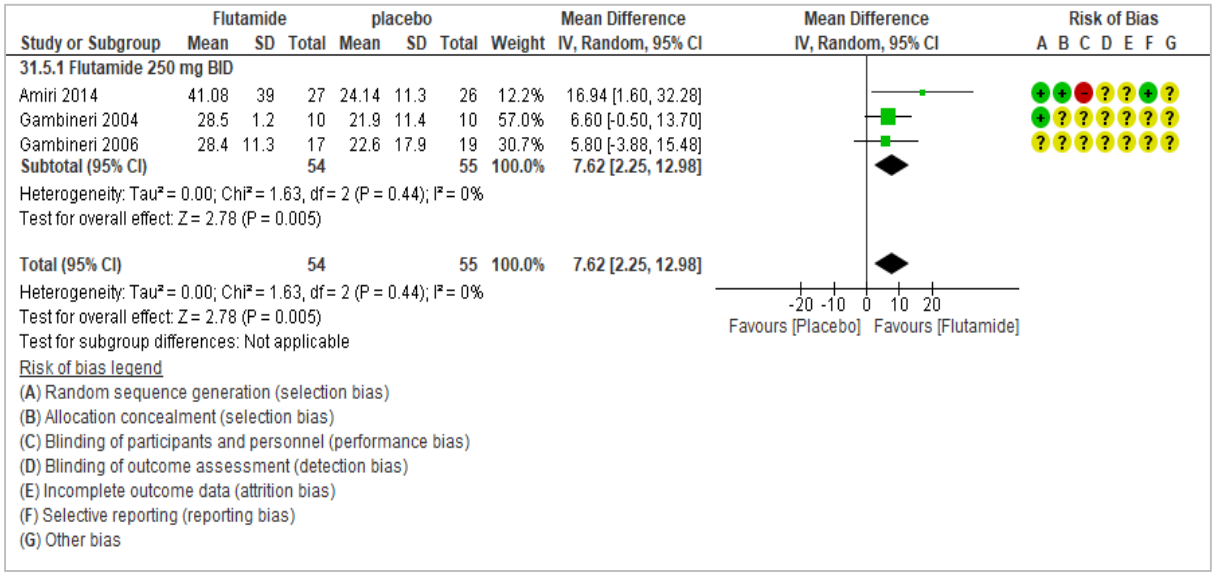
Figure 6-32: Forest plot of Pioglitazone versus placebo on SHBG (nmol/L)



6.3.9.2 Flutamide versus placebo

Three RCTs compared flutamide 250 mg BID with placebo that showed a significant increase in the level of SHBG (MD: 7.62 nmol/L; 95% CI: 2.25, 12.98) (Figure 6-33) (very low-grade evidence, table 10).

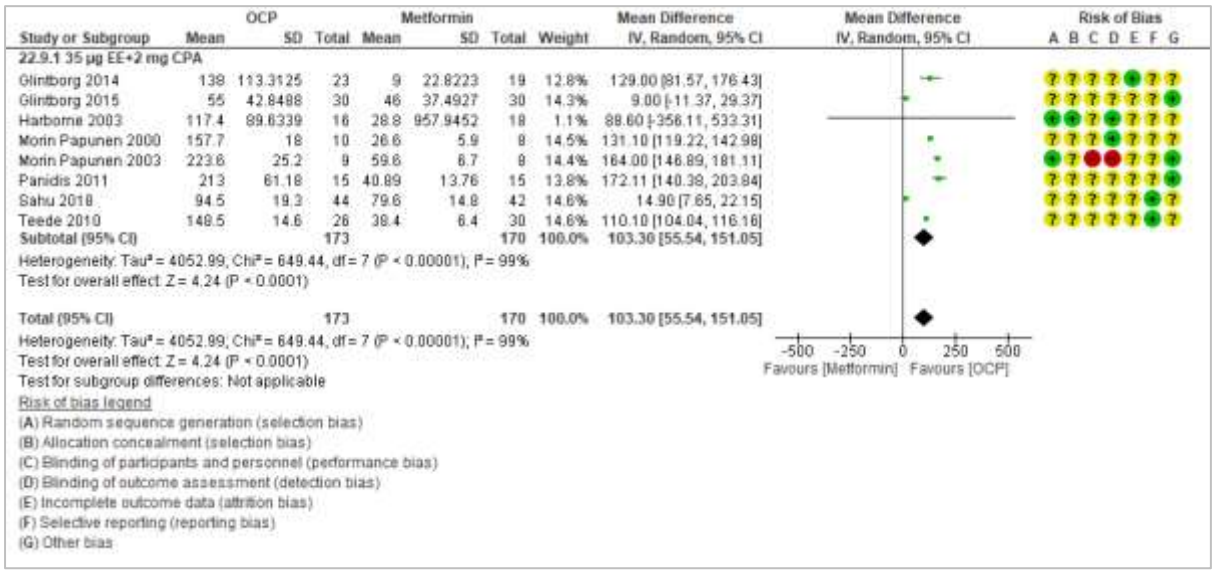
Figure 6-33: Forest plot of Flutamide versus placebo on the SHBG (nmol/L)



6.3.9.3 OCP versus Metformin

Eight RCTs compared OCP (35 µg EE/2 mg CPA) with metformin showed a significant increase in the level of SHBG (MD: 103.30 nmol/L; 95% CI: 55.54, 151.05) (Figure 6-34) (very low-grade evidence, table 10).

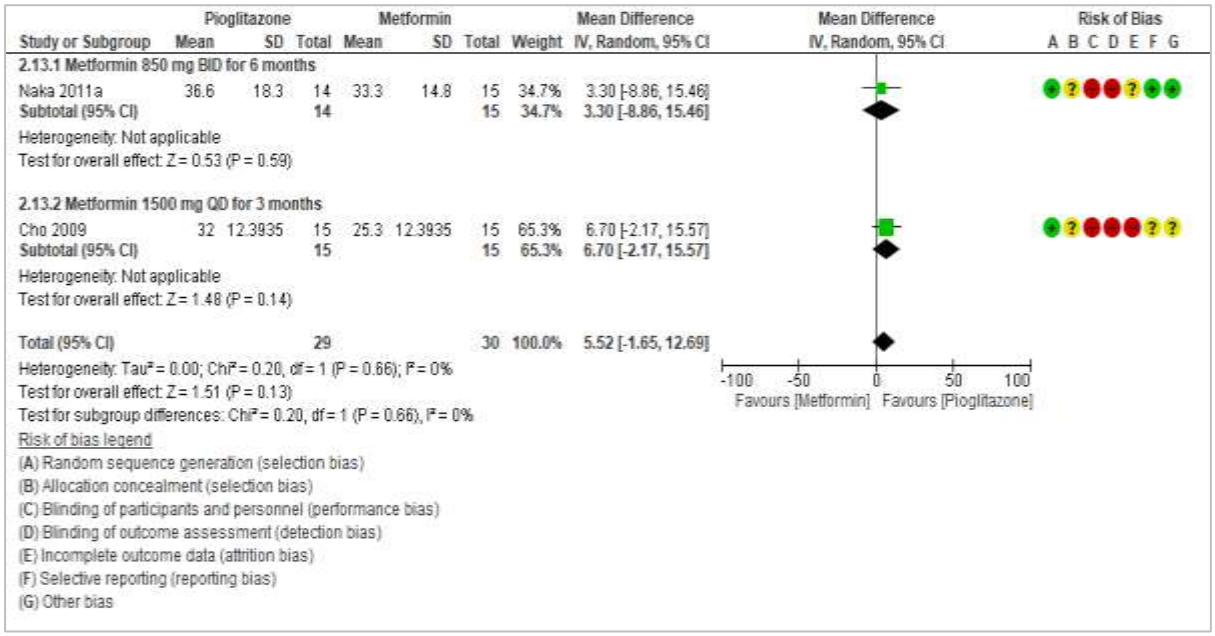
Figure 6-34: Forest plot of OCP versus Metformin on the SHBG (nmol/L)



6.3.9.4 Metformin versus Pioglitazone

Two RCTs compared metformin of various dosages and for the various duration with pioglitazone showed no effect on the SHBG (MD: 5.52 nmol/L; 95% CI: -1.65, 12.69) (Figure 6-35) (very low-grade evidence, table 10).

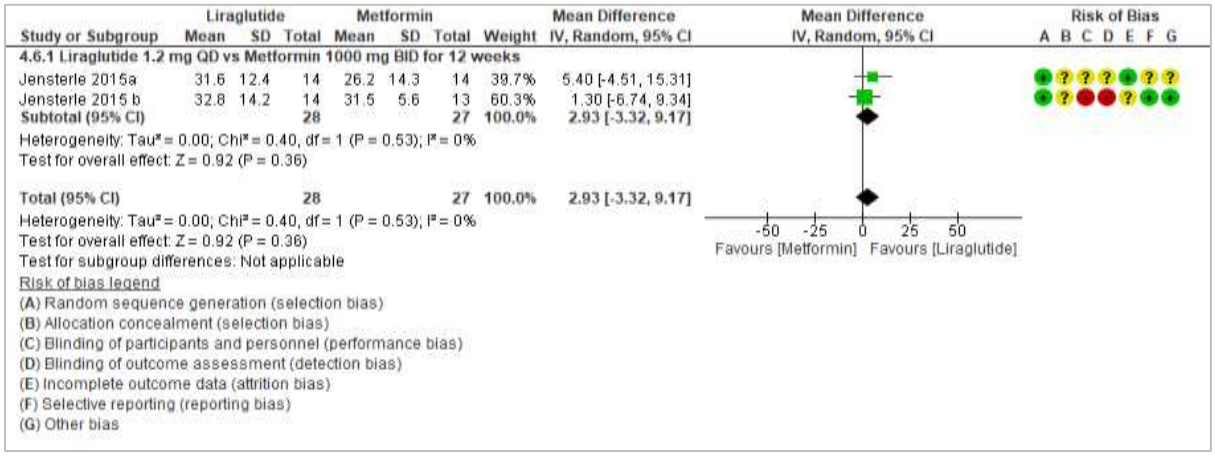
Figure 6-35: Forest plot of Metformin versus Pioglitazone on the SHBG (nmol/L)



6.3.9.5 Liraglutide versus Metformin

Two RCTs compared liraglutide 1.2 mg QD with metformin 1000 mg BID for 12 weeks showed no effect on the SHBG (MD: 2.93 nmol/L; 95% CI: -3.32, 9.17) (Figure 6-36) (very low-grade evidence, table 10).

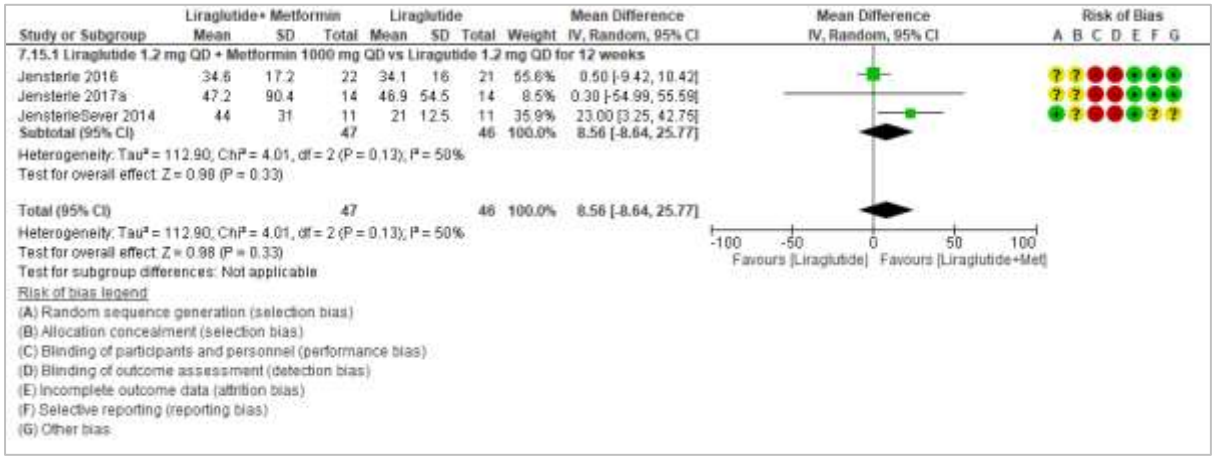
Figure 6-36: Forest plot of Liraglutide versus Metformin on the SHBG (nmol/L)



6.3.9.6 Liraglutide versus Liraglutide + Metformin

Three RCTs compared liraglutide 1.2 mg QD with liraglutide 1.2 mg QD added to metformin 1000 mg BID for 12 weeks showed no effect on the SHBG (MD: 8.56 nmol/L; 95% CI: -8.64, 25.77) (Figure 6-37) (very low-grade evidence, table 10).

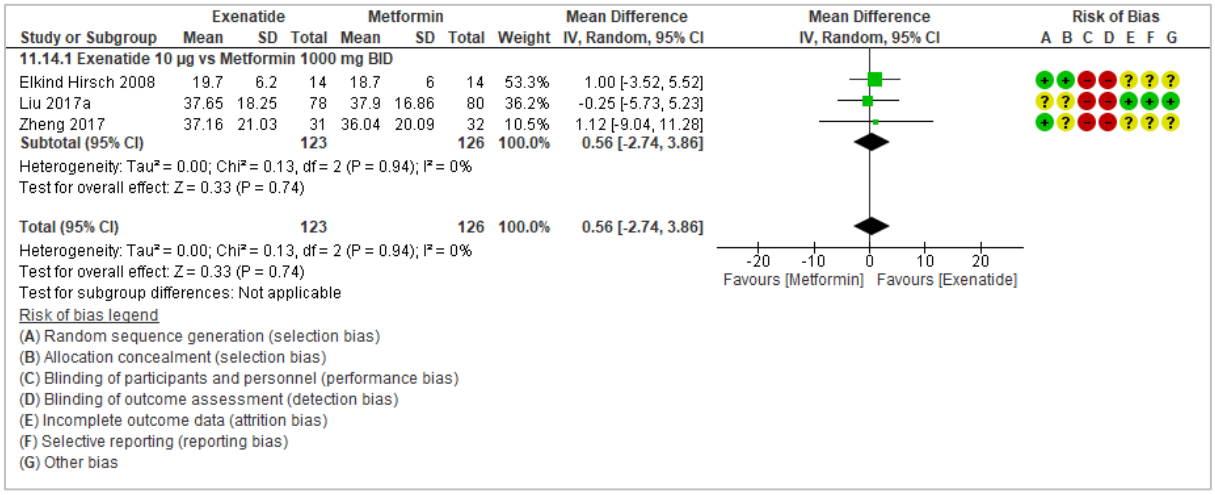
Figure 6-37: Forest plot of Liraglutide versus Liraglutide + Metformin on the SHBG (nmol/L)



6.3.9.7 Exenatide versus Metformin

Three RCTs compared exenatide 10 µg QD with metformin 1000 mg BID showed no effect on the SHBG (MD: 0.56 nmol/L; 95% CI: -2.74,3.86) (Figure 6-38) (very low-grade evidence, table 10).

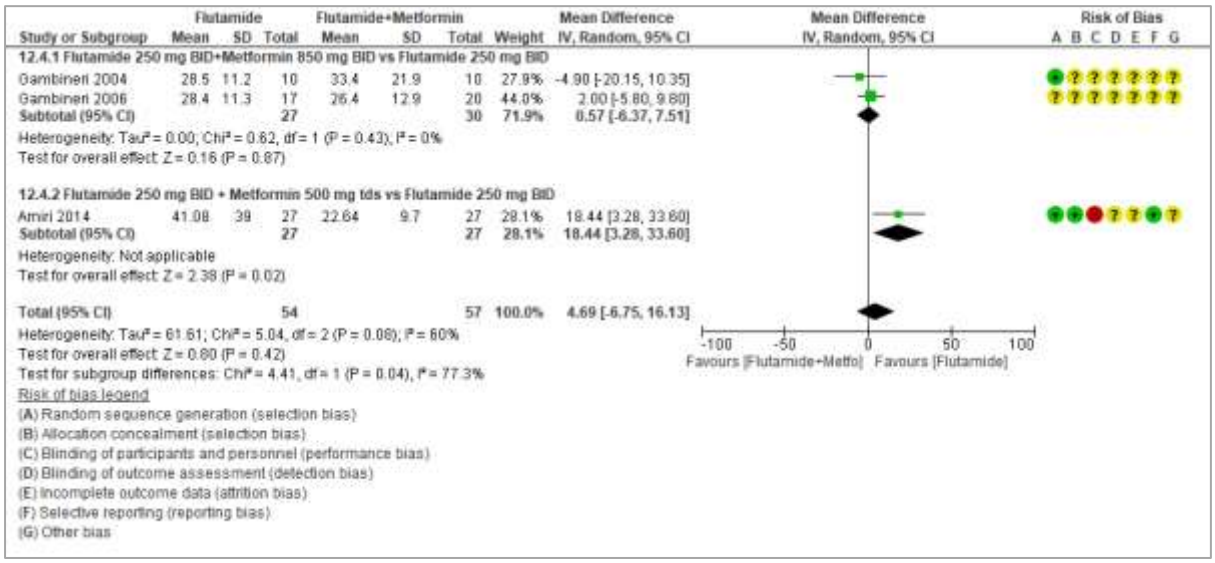
Figure 6-38: Forest plot of Exenatide versus Metformin on the SHBG (nmol/L)



6.3.9.8 Flutamide + Metformin versus Flutamide

Two RCTs compared flutamide 250 mg BID added to metformin 850 BID with flutamide 250 mg BID alone showed no effect on the SHBG (MD: 0.57 nmol/L; 95%CI: -6.37,7.51). One RCT compared flutamide 250 mg BID added to metformin 500 TDS with flutamide 250 mg BID alone showed significant increase in the SHBG (MD: 18.44 nmol/l; 95%CI: 3.28, 33.60). Overall, flutamide 250 mg BID added to metformin of various dosages compared with flutamide alone has no effect on the SHBG (MD: 4.69 nmol/L;95%CI: - 6.75, 16.13) (Figure 6-39) (very low-grade evidence, table 10).

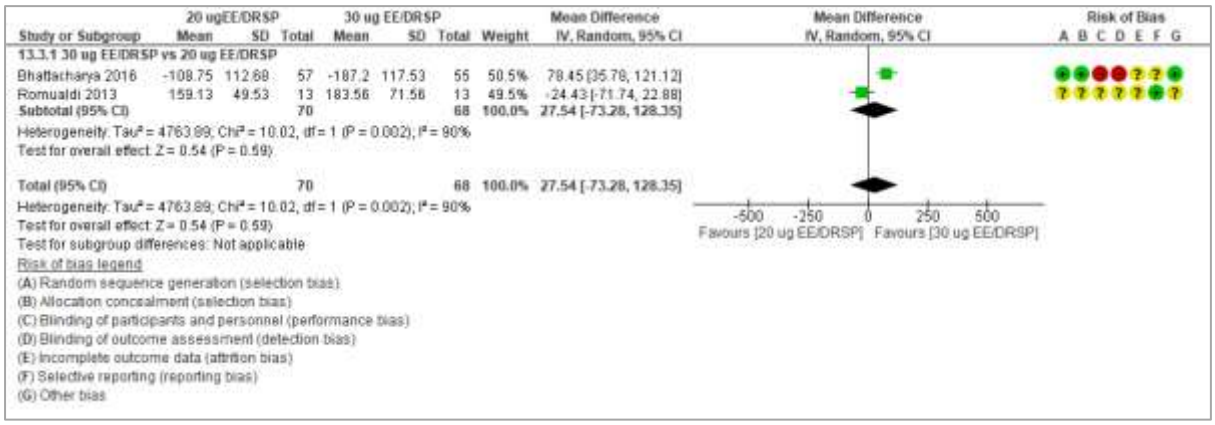
Figure 6-39: Forest plot of Flutamide + Metformin versus Flutamide on the SHBG (nmol/L)



6.3.9.9 OCP (30 µg EE/DRSP) versus OCP (20 µg EE/DRSP)

Two RCTs compared different dosages of OCP (EE/DRSP) showed no effect on the SHBG (MD: 27.54 nmol/L; 95% CI: -73.28, 128.35). (Figure 6-40) (very low-grade evidence, table 10).

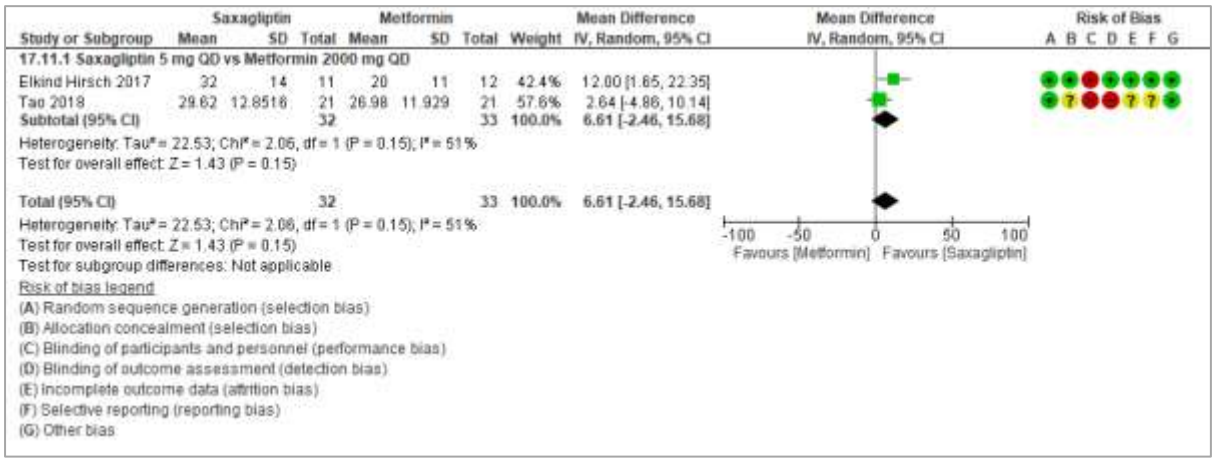
Figure 6-40: Forest plot of OCP (30 µg EE/DRSP) versus OCP (20 µg EE/DRSP) on the SHBG (nmol/L)



6.3.9.10 Saxagliptin versus Metformin

Two RCTs compared saxagliptin 5 mg QD with metformin 2000 mg QD showed no effect on the SHBG (MD: 6.61 nmol/L; 95%CI: -2.46,15.68) (Figure 6-41) (very low-grade evidence, table 10).

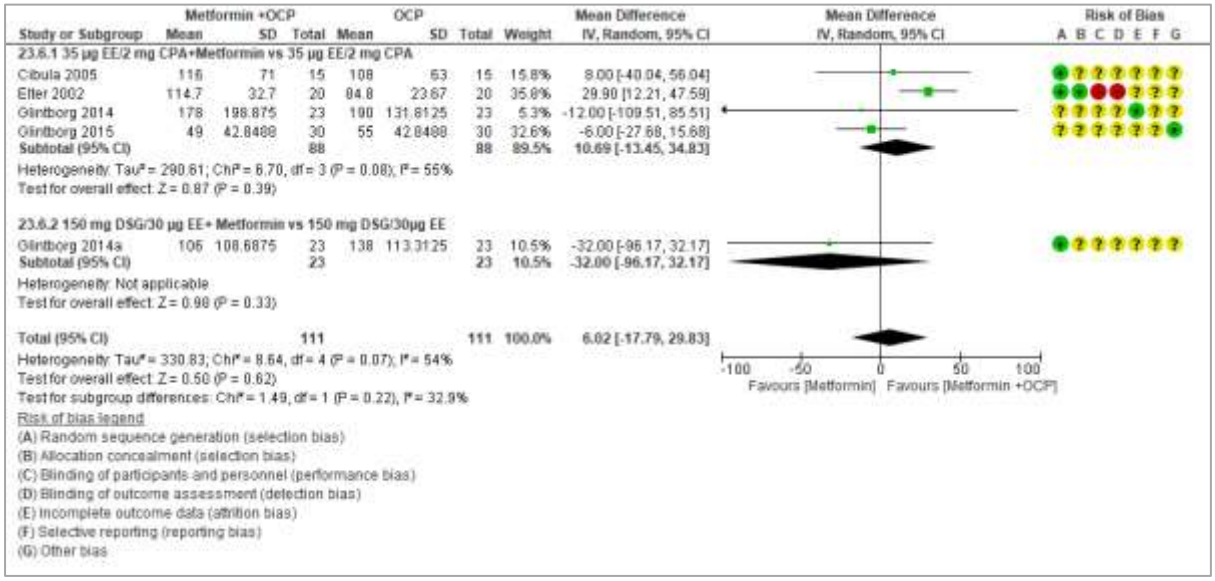
Figure 6-41: Forest plot of Saxagliptin versus Metformin on the SHBG (nmol/L)



6.3.9.11 Metformin + OCP versus OCP

Four RCTs compared metformin added to OCP (35µg EE/2 mg CPA) with OCP (35µg EE/2 mg CPA) alone showed no effect on the SHBG (MD: 10.69 nmol/L; 95%CI: -13.45, 34.83). In one RCT compared OCP (30 µg EE/ 150mg DSG) added to metformin with OCP (30 µg EE/ 150mg DSG) alone showed no effect on the SHBG (MD: -32.00 nmol/L; 95%CI: -96.17, 32.17). Overall, OCP of various forms and dosage alone or added to metformin has no effect on the SHBG (MD: 6.02 nmol/L; 95% CI: -17.79, 29.83) (Figure 6-42) (very low-grade evidence, table 10).

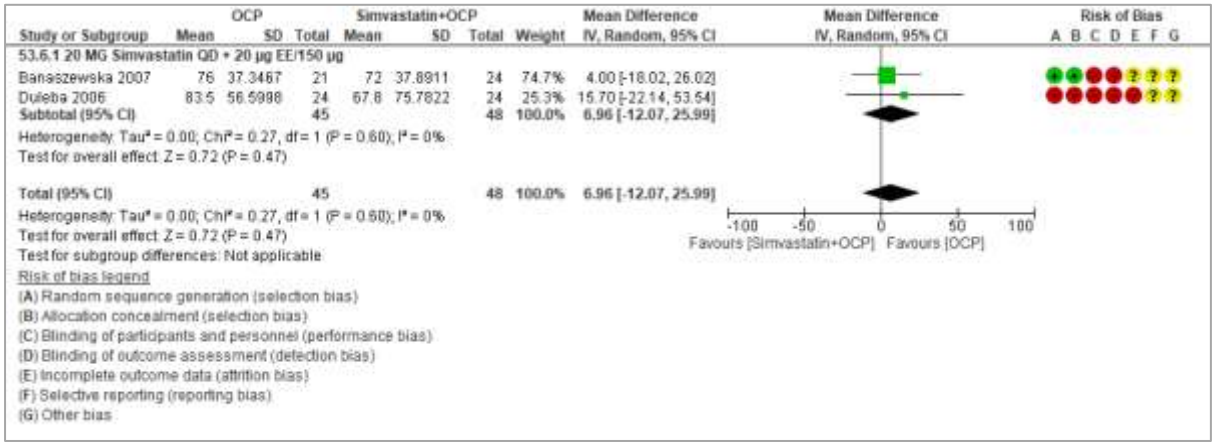
Figure 6-42: Forest plot of Metformin + OCP versus OCP on the SHBG (pmol/L)



6.3.9.12 Simvastatin + OCP versus OCP

Two RCTs compared simvastatin 20 mg QD added to OCP (20 µg EE/ 150 mg DSG) with OCP (20 µg EE/ 150 mg DSG) alone showed no effect on the SHBG (MD: 6.96 nmol/L;95% CI: -12.07, 25.99) (Figure 6-43) (very low-grade evidence, table 10).

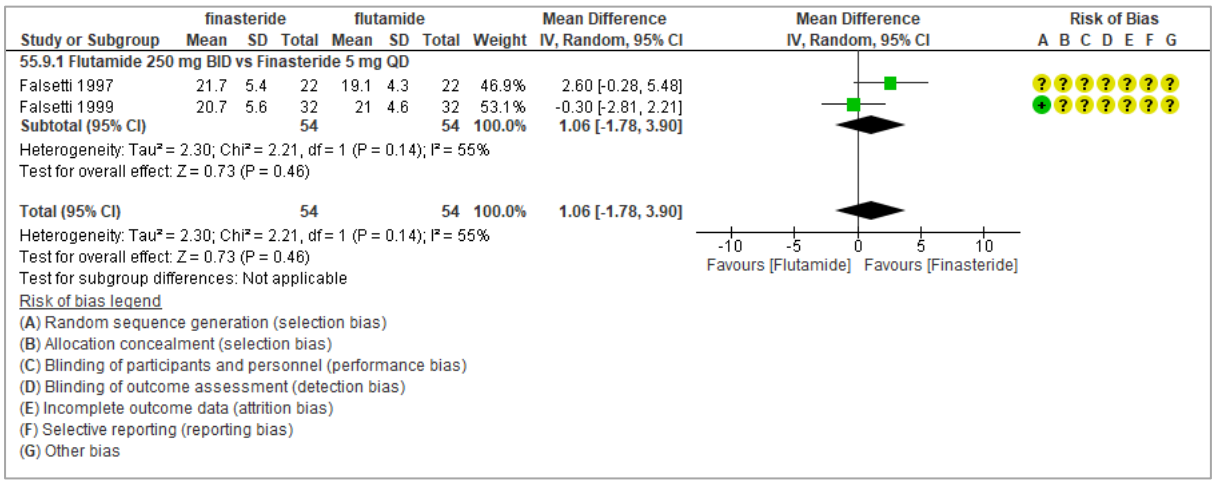
Figure 6-43: Forest plot of Simvastatin + OCP versus OCP on the SHBG (nmol/L)



6.3.9.13 Finasteride versus Flutamide

Two RCTs compared flutamide 250 mg BID with finasteride 5 mg BID showed no effect on the SHBG (MD: 1.06 nmol/L; 95% CI: -1.78, 3.90) (Figure 6-44) (very low-grade evidence, table 10).

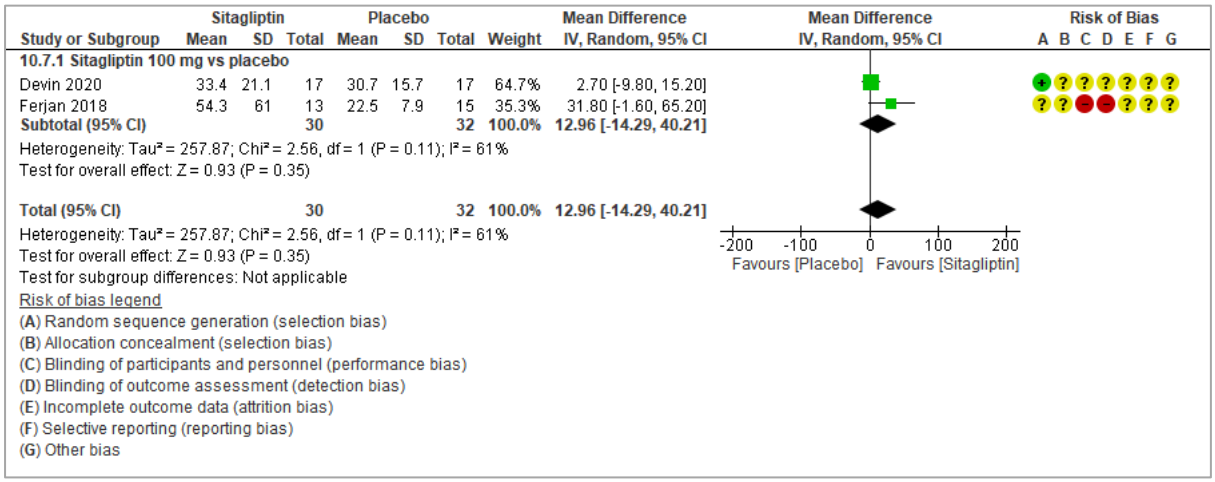
Figure 6-44: Forest plot of Finasteride versus Flutamide on the SHBG (nmol/L)



6.3.9.14 Sitagliptin versus placebo

Two RCTs compared sitagliptin 100 mg QD with placebo showed no effect on the SHBG (MD: 12.96 nmol/L; 95% CI: -14.29, 40.21) (Figure 6-45) (very low-grade evidence, table 10).

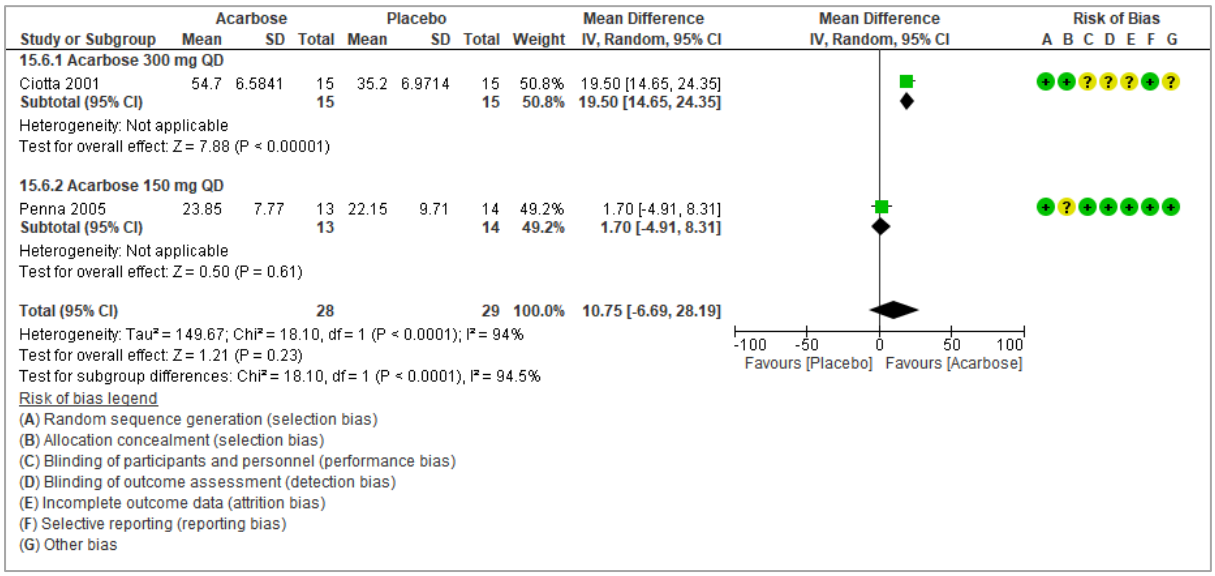
Figure 6-45: Forest plot of Sitagliptin versus placebo on the SHBG (nmol/L)



6.3.9.15 Acarbose versus placebo

One RCT compared acarbose 300 mg QD with placebo showed a significant increase in the level of SHBG (MD: 19.50 nmol/L; 95% CI: 14.65, 24.35), while another RCT compared acarbose 150 mg QD with placebo showed no effect on the SHBG (MD: 1.70 nmol/L; 95% CI: -4.91, 8.31). Overall, acarbose at various dosages has no effect on the SHBG when was compared with placebo (MD: 10.75 nmol/L; 95%CI: -6.69, 28.19) (Figure 6-46) (very low-grade evidence, table 10).

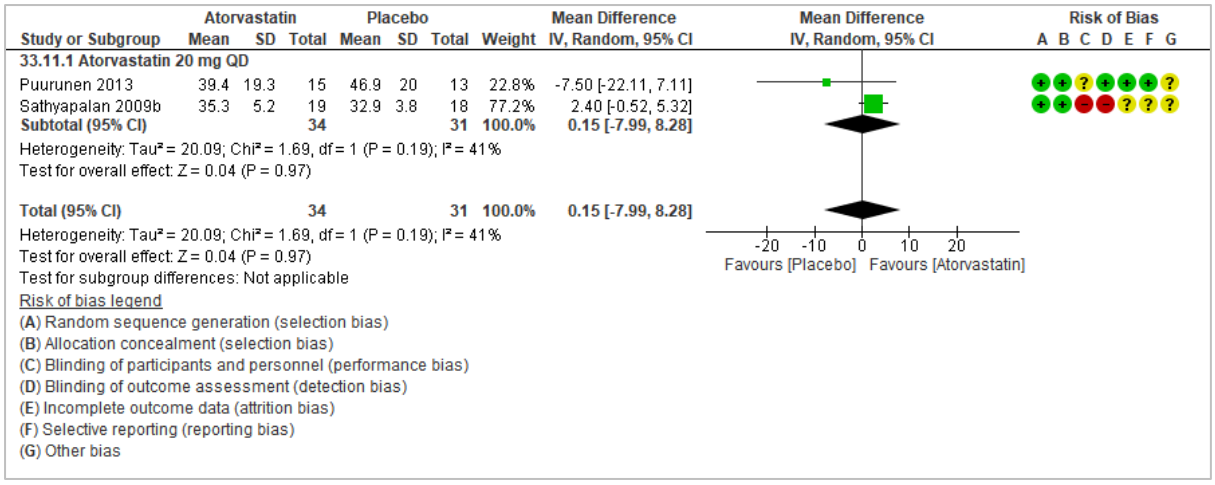
Figure 6-46: Forest plot of Acarbose versus placebo on the SHBG (nmol/L)



6.3.9.16 Atorvastatin versus placebo

Two RCTs compared atorvastatin 20 mg QD with placebo showed no effect on the SHBG (MD: 0.15 nmol/L; 95%CI: -7.99, 8.28) (Figure 6-47) (very low-grade evidence, table 10).

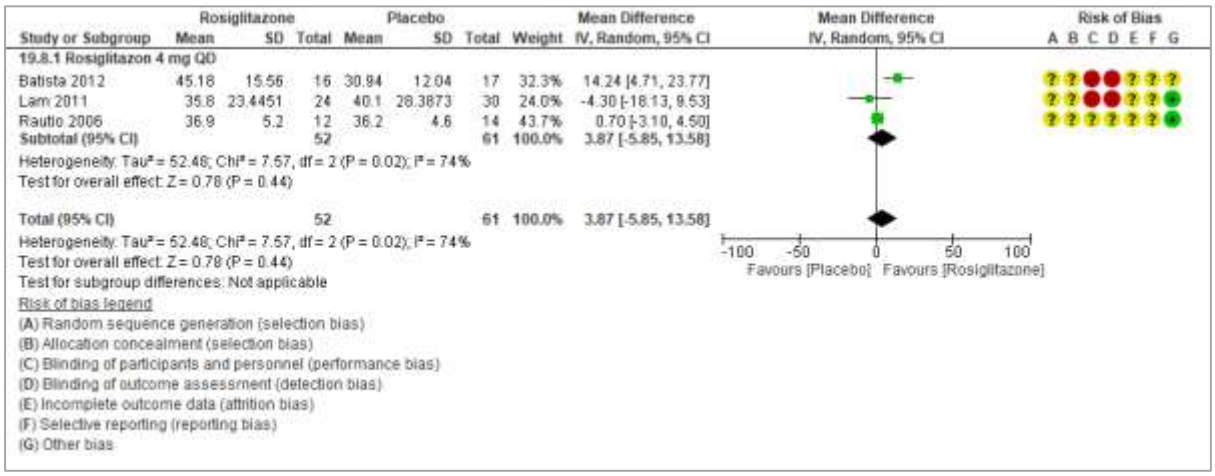
Figure 6-47: Forest plot of Atorvastatin versus placebo on the SHBG (nmol/L)



6.3.9.17 Rosiglitazone versus placebo

Three RCTs compared rosiglitazone 4 mg QD with placebo showed no effect on the SHBG (MD: 3.87 nmol/L; 95%CI: -5.85, 13.58) (Figure 6-48) (very low-grade evidence, table 10).

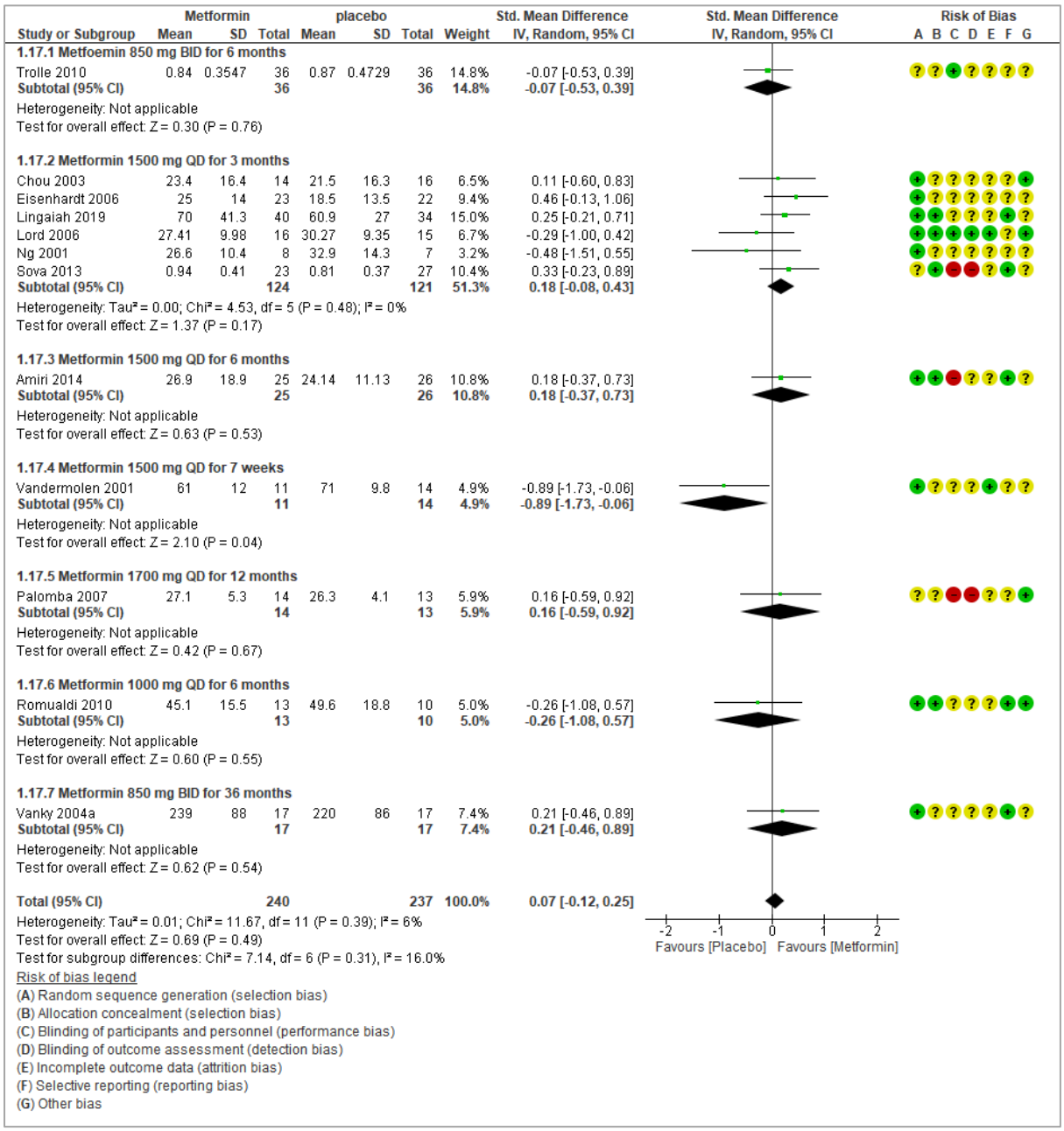
Figure 6-48: Forest plot of Rosiglitazone versus placebo on the SHBG (nmol/L)



6.3.9.18 Metformin versus placebo

When metformin of various dosages and for the various duration was compared with placebo, no effect on the SHBG was observed (SMD: 0.07; 95% CI: -0.12, 0.25) (Figure 6-49) (moderate grade evidence, table 10).

Figure 6-49: Forest plot of Metformin versus placebo on the SHBG

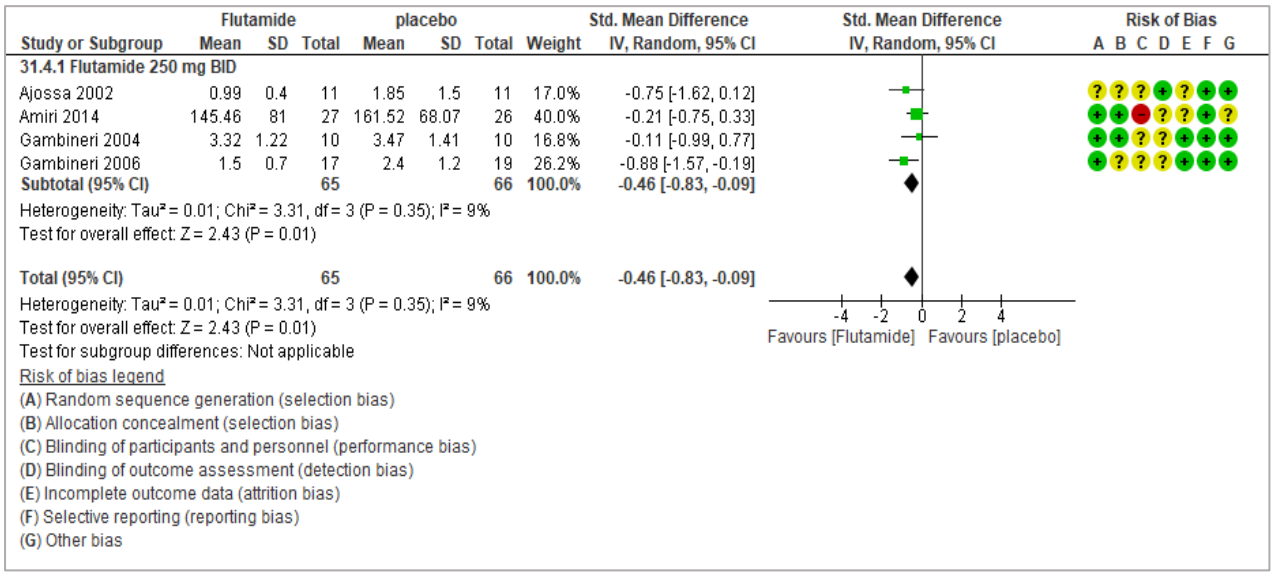


6.3.10 DHEAS

6.3.10.1 Flutamide versus placebo

Four RCTs compared flutamide 250 mg BID with placebo showed a significant reduction in DHEAS (SMD: -0.46; 95% CI: -0.83, -0.09) (Figure 6-50) (very low-grade evidence, table 10).

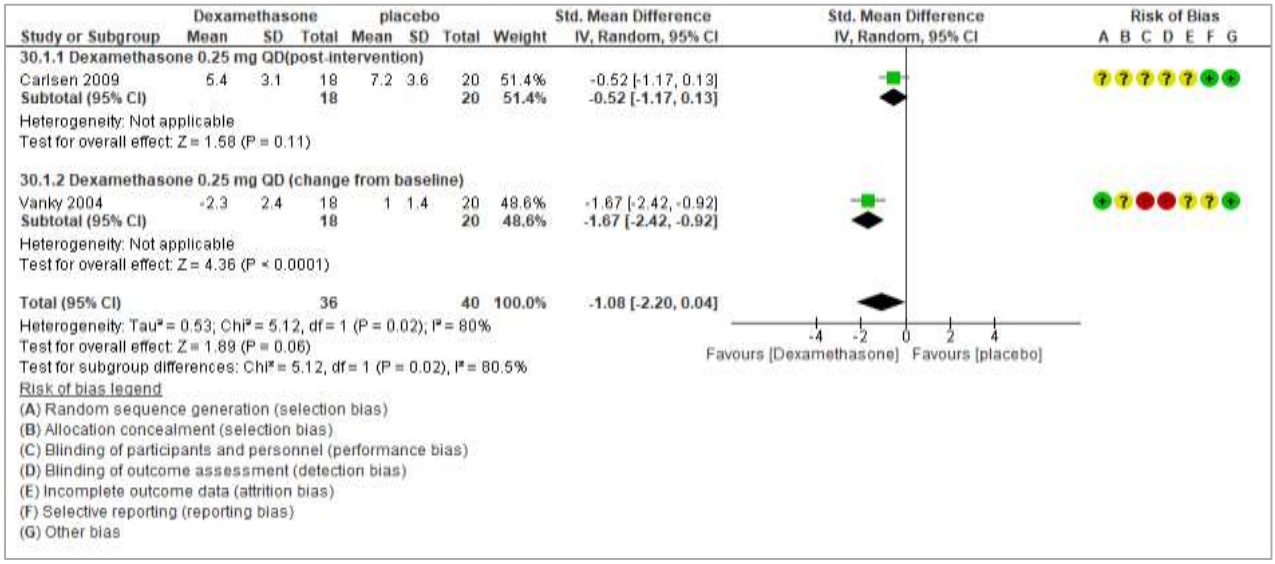
Figure 6-50: Forest plot of Flutamide versus placebo on the DHEAS



6.3.10.2 Dexamethasone versus placebo

In one RCT evaluated dexamethasone 0.25 mg QD, the post-intervention results showed no reduction in DHEAS (SMD: -0.51; 95% CI: -1.17, 0.13) while in another RCT change from baseline showed a significant reduction in DHEAS (SMD: -1.67; 95% CI: -2.42, -0.92). Overall, dexamethasone significantly reduced DHEAS when compared with placebo (SMD: -1.08; 95% CI: -2.20, 0.04) (Figure 6-51) (very low-grade evidence, table 10).

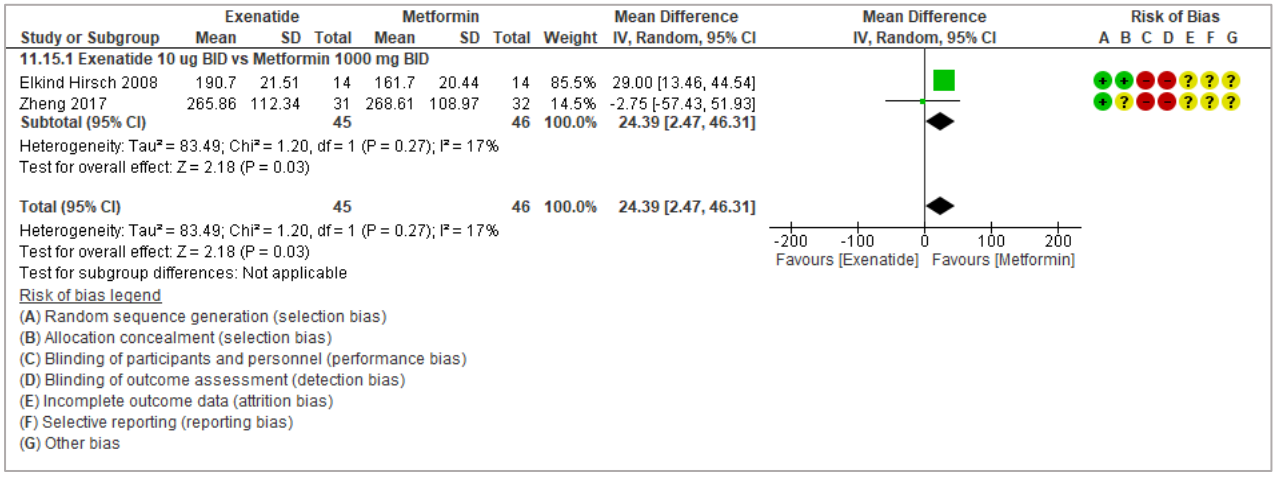
Figure 6-51: Forest plot of Dexamethasone versus placebo on the DHEAS



6.3.10.3 Exenatide versus Metformin

In two RCTs, exenatide 10 µg QD compared with metformin 1000 mg BID showed a significant increase in DHEAS (MD: 24.39 µg/dL; 95% CI: 2.47, 46.31) (Figure 6-52) (very low-grade evidence, table 10).

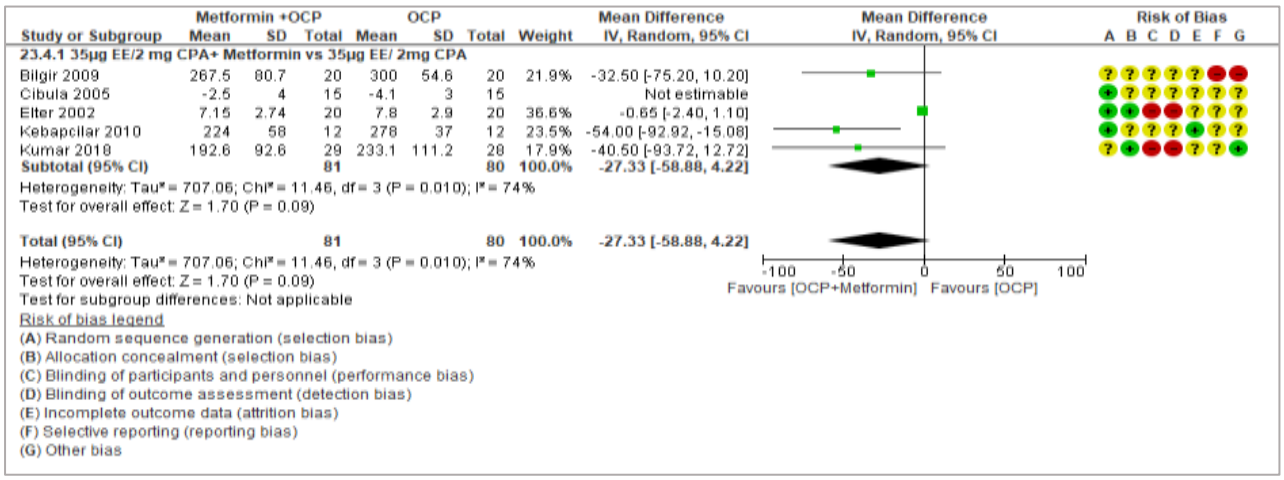
Figure 6-52: Forest plot of Exenatide versus Metformin on the DHEAS (µg/dL)



6.3.10.4 OCP+ Metformin versus OCP

In five RCTs, when OCP (35 µg EE/2 mg CPA) was added to metformin, compared with OCP (35 µg EE/2 mg CPA) alone, there was a significant reduction in DHEAS (MD: -27.33 µg/dL; 95% CI: -58.88, 4.22) (Figure 6-53) (very low-grade evidence, table 10).

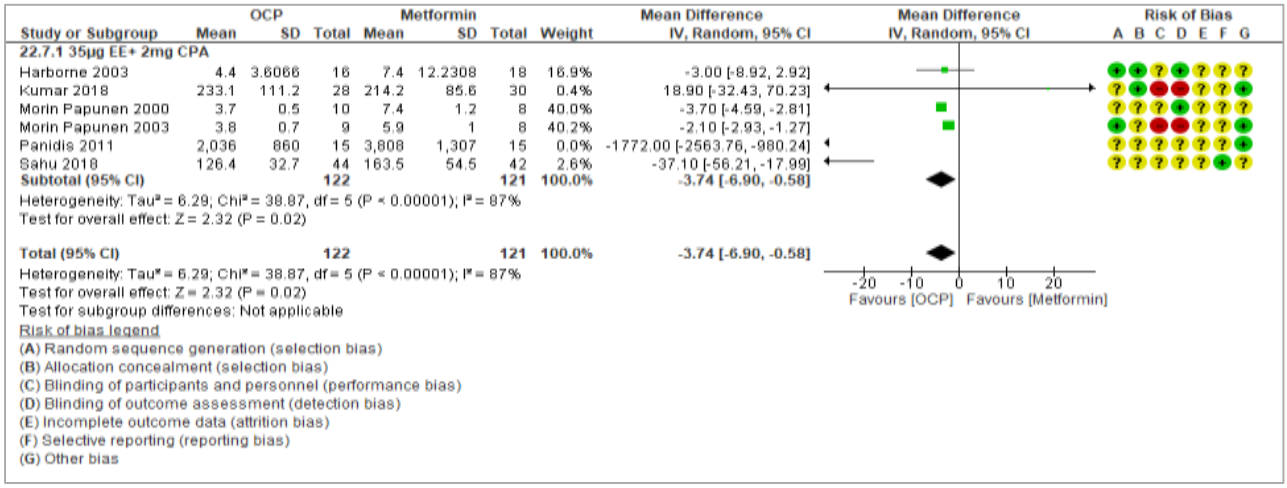
Figure 6-53: Forest plot of OCP+ Metformin versus OCP on the DHEAS (µg/dL)



6.3.10.5 OCP versus Metformin

Six RCTs compared OCP (35 µg EE/2 mg CPA) with metformin that showed a significant reduction in DHEAS (MD: -3.74 µg/dL; 95% CI: -6.90 ,-0.58) (Figure 6-54) (very low-grade evidence, table 10).

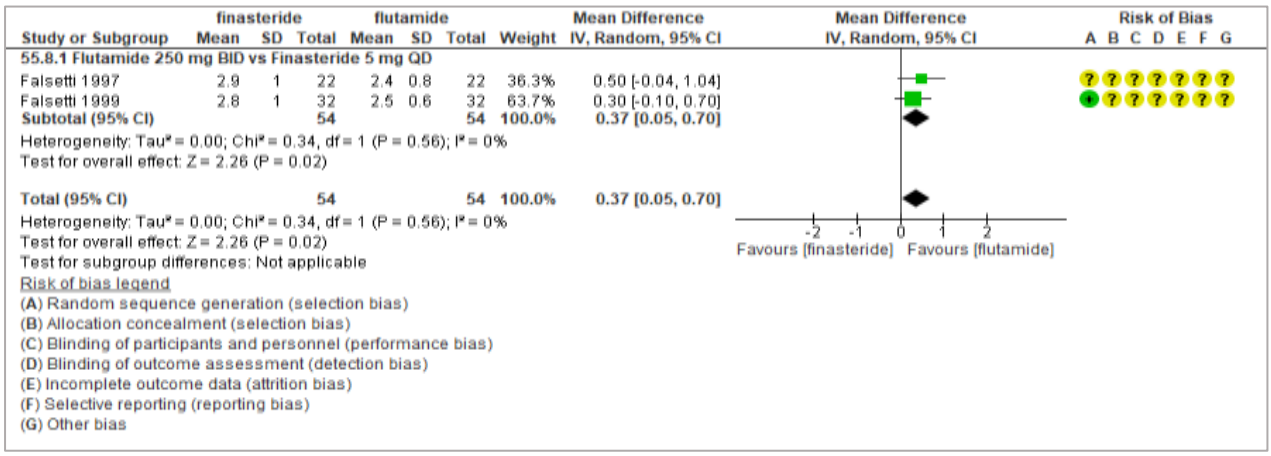
Figure 6-54: Forest plot of OCP versus Metformin on the DHEAS (µg/dL)



6.3.10.6 Flutamide versus Finasteride

Two RCTs compared flutamide 250 mg BID with finasteride 5 mg QD showed a significant increase in DHEAS (MD: 0.37 µg/dL; 95% CI: -0.05, -0.58) (Figure 6-55) (very low-grade evidence, table 10).

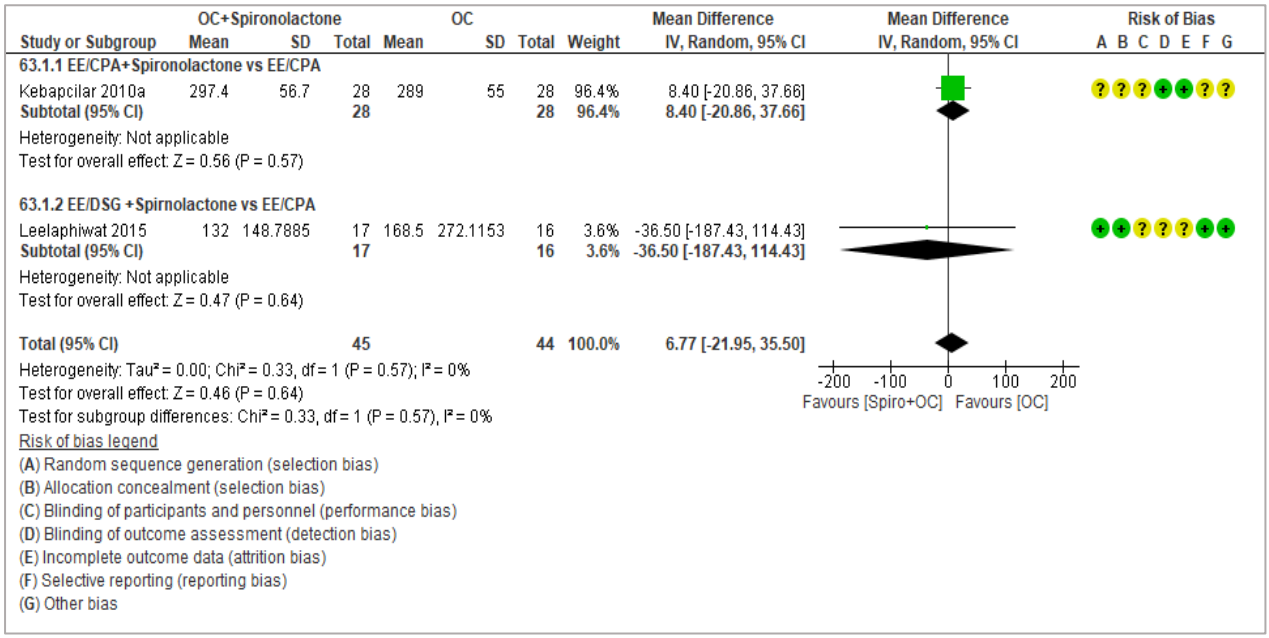
Figure 6-55: Forest plot of Finasteride versus Flutamide on the DHEAS (µg/dL)



6.3.10.7 OCP + Spironolactone versus OCP

One RCT compared OCP (EE/CPA) added to spironolactone with OCP (EE/CPA) alone showed no effect on the DHEAS (MD: 8.40 µg/dl; 95% CI: -20.88, 37.66). Another RCT compared OCP (EE/DSG) added to spironolactone with OCP (EE/DSG) alone showed no effect on the DHEAS (MD: -36.50 µg/dl; 95% CI: -187.43, 114.43). Overall, OCP added to spironolactone compared with OCP alone has no effect on the DHEAS (MD: 6.77 µg/dl; 95% CI: -21.95, 35.50) (Figure 6-56) (very low-grade evidence, table 10).

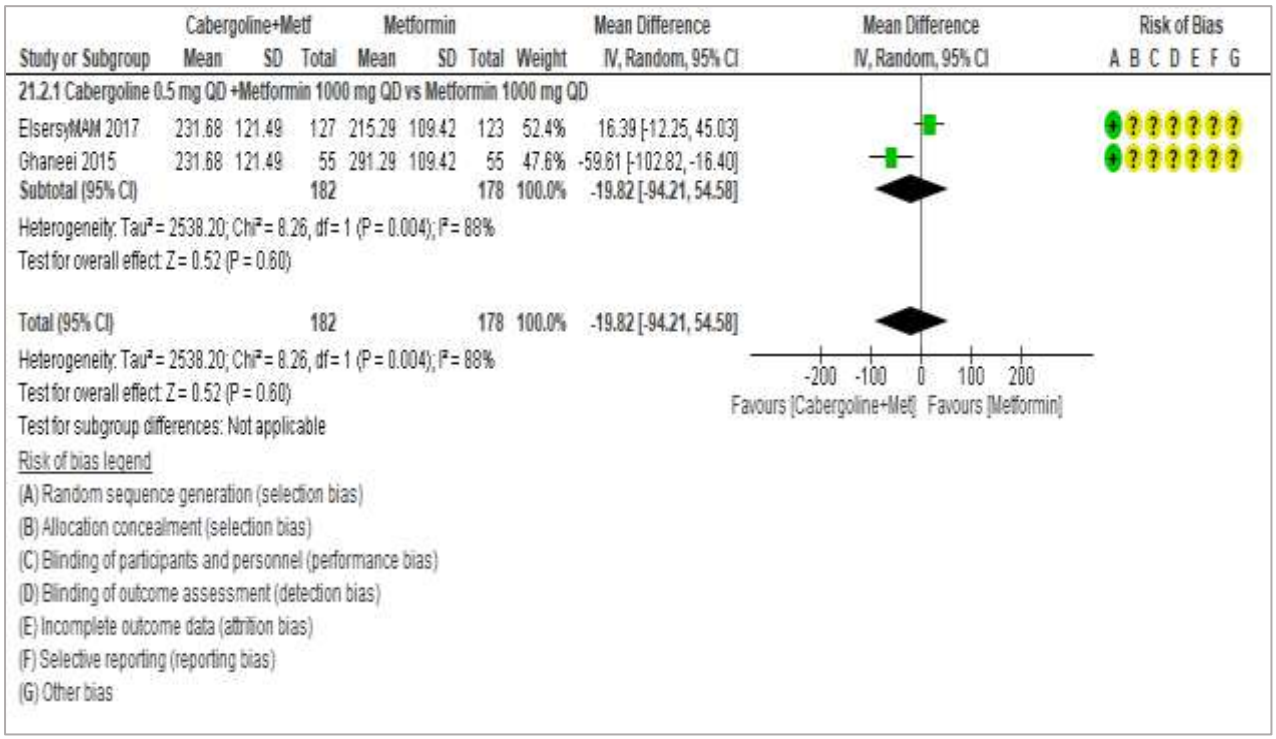
Figure 6-56: Forest plot of OCP + Spironolactone versus OCP on the DHEAS (µg/dL)



6.3.10.8 Cabergoline + Metformin versus Metformin

Two RCTs compared cabergoline 0.5 mg QD added to metformin 1000 mg QD with metformin 1000 mg QD alone showed no effect on the DHEAS (MD: -19.82 µg/dl; 95% CI: -94.21, 54.58) (Figure 6-57) (very low-grade evidence, table 10).

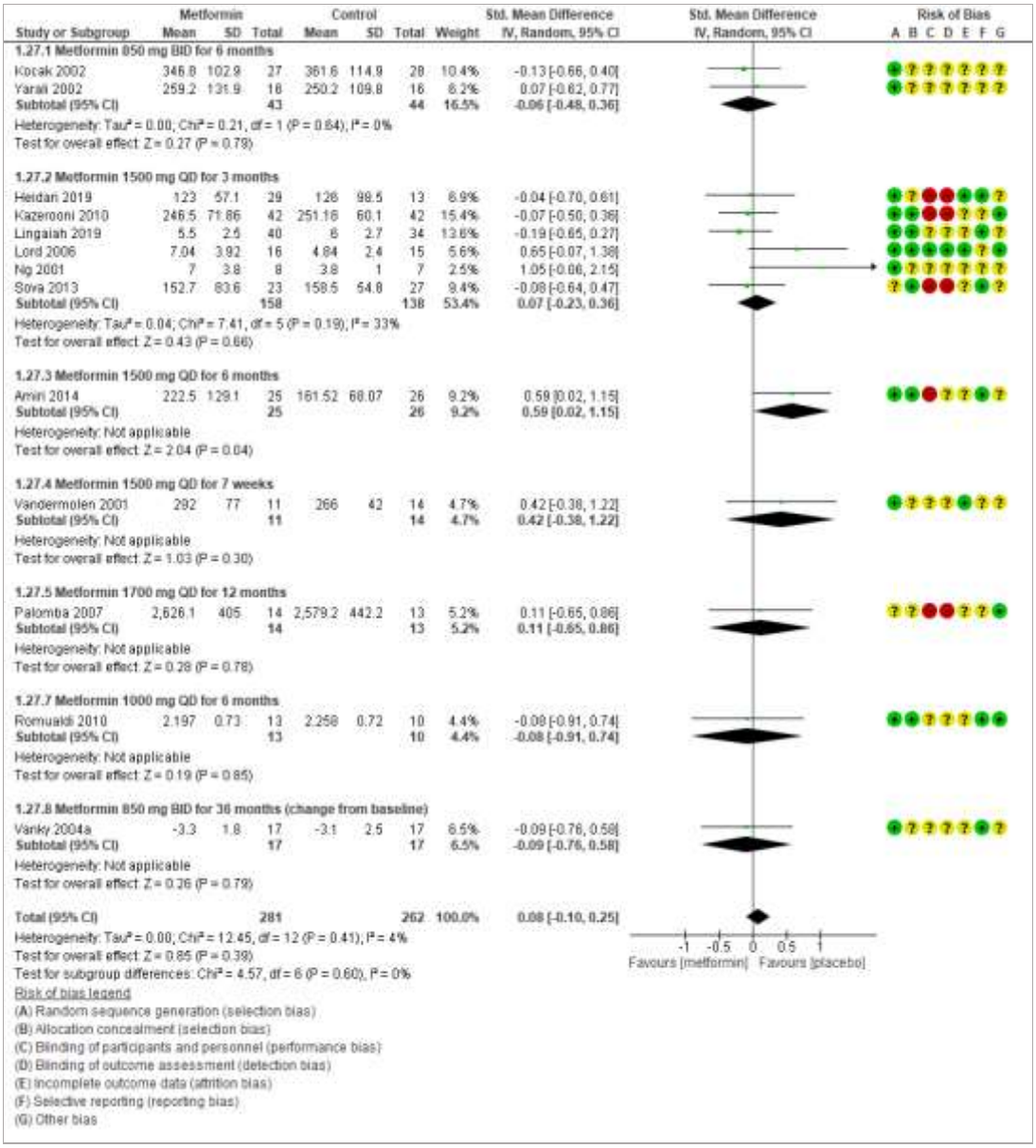
Figure 6-57: Forest plot of Cabergoline + Metformin versus Metformin on the DHEAS (µg/dL)



6.3.10.9 Metformin versus placebo

Thirteen RCTs compared metformin of various dosages and for the various duration with placebo showed no effect on DHEAS (SMD: 0.08; 95% CI: -0.10, 0.25) (Figure 6-58) (moderate grade evidence, table 10).

Figure 6-58: Forest plot of Metformin versus placebo on the DHEAS



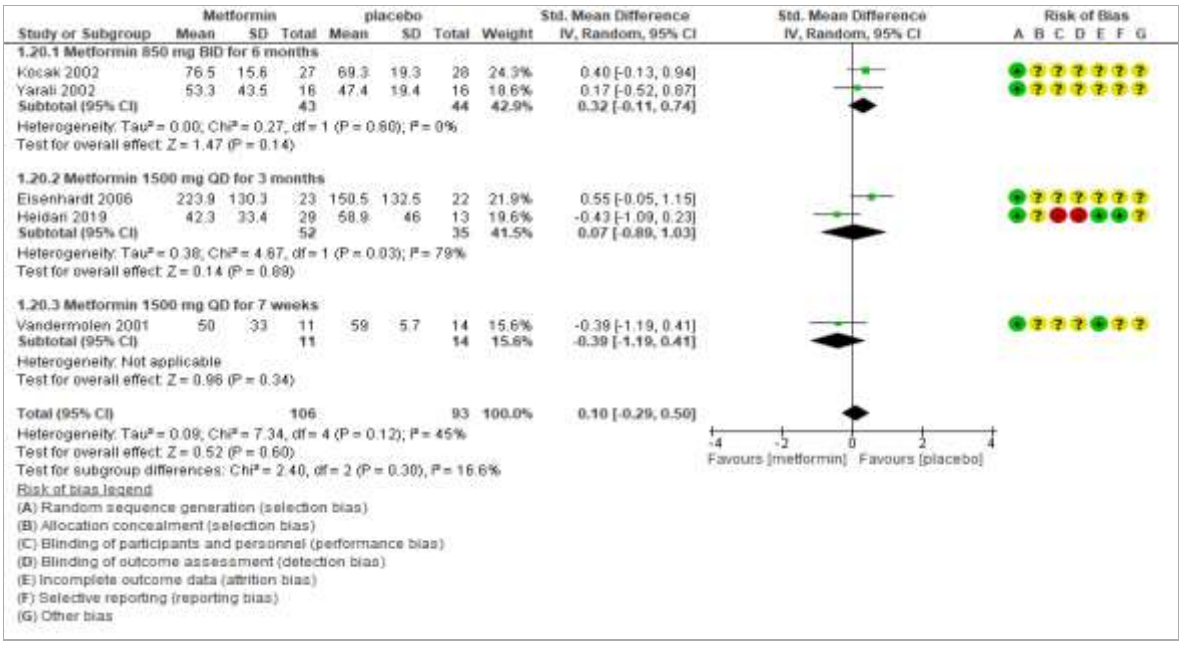
6.3.11 Oestradiol

6.3.11.1 Metformin versus placebo

In two RCTs, metformin 850 mg BID for six months had no effect on the serum oestradiol compared with placebo (SMD: 0.32; 95% CI: -0.11, 0.74). Likewise, two RCTs of metformin 1500 mg QD for three months (SMD: 0.07;95% CI: -0.89,1.03) and another one that compared metformin 1500 mg QD for seven weeks to placebo (SMD: -0.39; 95% CI: -1.19, 0.41) showed

no effects. Overall, metformin had no effect on the serum oestradiol when compared with placebo (SMD: 0.10; 95% CI: -0.29, 0.50) (Figure 6-59) (very low-grade evidence, table 10).

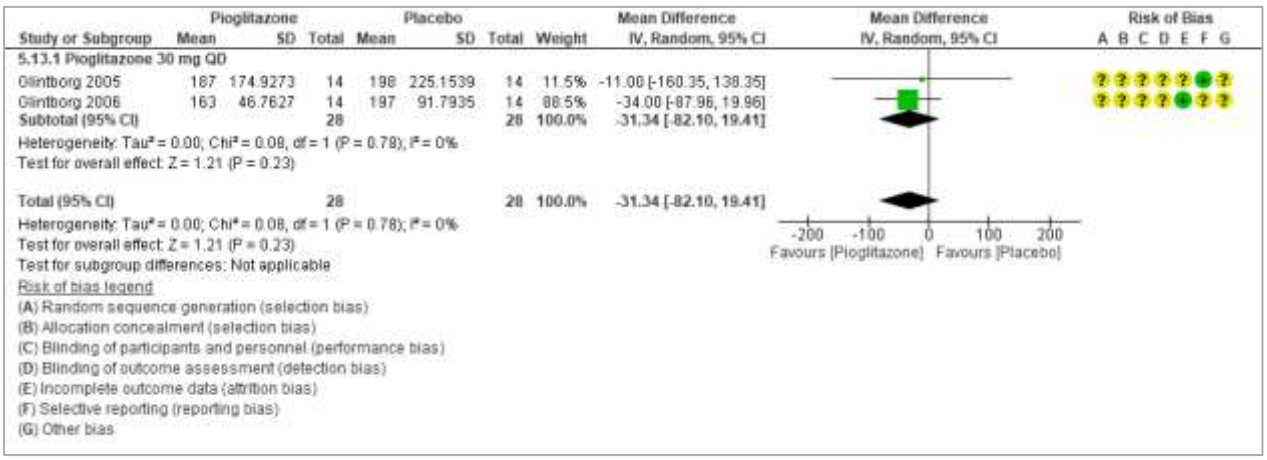
Figure 6-59: Forest plot of Metformin versus placebo on the oestradiol



6.3.11.2 Pioglitazone versus placebo

In two RCTs that compared pioglitazone 30 mg QD with placebo, there was no effect on the serum oestradiol (MD: -31.34 pg/mL; 95% CI: -82.10, 19.4) (Figure 6-60) (very low-grade evidence, table 10).

Figure 6-60: Forest plot of Pioglitazone versus placebo on the oestradiol (pg/mL)

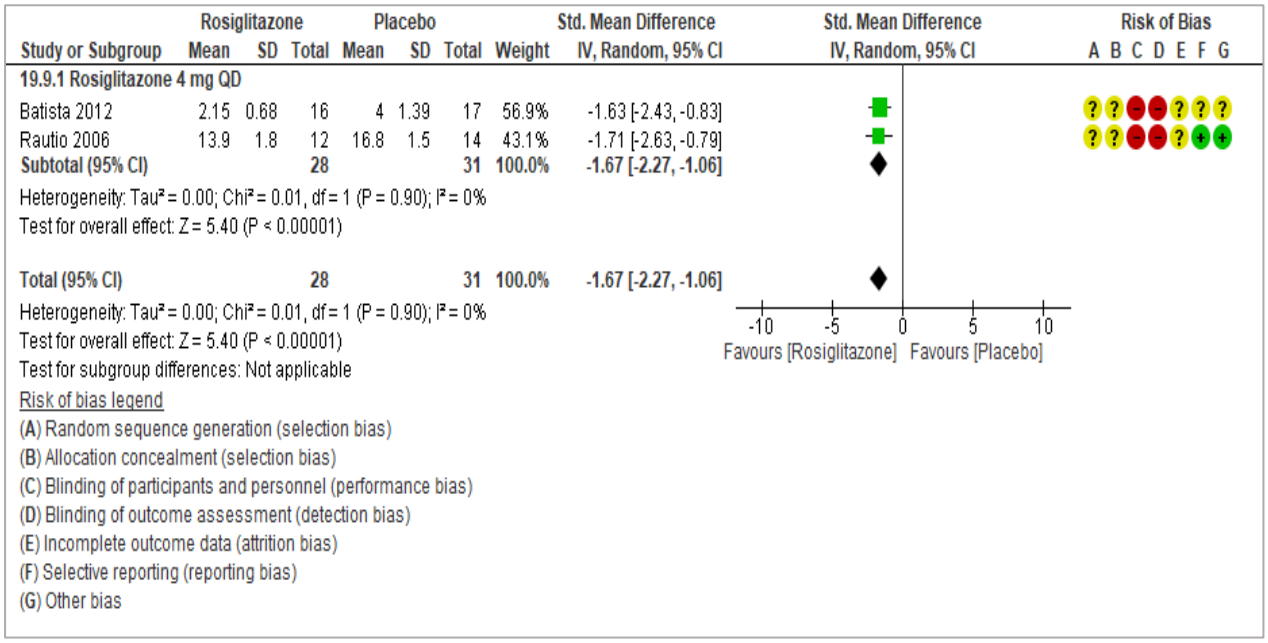


6.3.12 Androstenedione

6.3.12.1 Rosiglitazone versus placebo

Two RCTs compared rosiglitazone 4 mg QD with placebo showed a significant reduction in the serum androstenedione (SMD: -1.67; 95% CI: -2.27, -1.06) (Figure 6-61) (very low-grade evidence, table 10).

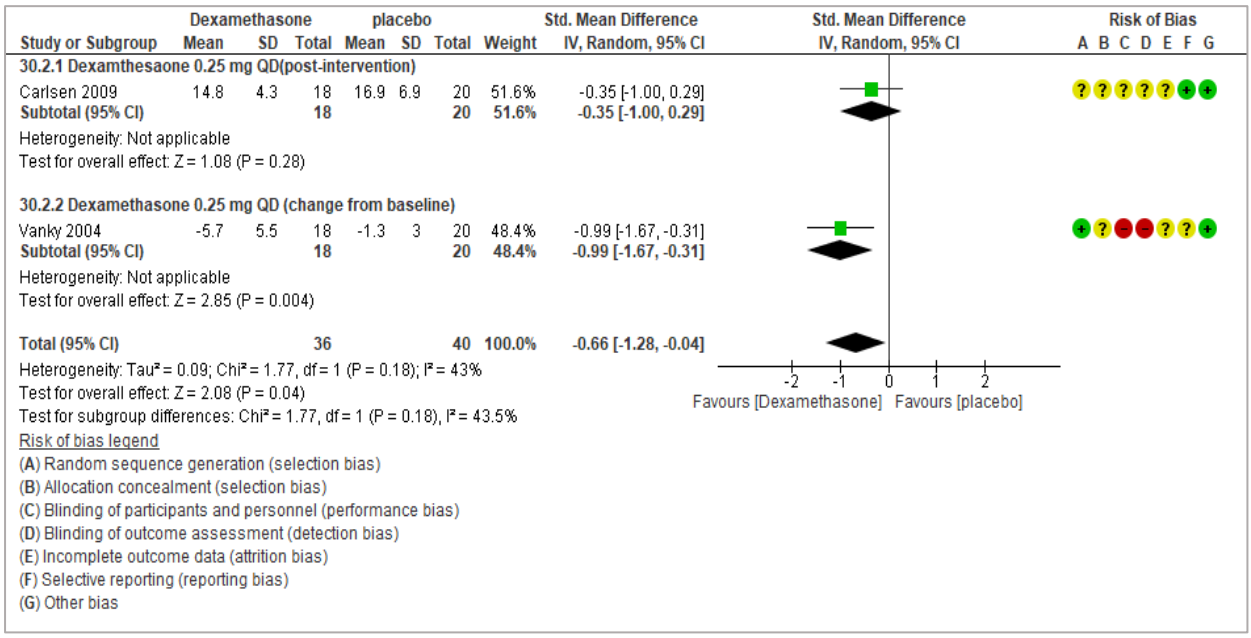
Figure 6-61: Forest plot of Rosiglitazone versus placebo on androstenedione



6.3.12.2 Dexamethasone versus placebo

One RCT assessed dexamethasone 0.25 mg QD showed no reduction in the level of androstenedione (SMD: -0.35; 95% CI: -1.00, 0.29), while the changes from baseline in another RCT showed significant reduction in the androstenedione (SMD: -0.99; 95%CI: -1.67 , -0.31). Overall, dexamethasone significantly reduced androstenedione when compared with placebo (SMD: -0.66; 95% CI: -1.28, -0.04) (Figure 6-62) (very low-grade evidence, table 10).

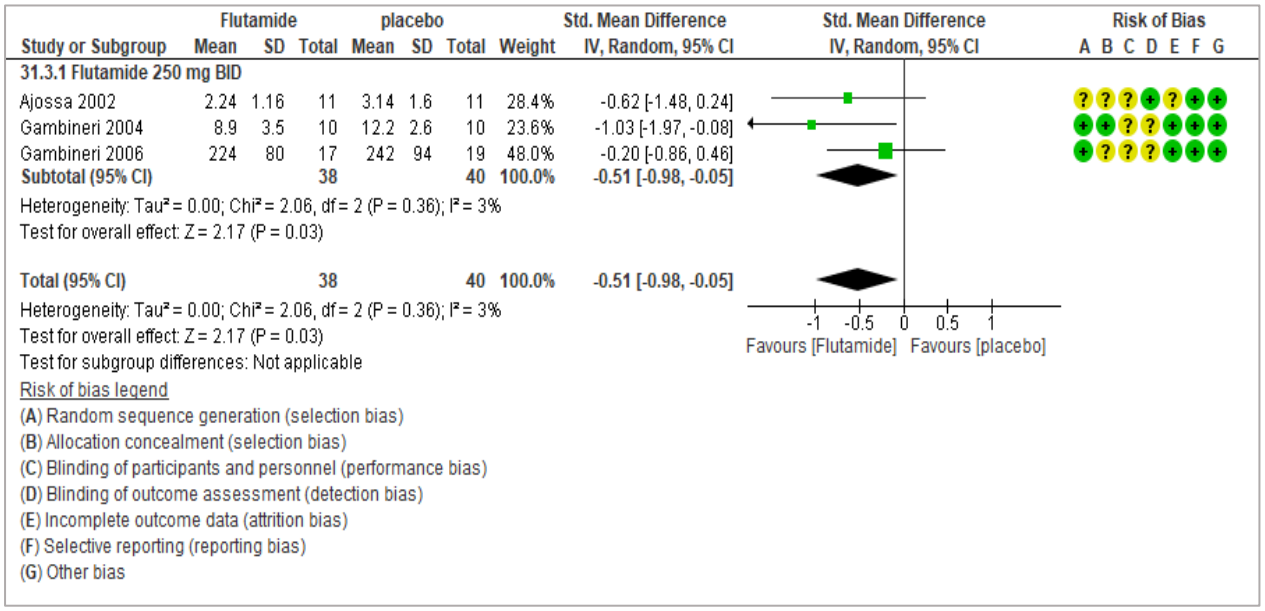
Figure 6-62: Forest plot of Dexamethasone versus placebo on androstenedione



6.3.12.3 Flutamide versus placebo

Three RCTs compared flutamide 250 mg BID with placebo showed a significant reduction in the level of androstenedione when compared with placebo (SMD: -0.51; 95% CI: -0.98,-0.05) (Figure 6-63) (very low-grade evidence, table 10).

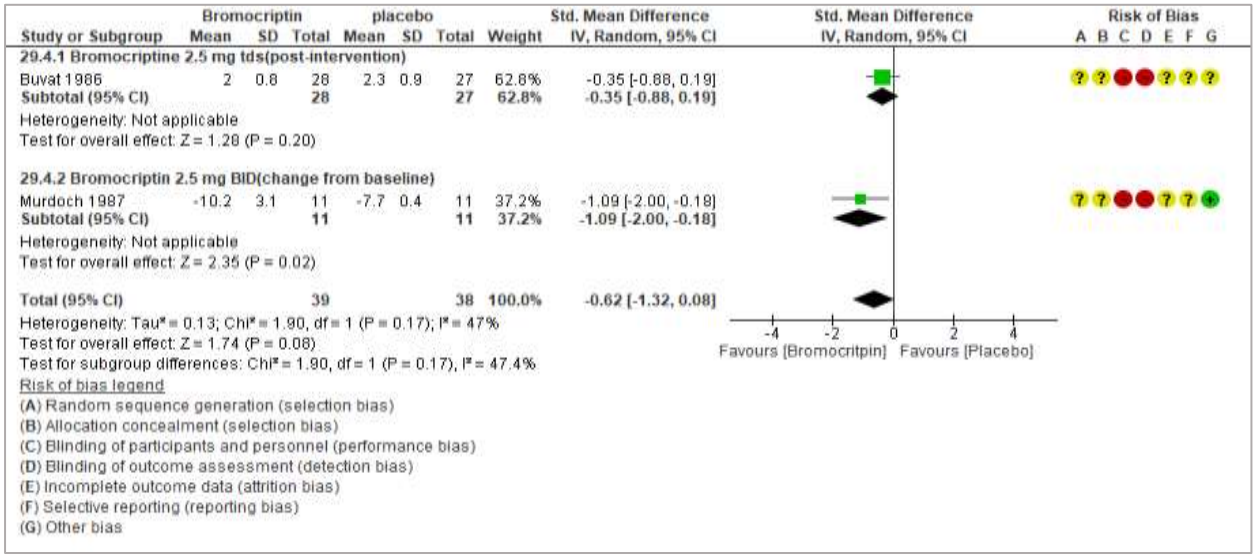
Figure 6-63: Forest plot of Flutamide versus placebo on the androstenedione



6.3.12.4 Bromocriptine versus placebo

Two RCTs compared bromocriptine 2.5 mg TDS with placebo showed a significant reduction in the level of androstenedione (SMD: -0.62; 95% CI: -1.32, 0.08) (Figure 6-64) (very low-grade evidence, table 10).

Figure 6-64: Forest plot of Bromocriptine versus placebo on androstenedione

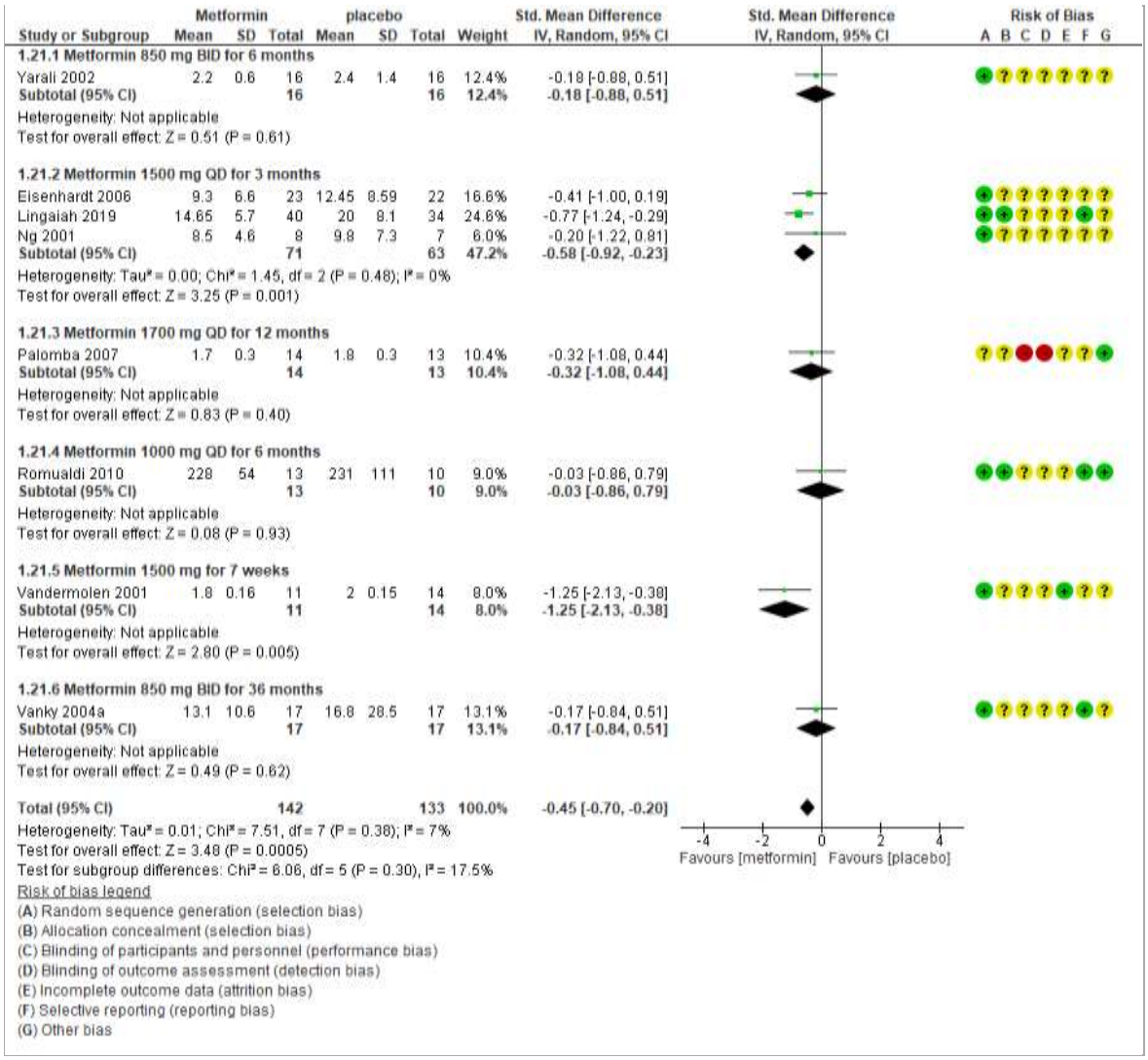


6.3.12.5 Metformin versus placebo

In one RCT, metformin 850 mg BID for six months had no effect on androstenedione (SMD: -0.18; 95% CI: -0.88, 0.51). Three RCTs compared metformin 1500 mg QD for three months showed a significant reduction in androstenedione (SMD: -0.58; 95% CI: -0.92, -0.23). One RCT compared metformin 1700 mg QD for 12 months showed no effect on androstenedione (SMD: -0.32; 95% CI: -1.08, 0.44). One RCT compared metformin 1000 mg QD for six months showed no effect on androstenedione. However, another RCT that compared metformin 1500 mg QD for seven weeks showed a significant reduction in the androstenedione (SMD: -1.25; 95% CI: -2.13, -0.38). One RCT compared metformin 850 mg BID for 36 months showed no reduction in the level of androstenedione (SMD: -0.17; 95% CI: -0.84, 0.51). Overall,

metformin of various dosages significantly reduced the level of androstadiene when compared with placebo (SMD: -0.45; 95% CI: -0.70,-0.20) (Figure 6-65) (very low-grade evidence, table 10).

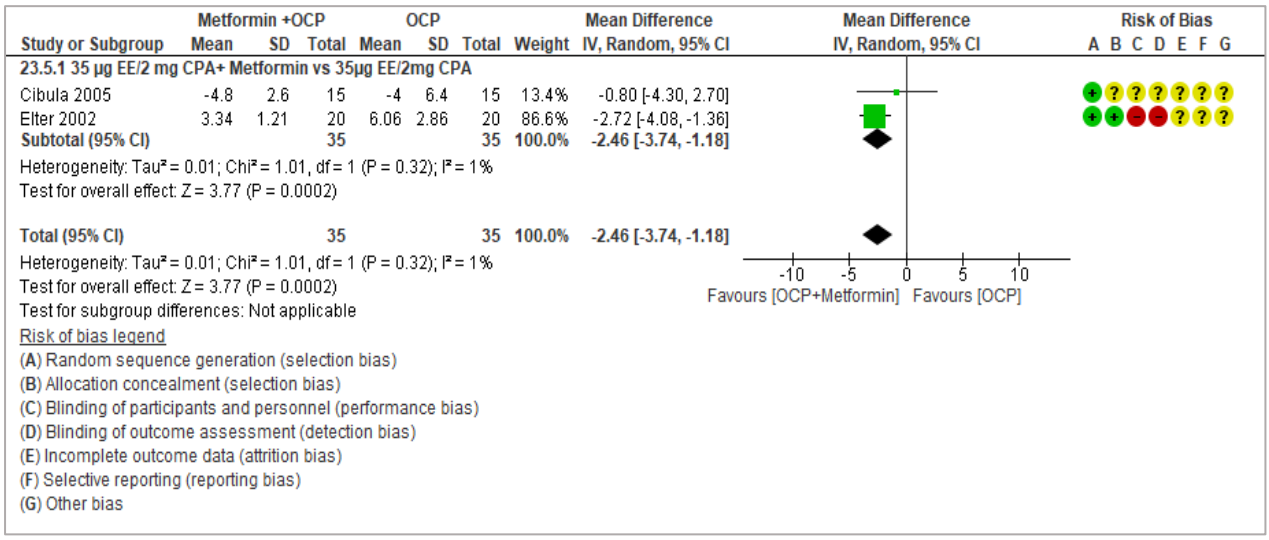
Figure 6-65: Forest plot of Metformin versus placebo on the androstenedione



6.3.12.6 OCP + Metformin versus OCP

Two RCTs compared OCP (35 µg EE/2 mg CPA) added to metformin with OCP (35 µg EE/2 mg CPA) alone showed a significant reduction in the level of androstenedione (MD: -2.46 nmol/L; 95% CI: -3.74, -1.16) (Figure 6-66) (very low-grade evidence, table 10).

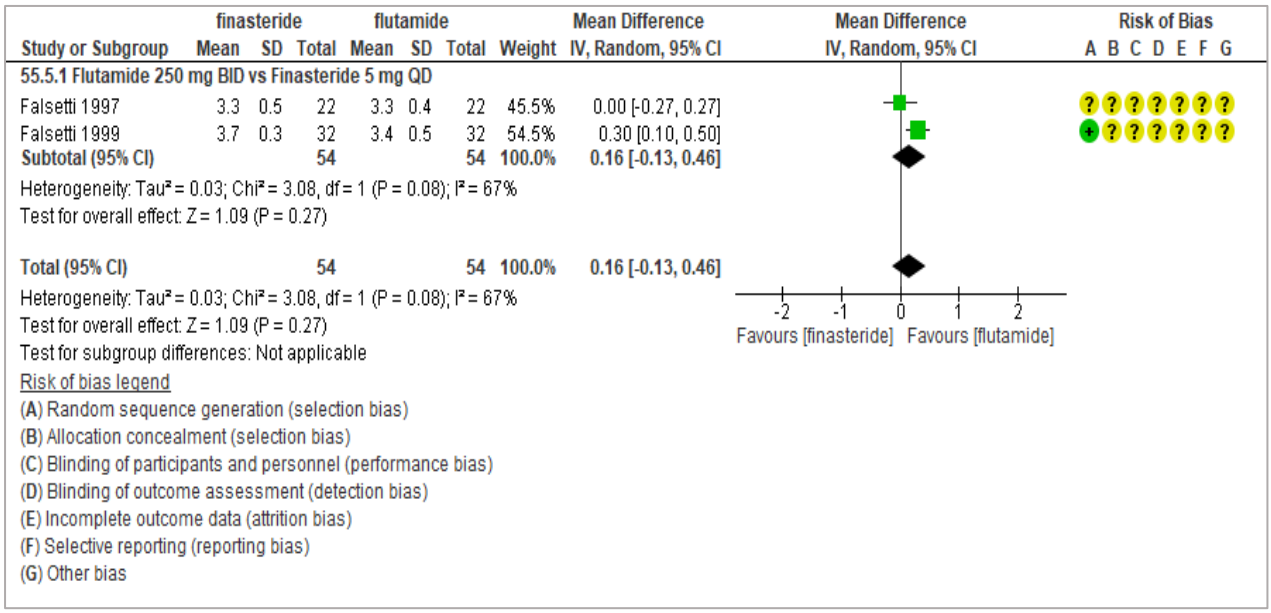
Figure 6-66: Forest plot of OCP+ Metformin versus OCP on the androstenedione (nmol/L)



6.3.12.7 Finasteride versus Flutamide

Finasteride 5 mg QD compared with flutamide 250 mg BID in two RCTs showed no effect on the level of androstenedione (MD: 0.16 nmol/L; 95%CI: -0.13, 0.46) (Figure 6-67) (very low-grade evidence, table 10).

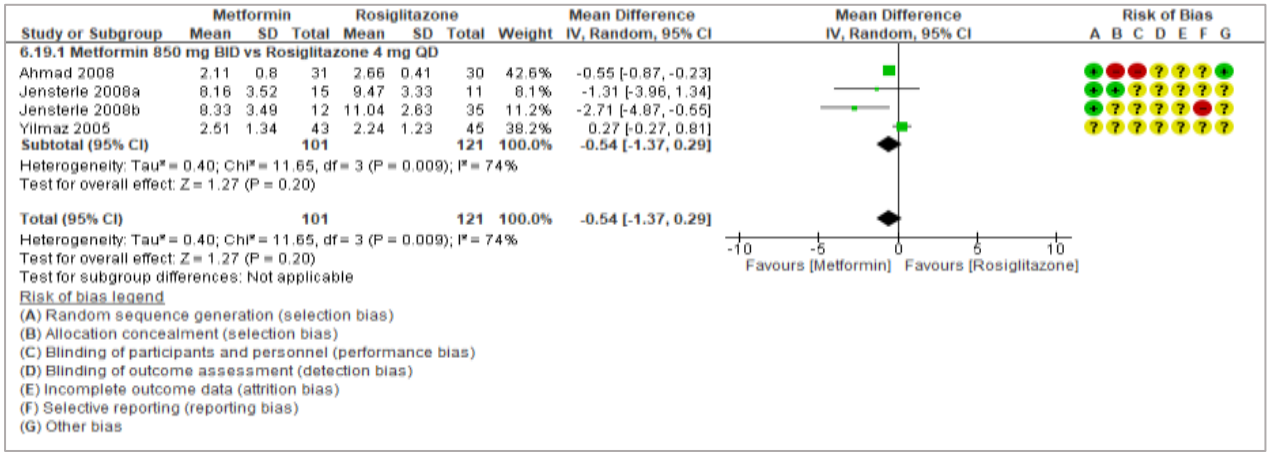
Figure 6-67: Forest plot of Flutamide versus Finasteride on the androstenedione (nmol/L)



6.3.12.8 Metformin versus Rosiglitazone

Four RCTs compared metformin 850 mg BID with rosiglitazone 4 mg DQ showed no effect on the serum level of androstenedione (MD: -0.54 nmol/L; 95% CI: -1.37, 0.29) (Figure 6-68) (very low-grade evidence, table 10).

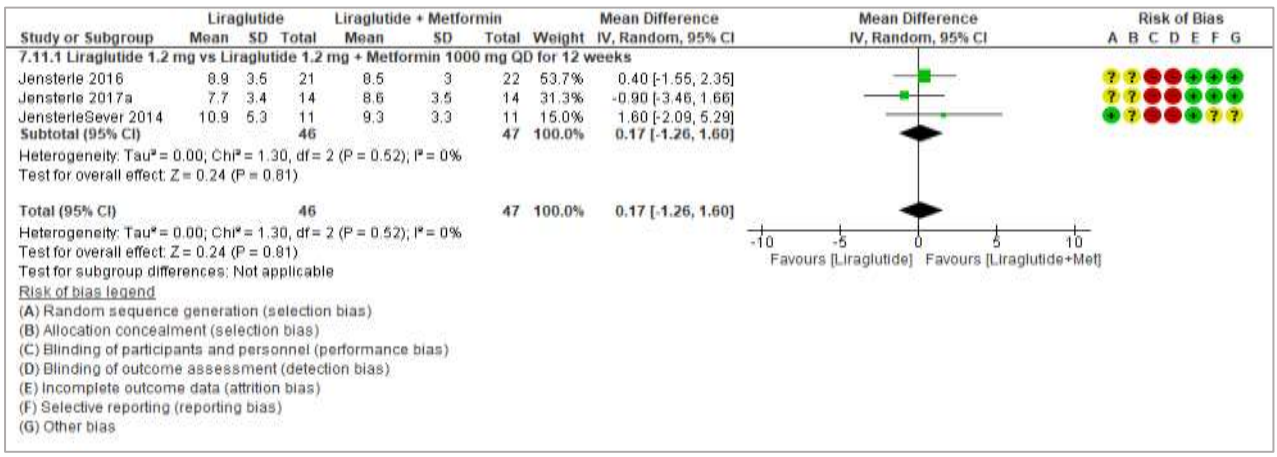
Figure 6-68: Forest plot of Metformin versus Rosiglitazone on the androstenedione (nmol/L)



6.3.12.9 Liraglutide versus Liraglutide + Metformin

Three RCTs compared liraglutide 1.2 mg QD with liraglutide 1.2 mg QD added to metformin 1000 mg QD for 12 weeks showed no effect on the level of androstenedione (MD: 0.17 nmol/L; 95% CI: -1.26, 1.60) (Figure 6-69) (very low-grade evidence, table 10).

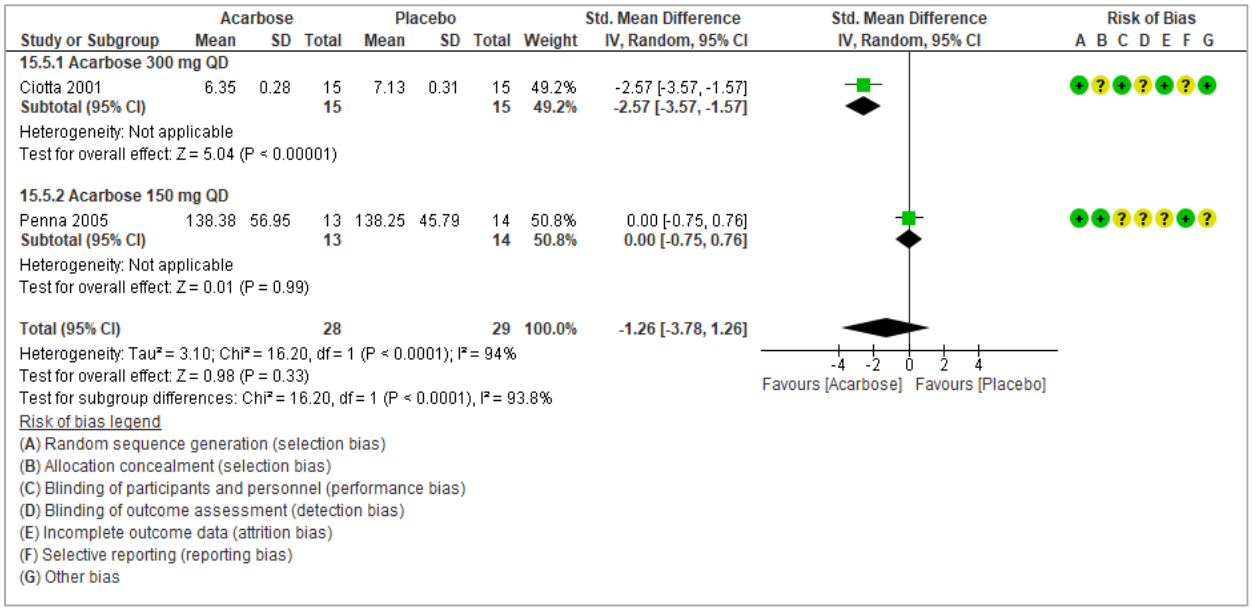
Figure 6-69: Forest plot of Liraglutide versus Liraglutide + Metformin on the androstenedione (nmol/L)



6.3.12.10 Acarbose versus placebo

One RCT compared acarbose 300 mg QD with placebo showed a significant reduction in the level of androstenedione (SMD: -2.57; 95%CI: -3.57, 1.57). Another RCT compared acarbose 150 mg QD with placebo showed no effect on the level of androstenedione (SMD: 0.00; 95% CI:-0.75,0.76). Overall, acarbose at various dosages has no effect on the level of androstenedione compared with placebo (SMD: -1.26; 95% CI: -3.78, 1.26) (Figure 6-70)(very low-grade evidence, table 10).

Figure 6-70: Forest plot of Acarbose versus placebo on the androstenedione

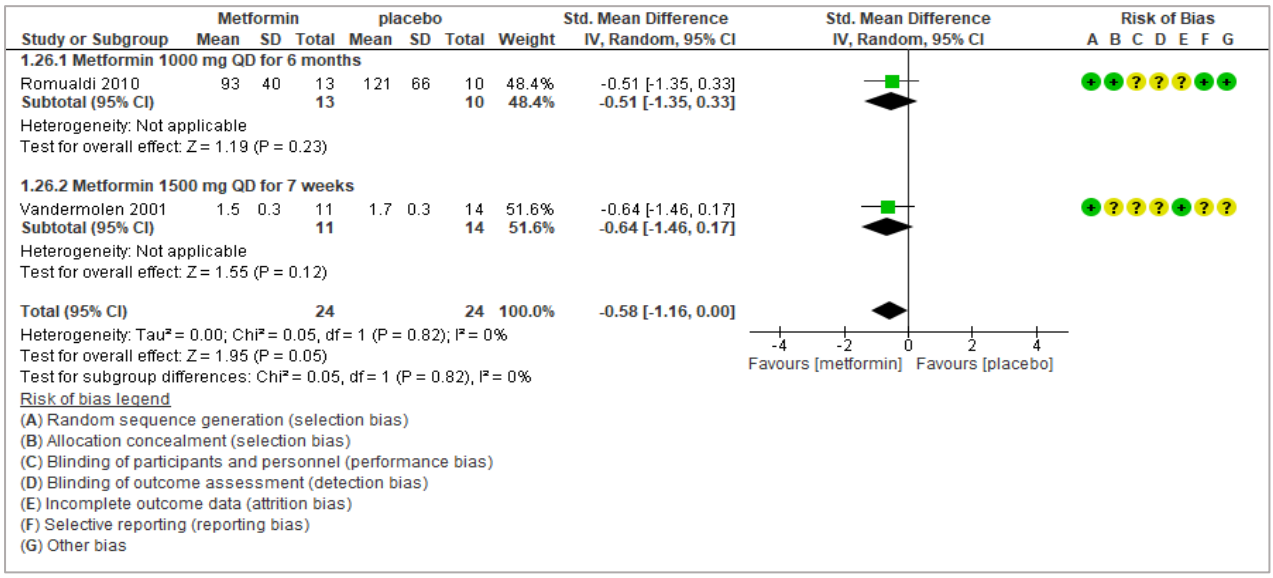


6.3.13 17-hydroxyprogesterone (17-OHP)

6.3.13.1 Metformin versus placebo

In one RCT, metformin 1000 mg QD for six months had no effect on 17-OHP compared with placebo (SMD: -0.51; 95% CI: -1.35, 0.33). In another RCT, metformin 1500 mg QD for seven weeks also has had no effect on 17-OHP (SMD: -0.64; 95% CI: -1.46, 0.17). However, the pooled estimate showed that metformin significantly reduced 17-OHP when compared with placebo (SMD: -0.58; 95%CI: -1.16, 0.00) (Figure 6-71) (very low-grade evidence, table 10).

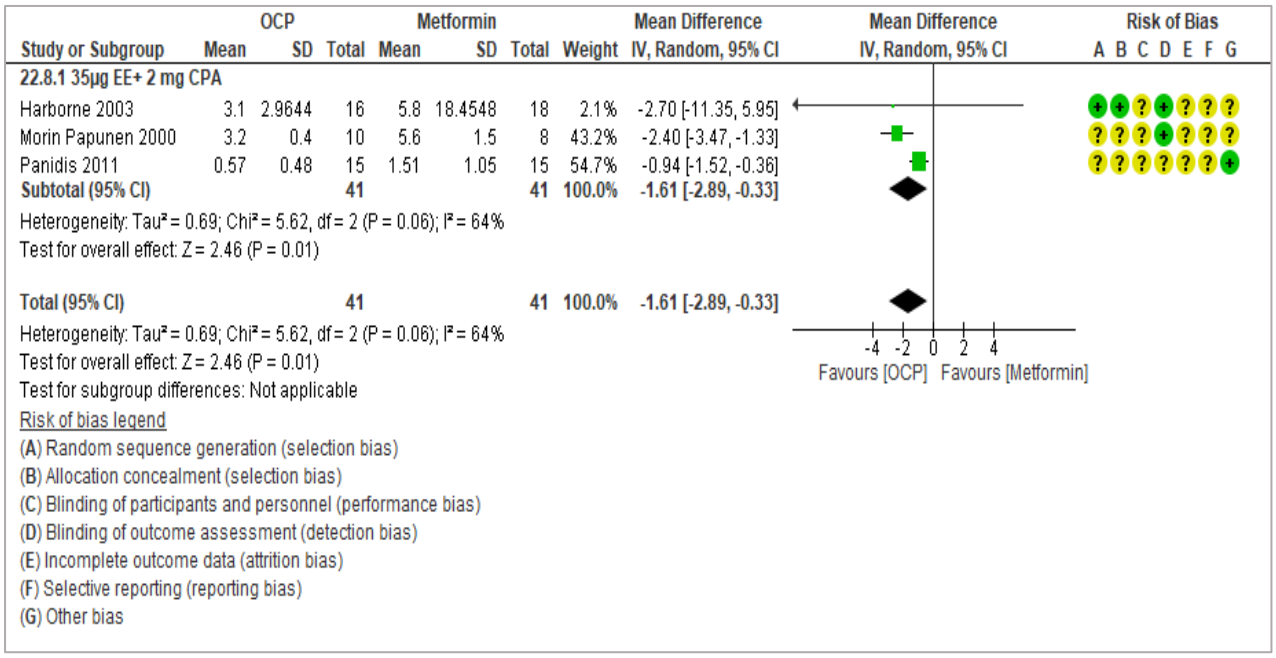
Figure 6-71: Forest plot of Metformin versus placebo on the 17-OHP



6.3.13.2 OCP versus Metformin

Three RCTs compared OCP (35µg EE/2 mg CPA) with metformin showed a significant reduction in 17-OHP (MD: -1.61 nmol/L; 95% CI: -2.89,-0.33) (Figure 6-72) (very low-grade evidence, table 10).

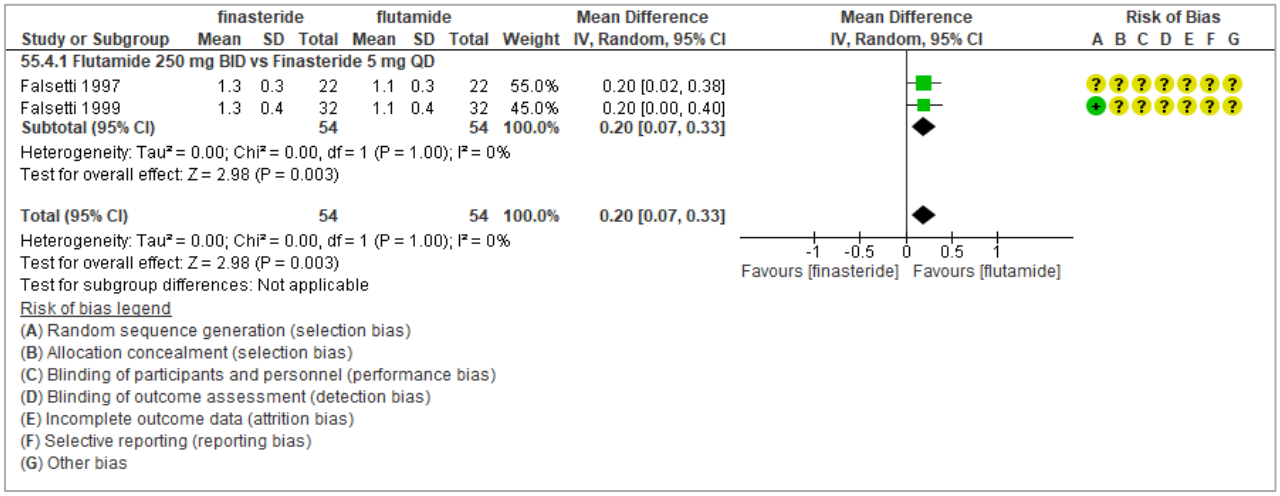
Figure 6-72: Forest plot of OCP versus Metformin on 17-OHP (nmol/L)



6.3.13.3 Flutamide versus Finasteride

In two RCTs, flutamide 250 mg BID significant increase in the 17-OHP when compared with finasteride 5 mg QD (MD: 0.20 nmol/L; 95% CI: -0.07,-0.33) (Figure 6-73) (very low-grade evidence, table 10).

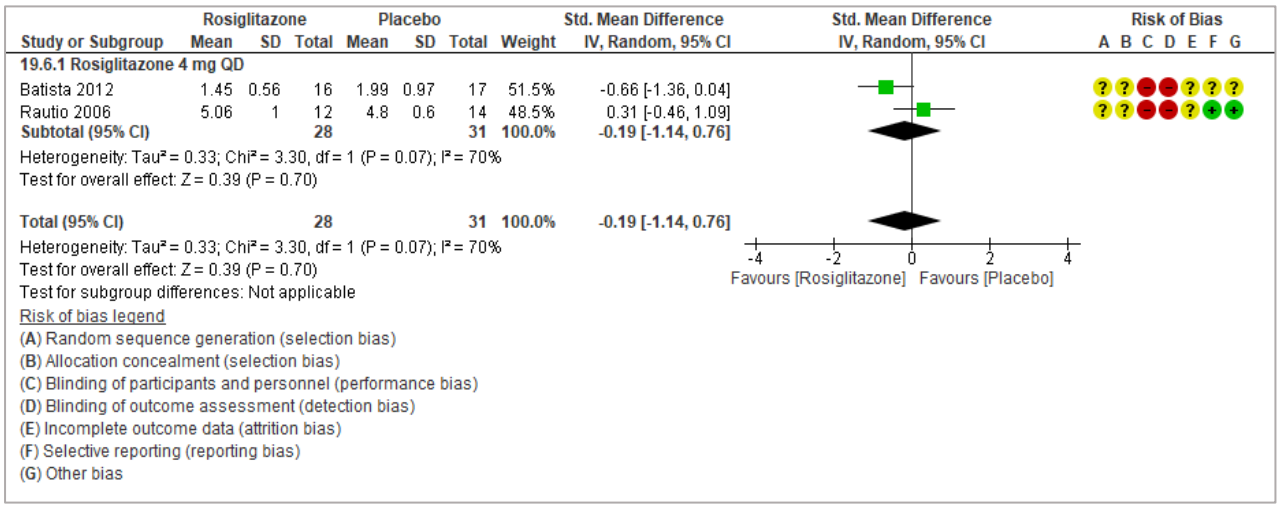
Figure 6-73: Forest plot of Finasteride versus Flutamide on 17-OHP (nmol/L)



6.3.13.4 Rosiglitazone versus placebo

Two RCTs compared rosiglitazone 4 mg QD with placebo showed no effect on the level of 17-OHP (SMD: -0.19;95% CI: -1.14, 0.76) (Figure 6-74) (very low-grade evidence, table 10).

Figure 6-74: Forest plot of Rosiglitazone versus placebo on 17-OHP

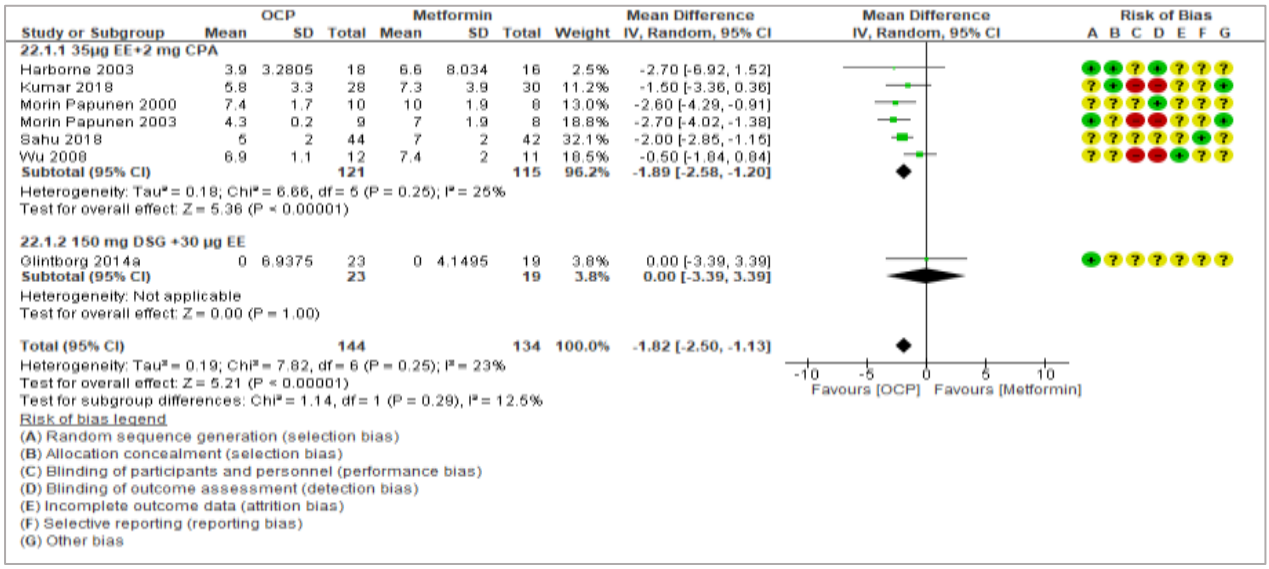


6.3.14 Hirsutism score

6.3.14.1 OCP versus Metformin

Six RCTs compared OCP (35 µg EE/2 mg CPA) with metformin showed significant reduction in the hirsutism score (MD: -1.89; 95% CI: -2.58, -1.20). One RCT compared OCP (150 DSG/ 30 µg DSG) with metformin showed no effect on the hirsutism score (MD: 0.00; 95% CI: -3.30, 3.39). Overall, OCP of various form and dosage compared with metformin significantly reduced the hirsutism score (MD: -1.82; 95% CI: -2.50, 1.13) (Figure 6-75) (very low-grade evidence, table 10).

Figure 6-75: Forest plot of OCP versus Metformin on hirsutism score

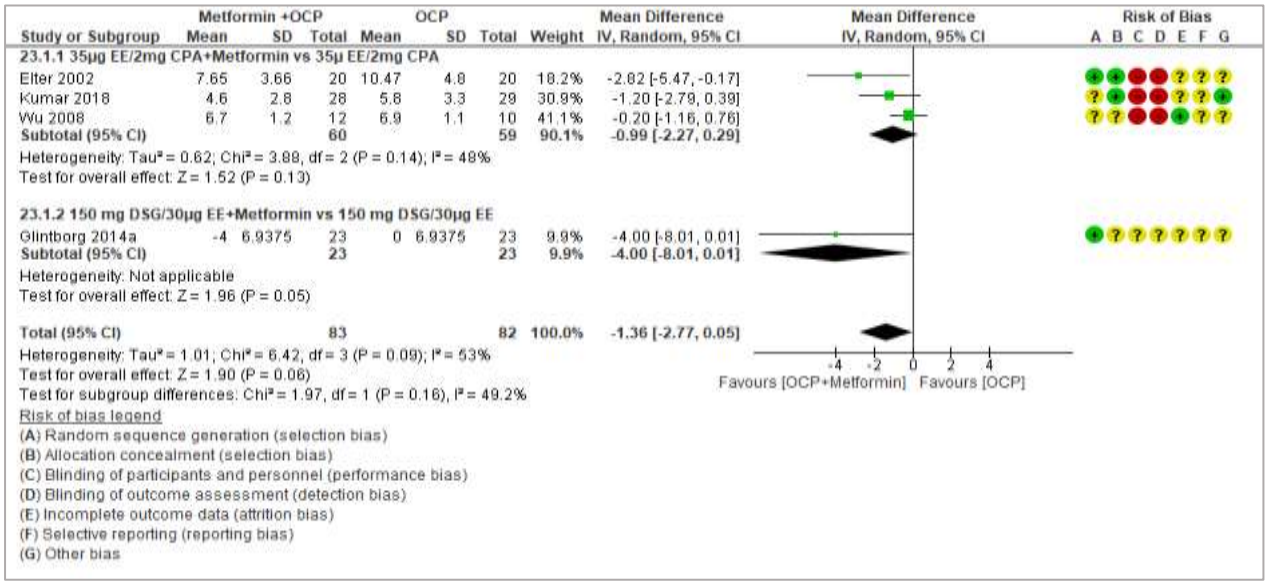


6.3.14.2 Metformin + OCP versus OCP

Three RCTs compared metformin added to OCP (35 µg EE/ 2 mg CPA) compared with OCP (35 µg EE/ 2 mg CPA) alone showed no effect on the hirsutism sore (MD: -0.99;95% CI; -2.27,0.29). One RCT compare OCP (150 mg DSG/ 30 µg EE) added to metformin with OCP (150 mg DSG/ 30 µg EE) alone showed significant reduction in the hirsutism score (MD: -4.00; 95% CI: -8.01,-0.01). Overall, regardless of the type or the dosage, OCP added to metformin significantly

reduced the hirsutism score compared with OCP alone (MD: -1.36; 95% CI: -2.77, 0.05) (Figure 6-76) (very low-grade evidence, table 10).

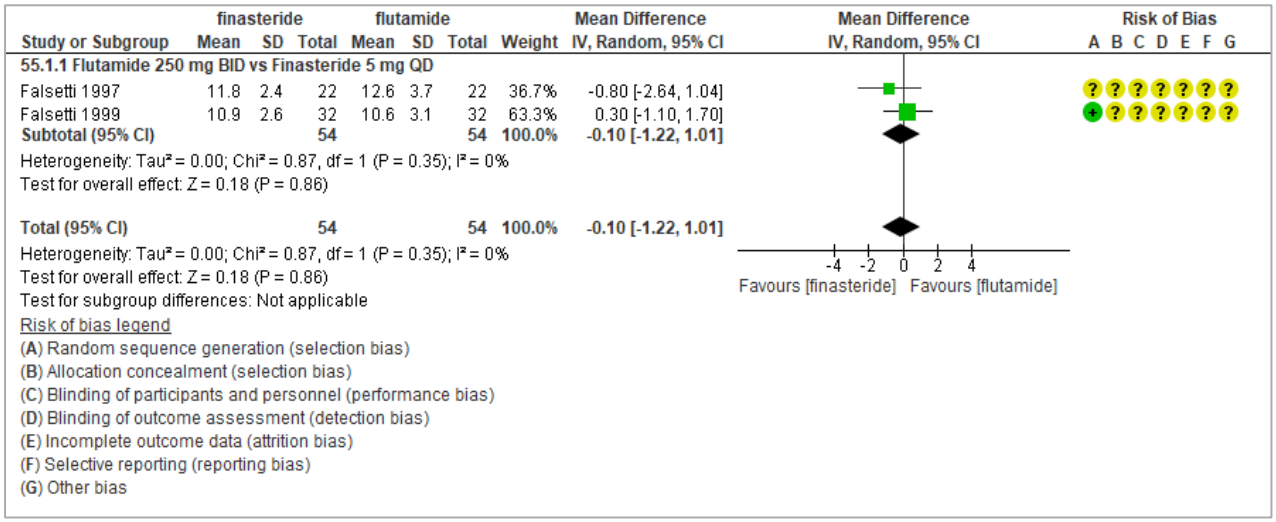
Figure 6-76: Forest plot of OCP + Metformin versus OCP on hirsutism score



6.3.14.3 Finasteride versus Flutamide

Two RCTs compared finasteride 5 mg QD with flutamide 250 mg BID showed no effect on the hirsutism score (MD: -0.10; 95% CI: -1.22, 1.01) (Figure 6-77) (very low-grade evidence, table 10).

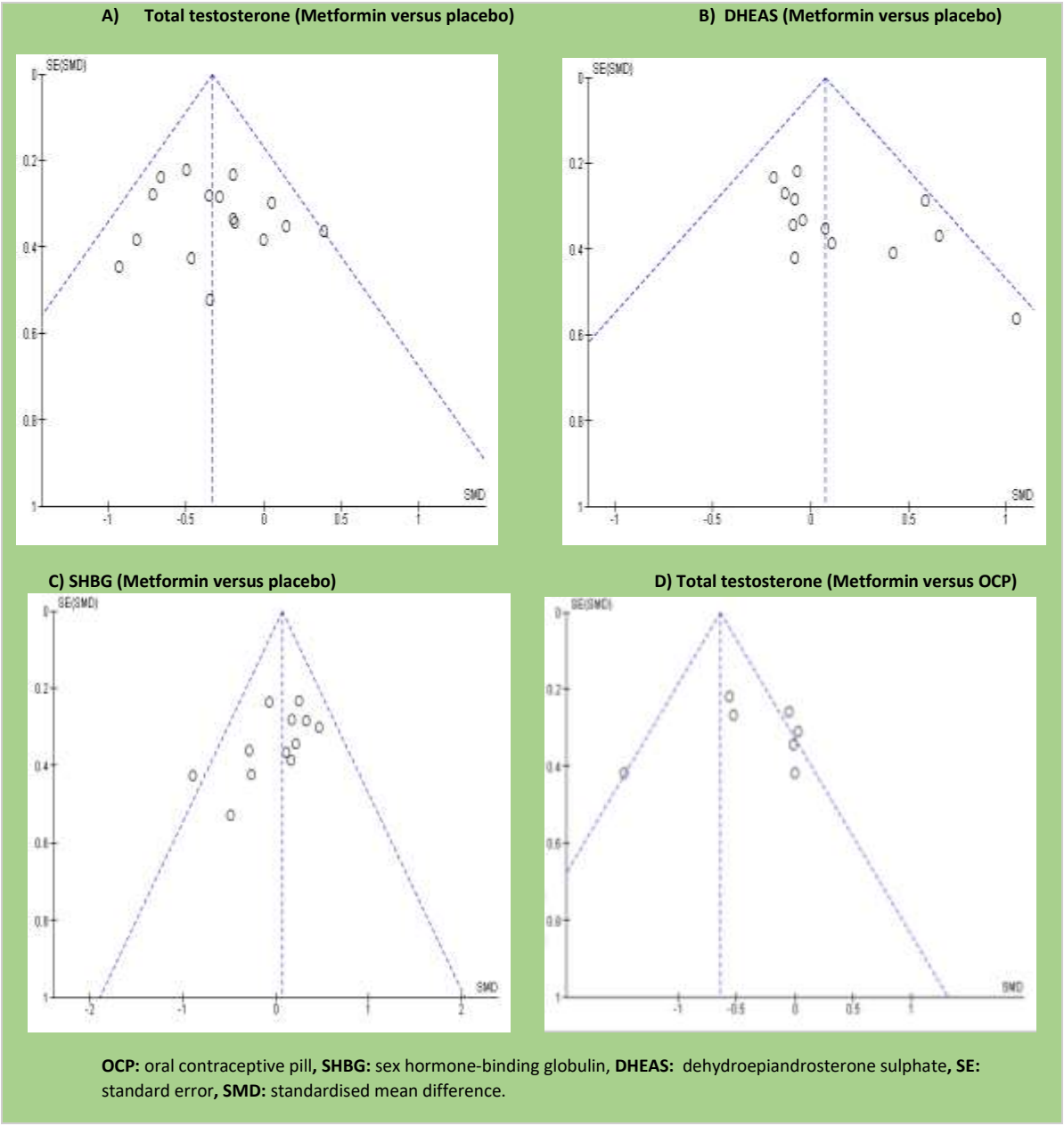
Figure 6-77: Forest plot of Flutamide versus Finasteride on hirsutism score



6.3.15 Assessment of publication bias

In two comparisons (metformin versus placebo and OCP versus metformin), there were ten or more RCTs. Thus, the funnel plot of the RevMan with standard error (SE) was used to assess publication bias in four outcomes: total testosterone-metformin versus placebo (Figure 6-78 A), DHEAS-metformin versus placebo (Figure 6-78 B), SHBG-metformin versus placebo (Figure 6-78 C) and total testosterone-OCP versus metformin (Figure 6-78 D). There was no significant asymmetry of the treatment effect for the assessed outcomes, which implied a low chance of publication bias. We did not assessed the publication bias in the other comparisons as they included fewer than 10 RCTs.

Figure 6-78: Funnel plots of comparisons



OCP: oral contraceptive pill**, SHBG:** sex hormone-binding globulin, **DHEAS:** dehydroepiandrosterone sulphate**, SE:** standard error**, SMD:** standardised mean difference.

6.3.16 Sensitivity analysis

Small sample-sized RCTs and those with high RoB were eliminated from the analysis while monitoring their impact on the final results. No significant effect was found, and hence no RCT was removed from the meta-analysis.

Table 10: Summary of findings

Patient or population: PCOS **Setting:** **Intervention: First treatment (T1)** **Comparison: Second treatment (T2)**					
Outcome	**№ of participants (studies)**	**Certainty of the evidence (GRADE)**	**Relative effect (95% CI)**	**Anticipated absolute effects**	
				Assumed risk	
				Risk difference with intervention	**Risk difference with comparison**
Meformin versus placebo					**(T1 minus T2)**
Total testosterone	690 (16 RCTs)	⊕⊕⊕◯ MODERATE	-	MD 0.33 lower (0.49 lower to 0.17 lower)	the mean total testosterone range 0.2-161
Free testosterone	108 (3 RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.88 lower (2.86 lower to 1.10 higher)	the mean free testosterone range 2.9-35
DHEAS	534 (13 RCTs)	⊕⊕⊕◯ MODERATE	-	MD 0.32 higher (0.53 lower to 1.17 higher)	the mean DHEAS range 7-251
SHBG	477 (12RCTs)	⊕⊕⊕◯ MODERATE	-	MD 0.05 higher (0.21 lower to 0.30 higher)	the mean SHBG range 0.8-60
Oestradiol	199 (5RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.31 lower (13.35 lower to 12.72 higher)	the mean Oestradiol range 47-150
A4	175 (6 RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.22 lower (0.51 lower to 0.07 higher)	the mean A4 range 1.2-4.2
FAI	165 (5 RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.07 lower (1.42 lower to 1.27 higher)	the mean FAI range 5.3-7.9
17-OHP	48 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 4.05 lower (22.88 lower to 14.77)	the mean 17-OHP range 1.7-121
Acarbose versus placebo					**(T1 minus T2)**
Total testosterone	57 (2 RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 1.22 lower (1.53 lower to 0.91 lower)	the mean total testosterone range 1.87-69
SHBG	57 (2 RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 10.75 higher (6.69 lower to 28.19 higher)	the mean SHBG range 22.15-35.4
A4	57 (2 RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.78 lower (1.60 lower to 0.04 higher)	the mean A4 range 7.13-138
Bromocriptine versus Placebo					**(T1 minus T2)**
Total testosterone	77 (2 RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.10 lower (0.21 lower to 0.01 higher)	the mean total testosterone range -0.9-0.6
A4	77 (2 RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.30 lower (0.75 lower to 0.15 higher)	the mean A4 range -7.7-2.3
Dexamethasone versus placebo					**(T1 minus T2)**
Total testosterone	76 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.86 lower (1.34 lower to 0.39 lower)	the mean total testosterone range -0.33-2.79
DHEAS	76 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	SMD 1.08 lower (2.20 lower to 0.04 lower)	the mean DHEAS range 1.0-7.1
A4	76 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 3.52 lower (5.76 lower to 1.27 lower)	the mean A4 range 1.3-16.9
Pioglitazone versus placebo					**(T1 minus T2)**
Total testosterone	114 (4 RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.12 lower (0.48 lower to 0.25 higher)	the mean total testosterone range 0.41-2.5
Free testosterone	107 (4 RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.01 higher (0.04 lower to 0.07 higher)	the mean free testosterone range 0.04-81
SHBG	114 (4RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 2.57 lower (4.35 lower to 0.79 lower)	the mean SHBG range 20.21-35.8
Oestradiol	56 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 31.34 lower (82.10 lower to 19.41 lower)	the mean oestradiol range 197-198
Sitagliptin versus placebo					**(T1 minus T2)**
Total testosterone	62 (2 RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.19 higher (0.82 lower to 1.20 higher)	the mean total testosterone range 1.5-48.4
Free testosterone	62 (2 RCTs)	⊕◯◯◯ VERY LOW a,c	-	SMD 0.47 lower (0.97 lower to 0.03 lower)	the mean free testosterone range 6.8-22.9
SHBG		⊕◯◯◯ VERY LOW a,c			

	62 (2 RCTs)		-	MD 12.96 lower (40.21 lower to 14.29 lower)	the mean SHBG range 33.4-54.3
Flutamide versus placebo					**(T1 minus T2)**
Total testosterone	131 (4 RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.02 higher (0.20 lower to 0.25 higher)	the mean total testosterone range 0.45-1.36
Free testosterone	95 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.13 higher (0.23 lower to 0.50 higher)	the mean free testosterone range 0.55-2.9
DHEAS	131 (4RCTs)	⊕◯◯◯ VERY LOW a,c	-	SMD 0.46 lower (0.83 lower to 0.09 lower)	the mean DHEAS range 1.85-166
SHBG	109 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 7.62 higher (2.25 higher to 12.98 higher)	the mean SHBG range 21.9-24.14
A4	78 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 1.68 lower (3.56 lower to 0.20 higher)	the mean A4 range 3.14-242
atorvastatin versus placebo					**(T1 minus T2)**
Total testosterone	65 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.40 lower (2.36 lower to 1.56 higher)	the mean total testosterone range 0.7-4.3
SHBG	65 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 3.87 higher (5.85 lower to 13.58 higher)	the mean SHBG range 30.94-40.1
FAI	65 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 1.13 lower (7.99 lower to 5.73 higher)	the FAI range 1.9-13.3
Rosiglitazone versus placebo					**(T1 minus T2)**
DHEAS	59 (2RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	MD 11.45 lower (47.16 lower to 24.27 higher)	the mean DHEAS range 5.4-215
SHBG	113 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 3.87 higher (5.85 lower to 13.58 higher)	the mean SHBG range 30.94-40.1
A4	59 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 2.24 lower (3.23 lower to 1.24 lower)	the mean A4 range 4-16.8
17-OHP	59 (2RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	MD 0.16 lower (0.94 lower to 0.62 higher)	the mean 17-OHP range
Metformin versus Pioglitazone					**(T1 minus T2)**
Total testosterone	129 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	SMD 0.12 lower (0.22 lower to 0.02 lower)	the mean total testosterone range 0.7-0.79
DHEAS	145 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.18 lower (0.43 lower to 0.08 higher)	the mean DHEAS range 1.6-221
SHBG	59 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 5.52 lower (12.69 lower to 1.65 lower)	the mean SHBG range 32-36.6
FAI	59 (2RCTS)	⊕◯◯◯ VERY LOW a,b,c	-	MD 1.35 higher (0.48 lower to 3.18 higher)	the mean FAI range 4.7-8.1
Liraglutide versus Metformin					**(T1 minus T2)**
Total testosterone	55 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.05 lower (0.57 lower to 0.47 higher)	the mean total testosterone range 1.5-2.1
Free testosterone	55 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.42 lower (1.52 lower to 0.68 higher)	the mean free testosterone range 4.3-4.7
SHBG	55 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 2.39 higher (3.32 lower to 9.17 higher)	the mean SHBG range 26.2-35.6
FAI	55 (2RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	MD 0.34 higher (1.85 lower to 2.54 higher)	the mean FAI range 4.8-9.6
Metformin vs Rosiglitazone					**(T1 minus T2)**
Free testosterone	237 (5RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.29 higher (1.72 lower to 2.30 higher)	the mean free testosterone range 2.19-10.13
Total testosterone	230 (6 RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.13 lower (0.31 lower to 0.06 higher)	the mean total testosterone range 0.53-2.96
DHEAS	283 (5 RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 1.50 lower (5.02 lower to 2.03 higher)	the mean DHEAS range 6.68-240.23
A4	223 (4 RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.54 lower (1.37 lower to 0.29 higher)	the mean A4 range 2.24-11.4
Liraglutide versus Liraglutide + Metformin					**(T1 minus T2)**
A4	93 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.17 higher (1.26 lower to 1.60 higher)	the mean A4 range 8.5-9.3
Total testosterone	93 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.07 lower (0.46 lower to 0.31 higher)	the mean total testosterone range 1.5-1.6
Free testosterone	93 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.34 higher (1.69 lower to 2.38 higher)	the mean free testosterone range 2.7-4.5
SHBG	93 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 8.56 lower (25.77 lower to 8.64 higher)	the mean range of SHBG 33.4-4
Exenatide versus Metformin					**(T1 minus T2)**
Total testosterone	149 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.33 higher (0.76 lower to 1.41 higher)	the mean total testosterone range 1.93-53.2
FAI	149 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.22 higher (0.57 lower to 1.01 higher)	the mean FAI range 7.25-11.4
SHBG	149 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.56 higher (2.74 lower to 3.86 higher)	the mean SHBG range 18.7-37.9

DHEAS	149 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 24.39 higher (2.47 higher to 46.31 higher)	the mean DHEAS range 161.7-268
Flutamide+ Metformin versus Flutamide					**(T1 minus T2)**
Total testosterone	111 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.05 lower (0.23 lower to 0.12 higher)	the mean total testosterone range 0.5-2
Free testosterone	111 (3RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	MD 0.07 lower (0.76 lower to 0.62 higher)	the mean free testosterone range 0.78-2.19
DHEAS	111 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.86 higher (0.80 lower to 2.52 higher)	the mean DHEAS range 1.5-145
SHBG	111 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 4.69 lower (16.13 lower to 6.75 higher)	the mean SHBG range 28.4-41.08
OCP (30 µg EE/DRSP) versus OCP (20 µg EE/DRSP)					**(T1 minus T2)**
Total testosterone	138(2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.06 higher (0.08 lower to 0.21 higher)	the mean total testosterone range 0-0.43
FAI	138(2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.07 higher (0.11 lower to 0.25 higher) MD	the mean FAI range 0.32-4.81
SHBG	138(2RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	27.54 lower (128.35 lower to 73.28higher)	the mean SHBG range 108-159.1
Saxagliptin versus Metformin					**(T1 minus T2)**
Total testosterone	65 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.18 higher (0.19 lower to 0.54 higher)	the mean total testosterone range 1.1-2.11
SHBG	65 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 6.61 higher (2.46 lower to 15.68 higher)	the mean SHBG range 20-26.98
FAI	65 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.55 lower (2.46 lower to 1.35 higher)	the mean FAI range 6.3-9.13
Cabergoline+ Metformin versus Cabergoline					**(T1 minus T2)**
Total testosterone	360 (2RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	MD 0.01 higher (0.24 lower to 0.27 higher)	the mean total testosterone range 0.87-0.9
DHEAS	360 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 19.82 lower (94.21 lower to 54.58 higher)	the mean DHEAS range 215.29-291.29
OCP versus Metformin					**(T1 minus T2)**
Total testosterone	266(11RCTs)	⊕◯◯◯ VERY LOW a,c	-	SMD 0.35 lower (0.55 to 0.15 lower)	the mean total testosterone range 0.8-76.5
SHBG	243 (8RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	MD 103.30 higher (55.54 higher to 151.05 higher)	the mean SHBG range 9-79.6
FAI	155 (5RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 6.08 lower (9.34 lower to 2.83 lower)	the mean FAI range 3.8-12.9
DHEAS	243(8RCTs)	⊕◯◯◯ VERY LOW a,c	-	SMD 3.74 lower (6.90 lower to 0.58 lower)	the mean DHEAS range 5.9-214
17-OHP	82 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 1.61 lower (2.89 lower to 0.33 lower)	the mean 17-OHP range 1.51-6.8
OCP+ Metformin versus OCP					**(T1 minus T2)**
Total testosterone	301(7RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.03 lower (0.15 lower to 0.09 higher)	the mean total testosterone 0.36-2.1
DHEAS	161(5RCTs)	⊕◯◯◯ VERY LOW a,c	-	SMD 27.33 lower (58.88 lower to 4.22 higher)	the mean DHEAS range 7.8-300
A4	70(2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 2.46 lower (3.74 lower to 1.18 lower)	the mean A4 range -4.6.6
SHBG	224 (5RCts)	⊕◯◯◯ VERY LOW a,c	-	MD 6.06 higher (17.79 lower to 29.83 higher)	the mean SHBG range 23-113
Free testosterone	104 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.04 lower (0.46 lower to 0.38 lower)	the mean free testosterone 3.7-8.9
EE/CPA versus EE/DRSP					**(T1 minus T2)**
Total testosterone	89(3 RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.01 lower (0.77 lower to 0.75 higher)	the mean total testosterone range 0.42-55
Simvastatin +OCP versus OCP					**(T1 minus T2)**
Total testosterone	93 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 10.44 lower (33.40 lower to 12.52 lower)	the mean total testosterone range 0.2-10.9
DHEAS	93 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.04 higher (0.24 lower to 0.32 higher)	the mean DHEAS range -0.80-0.96
SHBG	93 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 6.96 lower (25.99 lower to 12.07 higher)	the mean SHBG range 76-83.5
Flutamide versus Finasteride					**(T1 minus T2)**
17-OHP	108(2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.20 lower (0.7 lower to 0.33 higher)	the mean 17-OHP range 1.1-1.1
A4	108(2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.16 higher (0.13 lower to 0.46 higher)	the mean A4 range 3.3-3.4
Total testosterone	108(2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.46 lower (0.36 lower to 0.56 lower)	the mean total testosterone range 0.7-0.9

Free testosterone	108(2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 0.20 lower (0.48 lower to 0.08 higher)	the mean free testosterone range 3.-3.6
DHEAS	108(2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD -0.37 lower (0.05 lower to 0.70 lower)	the mean DHEAS range 2.4-2.5
SHBG	108(2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 1.06 higher (1.78 lower to 3.90 higher)	the mean SHBG range 19.1-21
OC+ Spironolactone versus OC					**(T1 minus T2)**
DHEAS	89 (2RCTs)	⊕◯◯◯ VERY LOW a,c	-	MD 6.77 higher (21.95 lower to 35.50 higher)	the mean DHEAS range 168-289

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **MD:** Mean difference, S**HBG:** sex hormone-binding globulin**, OCP:** oral contraceptive pill **LH:** luteinising hormone, **FSH:** follicular stimulating hormone, **17-OHP:** hydroxyprogesterone**, FAI:** free androgen index, **A4:** Androstenedione, **CI:** confidence interval**, RCTs:** randomised controlled trials, **T1:** first treatment, **T2:** second treatment

GRADE Working Group grades of evidence.

High certainty: We are very confident that the true effect lies close to that of the estimate of the effect.

Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.

Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

Explanations

a. Some studies have a high risk of performance bias and an unclear risk of bias across 5 out of the 7 domains. Thus, we downgraded one level.

b. A considerable level of heterogeneity across the studies.

c. small sample size with a wide confidence interval. We downgraded one level.

6.4 Discussion

This systematic review and meta-analysis have collated and analysed the available evidence on the effects of different pharmacological interventions on the androgenic hormonal profiles in women with PCOS. Metformin, dexamethasone, OCP (35 µg EE/2 mg CPA, 20 µg EE/150 ug DSG), rosiglitazone, pioglitazone and flutamide, administered alone or in combination with each other had a significant effect on the total testosterone, free testosterone, SHBG, DHEAS, A4, FAI and oestradiol levels. However, the strength of the evidence ranged from moderate to very low-grade across the outcomes reported in this review.

This review found beneficial effects on the androgen hormones. Regardless of the dosage and the duration that metformin significantly reduced the level of total testosterone compared with placebo. A similar trend in the testosterone level was reported in a recent systematic review and meta-analysis of nine RCTs with 458 women with PCOS (WMD: −8.96, 95% CI: −12.30, −5.62; $p < 0.0001$)(638). Our study also found that metformin reduced the levels of free testosterone, FSH, LH, DHEAS when compared with OCP. This significant effect was also reported in a systematic review and meta-analysis in which metformin was compared with OCP and significantly reduced FAI ($p = 0.001$) and total testosterone ($p = 0.004$). At the same time, increased SHBG ($p = 0.0001$)(781). A similar significant effect was also evident when metformin was combined with an OCP. However, a recent systematic review and meta-analysis that assessed the effect of metformin versus OCP in women with PCOS did not find any effect on the total testosterone (782). Flutamide has also significantly reduced the level of total testosterone and DHEAS compared with placebo or finasteride; however, the results from the most recent systematic review did not find any effect on total testosterone (783). We also found that thiazolidinedione (TZD) alone and combined with metformin significantly

reduced androgen hormones. These results are in accord with the findings from a previous meta-analysis that compared the effect of TZD with metformin in PCOS, which reported that TZD significantly reduced A4 and free testosterone but had no effect on other androgen hormones (700).

A strength of this study is that we only included RCTs and excluded all narrative reviews, observational studies and non-RCTs to reduce the risk of bias. The included RCT's quality was assessed using the GRADE system to determine the strength of evidence. Several weaknesses were identified, including the high heterogeneity among the RCTs in reporting the outcomes and using unified measuring scales. However, this was mitigated by using an appropriate statistical method to pool and interpret the overall effect estimates. In addition, we used a language filter. As a result, only RCTs reported in the English language were included; therefore, several studies in foreign languages may not have been retrieved that may have had an impact, though, in mitigation, the assessment for publication bias showed no bias. Pharmacological interventions used in the management of PCOS, including metformin and OCP, are associated with a reduction in the androgen hormones improving the clinical symptoms of PCOS. Furthermore, such interventions enhance reproductive function and prevent the long-term health risk associated with PCOS.

6.5 Conclusion

The available data showed that pharmacological interventions in women with PCOS improve the parameters of the androgen hormones. For example, metformin, OCP, flutamide, TZD, dexamethasone and acarbose significantly affect the total testosterone, free testosterone, FAI, SHBG and DHEAS.

7 Chapter 7: Impact of pharmacological interventions on fertility outcomes in women with polycystic ovary syndrome: a systematic review and meta-analysis of randomised controlled trials

7.1 Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine condition that affects around 20 % of women of reproductive age and accounts for up to 80% of anovulatory infertility (110, 506). One of the diagnostic criteria for PCOS is the Rotterdam criteria which require the presence of at least two out of the following: anovulation (irregular period), biochemical and clinical evidence of hyperandrogenaemia, and polycystic ovarian morphology on pelvis ultrasound (31). The clinical features are heterogeneous and include infertility, pregnancy-related complications, obesity, insulin resistance, type 2 diabetes mellitus (T2DM) and increased risk of cardiovascular disease (CVD)(784). In PCOS, several factors influence ovarian function, including insulin resistance. Hyperinsulinemia contributes to excess ovarian androgen production and positively correlates with insulin levels (55). Insulin promotes steroidogenesis in steroidogenic tissues such as the ovaries and the adrenal glands (785). Androgen actions are mediated by its receptors widely expressed in ovarian granulosa cells. Theca cells excessively produce ovarian androgen, which is converted to estrogen via the action of the follicular stimulating hormone (FSH) augmented aromatase enzyme (55). An increase in estrogen secretion inhibits the FSH through negative feedback to the pituitary gland; this leads to follicular atresia, thereby increasing the secretion and the responsiveness to the luteinising hormone (LH) (786).

Fertility treatment in PCOS includes oral agents such as clomiphene citrate (CC; antioestrogen), letrozole (aromatase inhibitors) and parental therapy such as gonadotropin treatment. However, as insulin resistance is one of the leading causes of infertility in PCOS, it is plausible to add an insulin sensitiser like metformin (436). Clomiphene citrate is a selective estrogen receptor modulator that blocks the estrogen receptor and increases the release of the gonadotropin-releasing hormone (GnRH), subsequently increasing the level of FSH and LH, thereby stimulating the follicular maturation and inducing ovulation in up to 90% of patients (787). As the aromatase enzyme is responsible for the androgen to estrogen conversion, letrozole, a selective aromatase inhibitor, suppresses the ovarian estradiol secretion with a subsequent increase in FSH and ovulatory rate (788). Gonadotropin injection therapy is considered a second-line infertility treatment for women who fail to respond to oral therapy. It is associated with a higher ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies. Exogenous gonadotropin FSH and the human menopausal gonadotropin stimulate follicular proliferation and growth (447). Metformin increases the peripheral glucose uptake and enhances insulin sensitivity. It also acts directly in the ovary and reduces androgen production by theca cells (445). However, the exact role of metformin in the management of infertility in PCOS is still controversial.

Several randomised controlled trials (RCTs) have examined different therapeutics and provided mixed results. Thus, the current study aimed to systematically search and review the literature and perform a meta-analysis for the effectiveness of the pharmacological interventions on the fertility outcomes in women with PCOS.

7.2 Methods and materials

7.2.1 Protocol and registration

The protocol and the registration of this systematic review and meta-analysis are explained in chapter 2, section 2.1.1.1.

7.2.2 Eligibility criteria for the included studies

The eligibility criteria, including inclusion/exclusion criteria, is explained in chapter 2, section 2.1.1.2.

7.2.3 Literature search

The search strategy, including the search in the medical databases, is explained in chapter 2, section 2.1.1.3.

7.2.4 Study selection

The study selection, including screening titles and abstracts of the retrieved studies, is explained in chapter 2, section 2.1.1.4.

7.2.5 Data extraction

The method used to extract information from the included studies is explained in chapter 2, section 2.1.1.5.

7.2.6 Risk of bias assessment in the included studies

The RoB assessment for the included studies is explained in chapter 2, section 2.1.1.6.

7.2.7 GRADE scoring

The robustness of evidence for each chosen outcome is assessed using the GRADE scoring system and is explained in chapter 2, section 2.1.1.7.

7.2.8 Statistical analysis

The statistical method used to estimate the pooled effects of the various interventions is explained in chapter 2, section 2.1.1.12.

7.2.9 Assessment of heterogeneity

Heterogeneity for the outcomes across each RCT was evaluated and explained in chapter 2, section 2.1.1.8.

7.2.10 Subgroup analysis

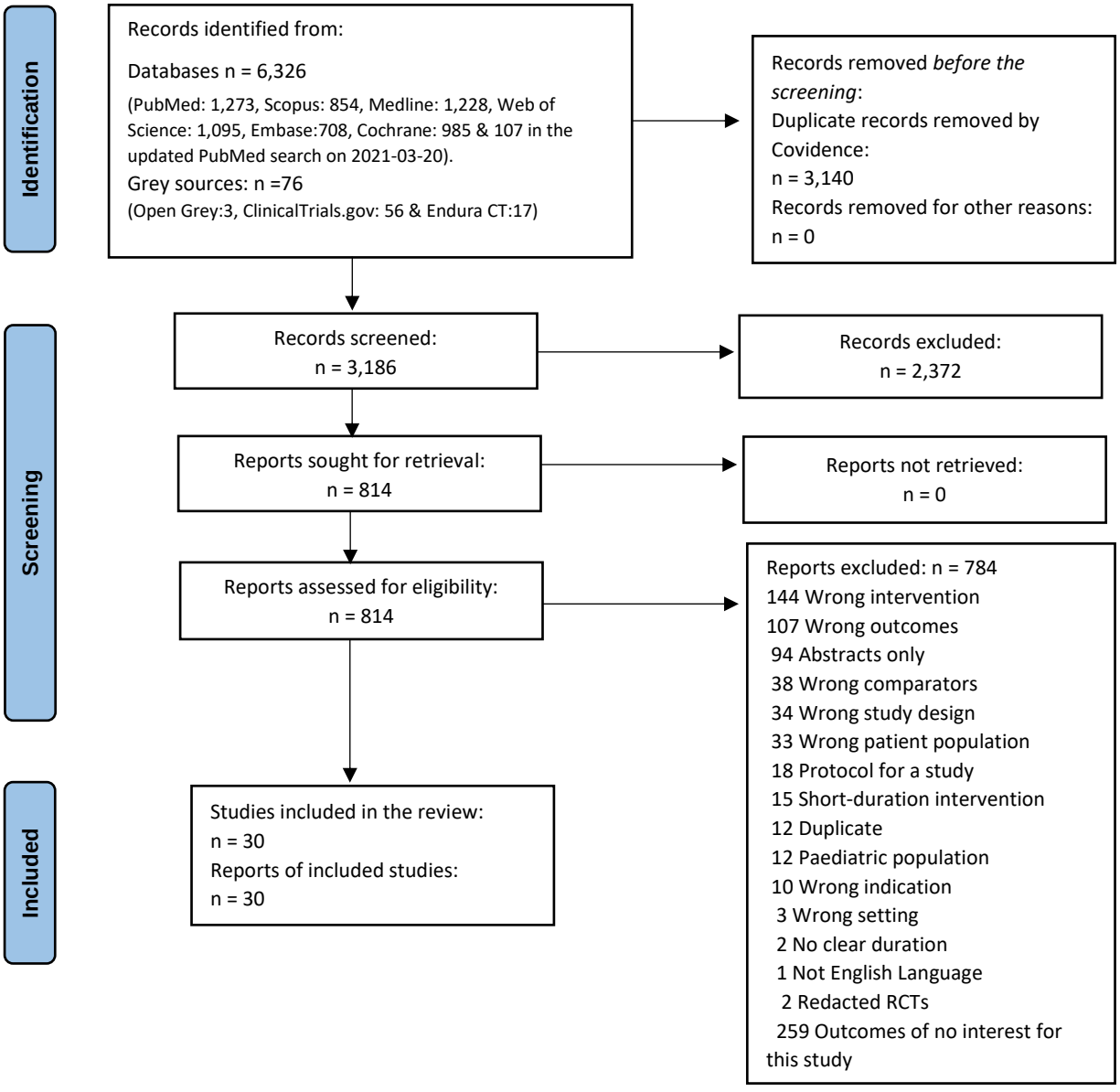
Subgroup analysis was conducted for the included RCTs and explained in chapter 2, section 2.1.1.9.

7.3 Results

7.3.1 Search results

Initially, 6,326 records were found in the searched databases; 3,186 were then screened after duplicates were removed. 814 articles were retrieved for full-text screening, of which 784 articles were excluded due to reasons presented in the PRISMA flow diagram. Figure 7-1. Finally, 30 RCTs met the eligibility criteria and were included in the meta-analysis.

Figure 7-1: PRISMA flow diagram



7.3.2 Characteristics of the included RCTs

Twenty RCTs (628, 676, 680, 758, 789-800) were diagnosed PCOS based on the Rotterdam diagnostic criteria. One RCT (801) used WHO type 2 criteria. One RCT (684) used the National Institute of Child Health (NICHD) criteria. No diagnostic criteria were specified for the remaining RCTs. 12 RCTs (789-791, 793, 795, 797, 800, 802-805) evaluated letrozole versus clomiphene citrate. 13 RCTs (758, 776, 792, 794, 798, 799, 801, 806-809) evaluated metformin versus clomiphene citrate. Three RCTs (676, 680, 684) examined metformin versus acarbose. Three RCTs (628, 687, 688) compared metformin versus placebo. Six RCTs (758, 789, 798, 804, 808, 809) reported live birth outcome. Twenty-four RCTs (628, 687, 688, 711, 758, 776, 789, 791-797, 800-806, 808, 809) reported pregnancy rate. Twenty-five RCTs reported ovulation rate (628, 676, 680, 684, 687, 688, 758, 776, 789, 790, 792-796, 798-801, 803, 804, 806-809).

Table 11: Characteristics of the RCTs included in the systematic review and meta-analysis

Author	Country	PCOS diagnostic criteria	PCOS patient's age (Mean±SD)	Patients, n (PCOS)	Patients, n (control)	Interventions	Duration	outcomes
Amer et al. (789)	UK	Rotterdam	28.3 ±4.4	80	79	Letrozole, CC	Six cycles	Pregnancy rate, Live birth
Ayaz et al. (806)	KSA	-	32 ± 3.5	21	21	Metformin + CC, CC	Three cycles	Ovulation rate
BanerjeeRay et al. (790)	India	Rotterdam	29±0	78	69	CC, Letrozole	Seven cycles	Ovulation rate
Baruah et al. (791)	India	Rotterdam	29.7 ± 0.5	25	25	Letrozole, CC	56- 58 cycles	Pregnancy rate
Basirat et al. (792)	Iran	Rotterdam	25.26±4.32	167	167	CC, Metformin + CC	Three cycles	Ovulation rate
Bayar et al. (793)	Turkey	Rotterdam	-	38	36	Letrozole, CC	95 - 99 cycles	Pregnancy rate
Behnoud et al. (802)	Iran	-	29.92 ± 6.97	40	40	Letrozole, CC	Three months	Pregnancy rate
Chen et al 2016(800)	China	Rotterdam	26.4±4.2	52	52	Letrozole, CC	Four to six cycles	Pregnancy rate, Ovulation rate
Dasari et al (794)	India	Rotterdam	-	24	16	CC, CC+ Metformin	Six cycles	Pregnancy rate, Ovulation rate
Dehbashi et al. (803)	Iran	-	23.62±2.92	50	50	Letrozole, CC	-	Pregnancy rate, Ovulation rate
El-khayat et al (795)	Egypt	Rotterdam	26.58 ± 2.93	50	50	Letrozole, CC	-	Pregnancy rate
Ganesh et al (796)	India	Rotterdam	30.25±4.90	372	669	Letrozole, CC-rFSH, rFSH	-	Pregnancy rate, Ovulation rate
Ghahiri et al.(797)	Iran	Rotterdam	25.63 ± 4.41	101	-	Letrozole, CC	-	Pregnancy rate
Hanjalic Beck et al.(680)	Germany	Rotterdam	-	62	-	Metformin, Acarbose	12 weeks	Ovulation rate
Kar et al. (798)	India	Rotterdam	25.8±2.46	32	24	CC, Metformin, CC+ Metformin	Six months	Ovulation rate, live birth
Katica et al.(807)	Bosnia and Herzegovina	-	32.8 ± 3.04	10	10	CC, Metformin, Metformin+ CC	-	Ovulation rate
Legro et al. (808)	USA	-	27.9±4.0	55	72	CC, Metformin, CC + Metformin	Six months	Pregnancy rate, live birth

Legro et al.(804)	USA	-	28.8±4.0	376	374	CC, Letrozole	-	Ovulation rate, Live birth
Liu et al.(799)	China	Rotterdam	27.69 ± 3.80	158	-	Letrozole, placebo	12 weeks	Ovulation rate
Lord et al. (628)	UK	Rotterdam	27.69 ± 3.80	16	-	Metformin, placebo	12 weeks	Pregnancy rate
Malkawi et al. (776)	Jordan	-	29 ± 3.1	16	12	Metformin /CC, placebo/CC	-	Ovulation rate
Moll et al. (801)	The Netherlands	WHO type 2 criteria	-	111	114	Metformin, placebo, CC	-	Ovulation rate
Morin papunen et al. (711)	Finland	-	28.2 ±1.4	17	-	Metformin, placebo	Three months	Pregnancy rate
Najafi et al. (805)	Iran	-	26.2±3.6	110	110	Letrozole, CC	-	Pregnancy rate
Rezai et al.(676)	Iran	Rotterdam	-	30	30	Acarbose, Metformin	Three months	Pregnancy rate, Ovulation rate
Sahin et al. (809)	Turkey	-	27±0	11	10	Metformin +CC, CC	Six cycles	Ovulation rate, Live birth
Sonmez et al. (684)	Turkey	NICHD	26.13±5.08	30	-	Metformin, Acarbose	Three months	Ovulation rate
Vandermolen et al. (688)	USA	-	29 6 ±1.2	11	14	Metformin, placebo	Seven weeks	Pregnancy rate
Yarali et al. (687)	Turkey	-	29.7±5.6	16	16	Metformin, placebo	Six weeks	Pregnancy rate
Zain et al. (758)	Australia	Rotterdam	27.8 ±3.6	115	-	Metformin , CC, Metformin + CC	Six months	Ovulation rate, Live birth

PCOS: polycystic ovary syndrome, **NIH:** national institute of health, **CC:** clomiphene citrate, **NICHD:** national institute of child health, **USA:** United States of America, **UK:** United Kingdom, **KSA:** Kingdom of Saudi Arabia, **SD:** standard deviation, **WHO:** world health organisation.

7.3.3 Risk of bias and quality assessment

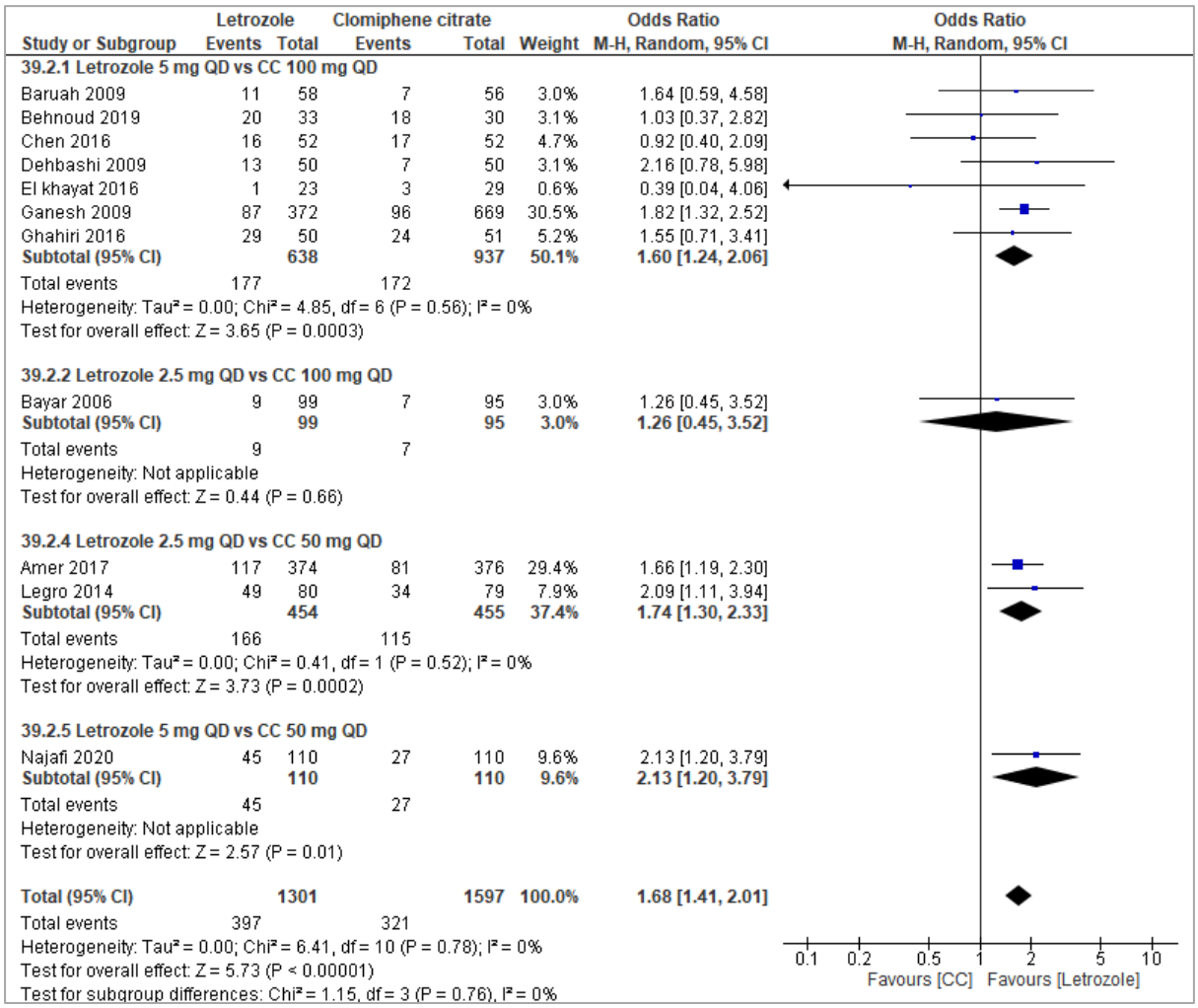
Most RCTs exhibited at least three or more unclear RoB across the assessed domains with allocation concealment, blinding participants and incomplete outcomes. The quality of the evidence for the outcomes was assessed using GRADEpro and is shown in table 12.

7.3.4 Pregnancy rate

7.3.4.1 Letrozole versus CC

In seven RCTs (791, 795-797, 800, 802, 803), letrozole 5 mg QD significantly increased the pregnancy rate compared with CC 100 mg QD (Odds ratio) (OR): 1.60; 95% CI: 1.24, 2.06). One RCT (793) compared letrozole 2.5 mg QD with CC 100 mg QD and showed no effect on the pregnancy rate (OR: 1.26; 95%CI: 0.45, 3.52). One RCT, letrozole 2.5 mg QD compared with CC 150 mg QD and showed no effect on the pregnancy rate (OR: 1.02; 95%CI: 0.64, 1.62). Two RCTs (789, 804) compared letrozole 2.5 mg QD with CC 50 mg QD significantly increased the pregnancy rate with letrozole (OR: 1.74; 95%CI: 1.30, 2.33). One RCT (805) compared letrozole 5 mg QD with CC 50 mg QD, letrozole significantly increased the rate of pregnancy (OR: 2.13; 95%CI: 1.20,3.79). Overall, regardless the administered dosage, letrozole significantly increased the pregnancy rate compared with CC (OR: 1.68; 95%CI: 1.41,2.01, I^2= *0%, p < 0.00001*) (Figure 7-2) (low grade evidence, table 12).

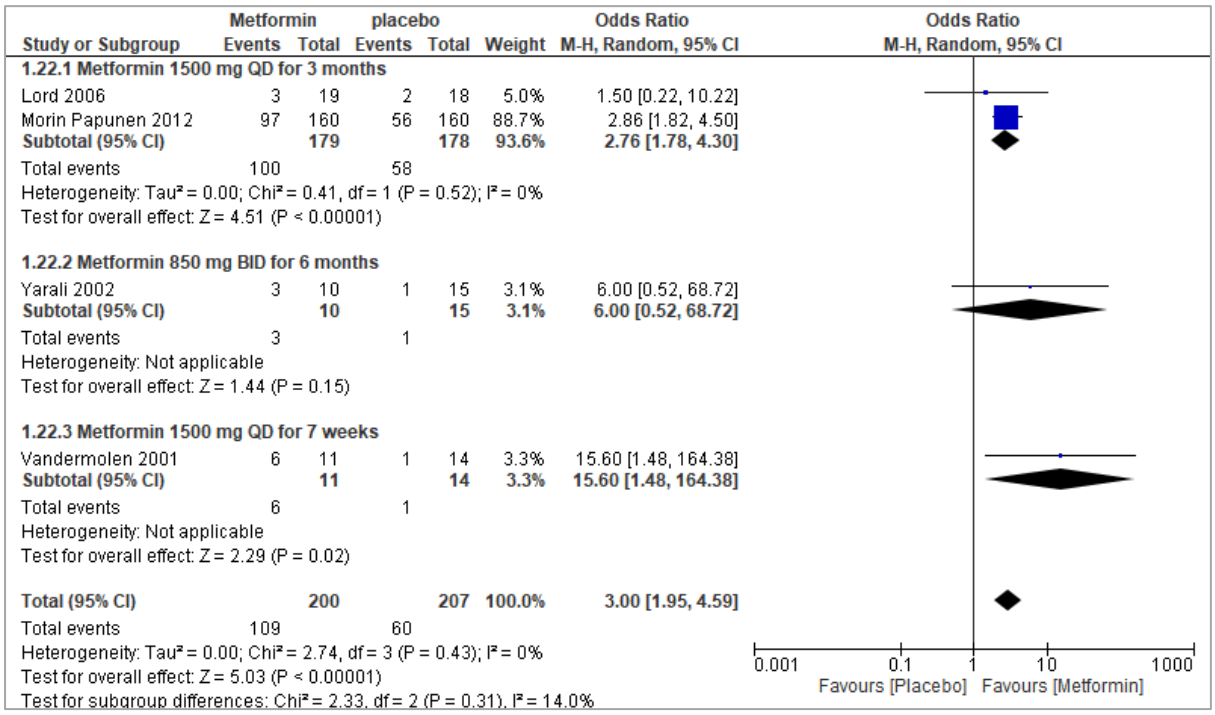
Figure 7-2: Forest plot of Letrozole versus CC on pregnancy rate



7.3.4.2 Metformin versus placebo

In two RCTs (628, 711), metformin 1500 mg QD for three months significantly increased the pregnancy rate (OR: 2.76; 95%CI: 1.78, 4.30). In one RCT (687), metformin 850 mg BID for six months did not affect the pregnancy rate (OR: 6.0;95%CI: 0.52, 68.72). Another RCT (688) of metformin 1500 mg QD for seven weeks significantly increased the pregnancy rate (OR: 15.60; 95%CI: 1.48,164.38). Overall, regardless of the administered dosage or the duration, metformin significantly increased the rate of pregnancy (OR: 3.00; 95%CI: 1.95, 4.59, I^2= *0%, p < 0.00001)* (Figure 7-3) *(*very low-grade evidence, table 12).

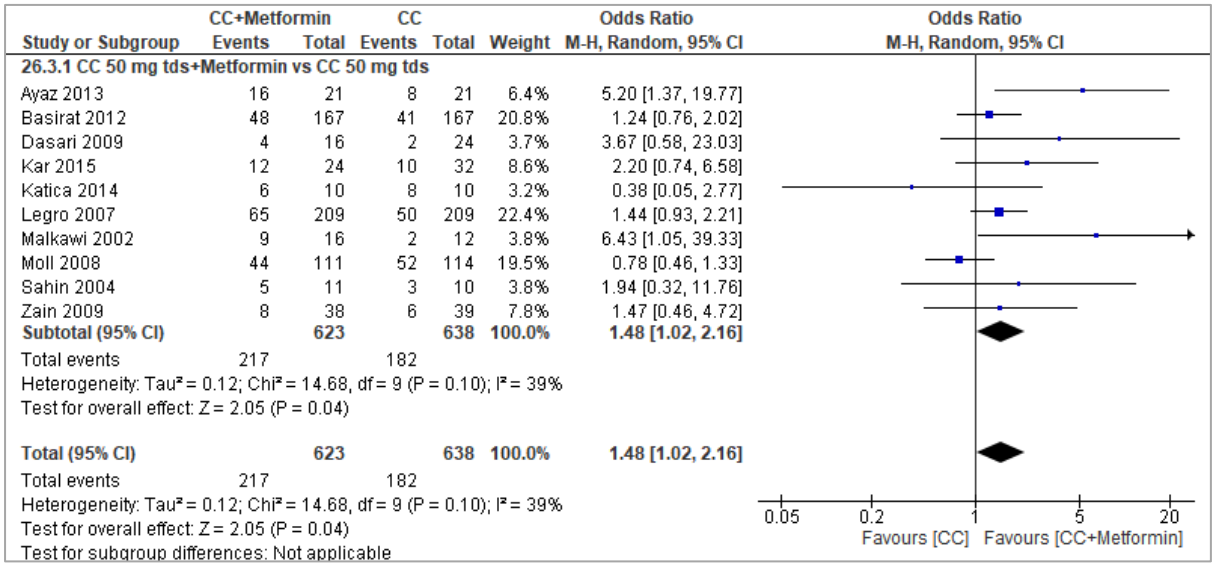
Figure 7-3: Forest plot of Metformin versus placebo on pregnancy rate



7.3.4.3 CC+ Metformin versus CC

Ten RCTs (758, 776, 792, 794, 798, 801, 806-809) compared CC 50 mg TDS added to metformin with CC 50 mg TDS alone showed a significant increase in the pregnancy rate (OR: 1.48; 95%CI: 1.02, 2.16, I^2= 39%, p = 0.04) (Figure 7-4) (low grade evidence, table 12).

Figure 7-4: Forest plot of CC + Metformin versus CC on pregnancy rate

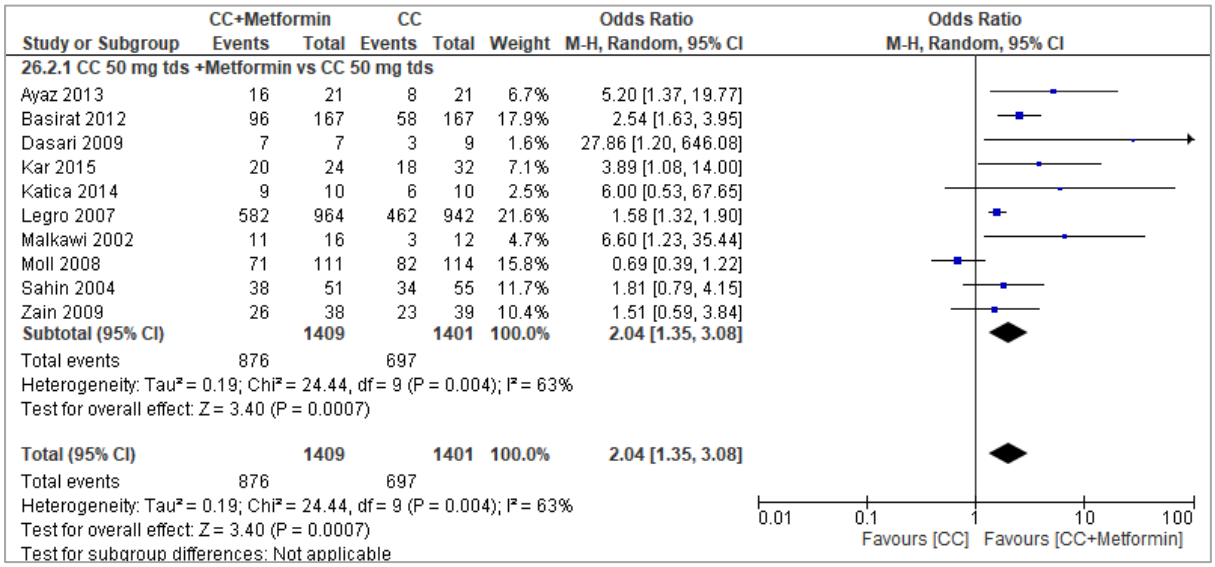


7.3.5 Ovulation rate

7.3.5.1 CC+ Metformin versus CC

In ten RCTs compared CC 150 mg QD with metformin added to CC 50 mg TDs showed a significant increase in the ovulation rate (OR: 2.04; 95%CI: 1.35, 3.08, I^2 = 63%, *p = 0.0007)* (Figure 7-5) *(*low grade evidence, table 12).

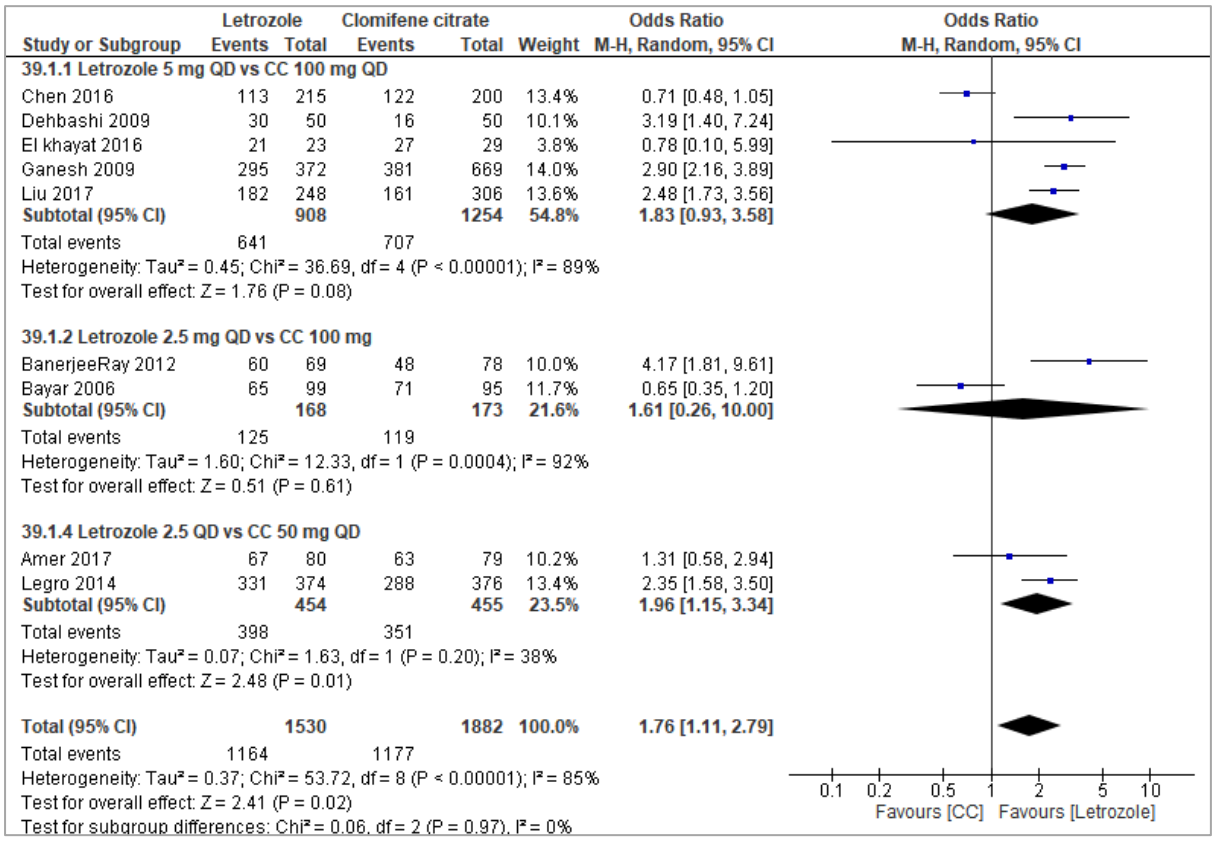
Figure 7-5: Forest plot of CC + Metformin versus CC on ovulation rate



7.3.5.2 Letrozole versus CC

In five RCTs (795, 796, 799, 800, 803) letrozole 5 mg QD significantly increased the rate of ovulation compared with CC 100 mg QD (OR: 1.83; 95%CI: 0.93,3.58). In two RCTs (790, 793) compared letrozole 2.5 mg QD with CC 100 mg showed no effect on the ovulation rate (OR: 1.61; 95%CI: 0.26,10.0). In two RCTs (789, 804), letrozole 2.5 mg QD significantly increased ovulation rate compared with CC 50 mg QD (OR: 1.96; 95%CI: 1.15,3.34). Overall, there was a significant increase in the ovulation rate when letrozole at various doses was compared with CC (OR: 1.76; 95%CI: 1.11,2.79, *I^2= 85%, p = 0.02)* (Figure7-6) *(*low grade evidence, table 12).

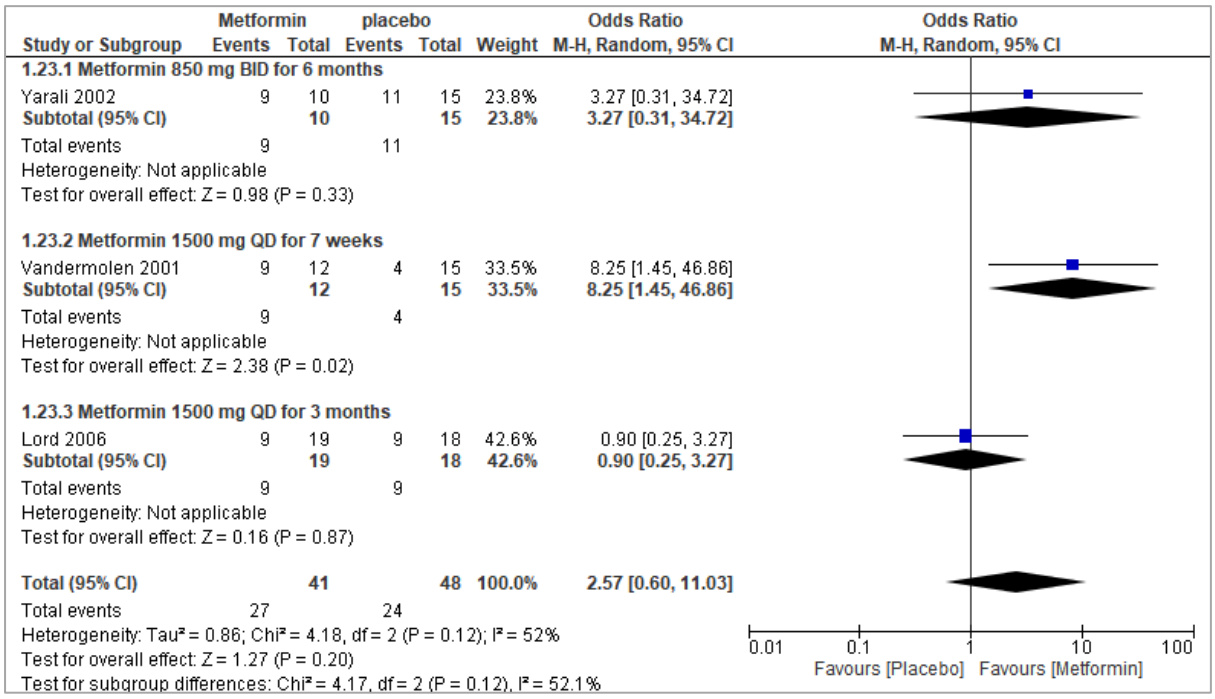
Figure 7-6: Forest plot of Letrozole versus CC on ovulation rate



7.3.5.3 Metformin versus placebo

One RCT compared metformin 850 mg BID with placebo for six months showed no effect on the ovulation rate (OR: 3.27;95%CI 0.31,34.72). Another RCT compared metformin 1500 mg QD with placebo for seven weeks showed a significant increase in the ovulation rate (OR: 8.25: 95%CI: 1.45, 46.86). One RCT compared metformin 1500 mg QD for three months with placebo showed no effect on the ovulation rate (OR: 0.90; 95%CI: 0.25, 3.27). Overall, metformin of various dosage for various duration compared with placebo has no effect on the ovulation rate (OR: 2.57; 95%CI: 0.60,11.03, p = 0.20, I^2= 52%) (Figure 7-7) (very low-grade evidence, table 12).

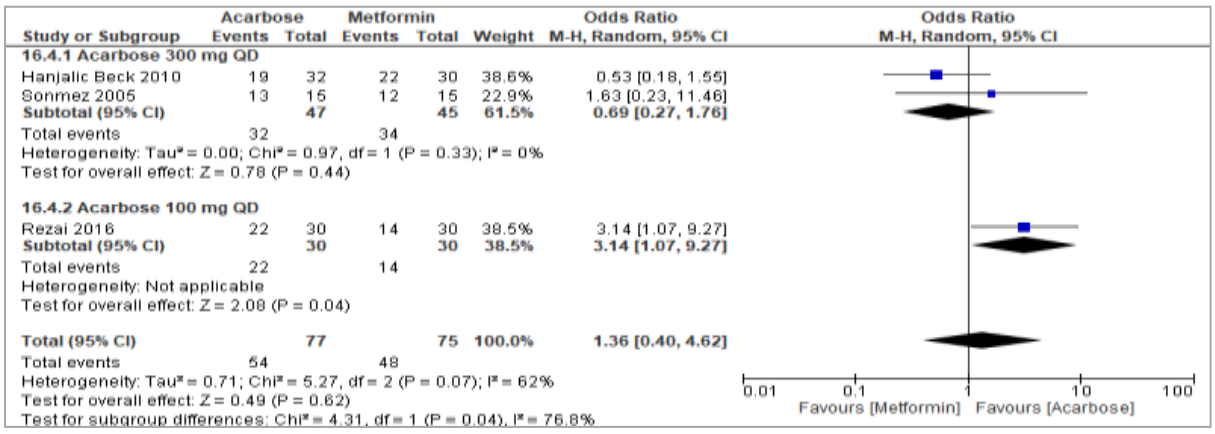
Figure 7-7: Forest plot of Metformin versus placebo on ovulation rate



7.3.5.4 Acarbose versus Metformin

Two RCTs compared acarbose 300 mg QD with metformin showed no effect on the ovulation rate (OR: 0.69; 95%CI: 0.27,1.76). Another RCT compared acarbose 100 mg QD with metformin showed a significant increase in the ovulation rate (OR: 3.14; 95%CI: 1.07, 9.27). Overall, acarbose at various dosage compared with metformin has no effect on the ovulation rate (OR: 1.36; 95%CI: 0.40,4.62, p= 0.62, I^2= 62%) (Figure 7-8) (low-grade evidence, table 12).

Figure 7-8: Forest plot of Acarbose versus Metformin on ovulation rate

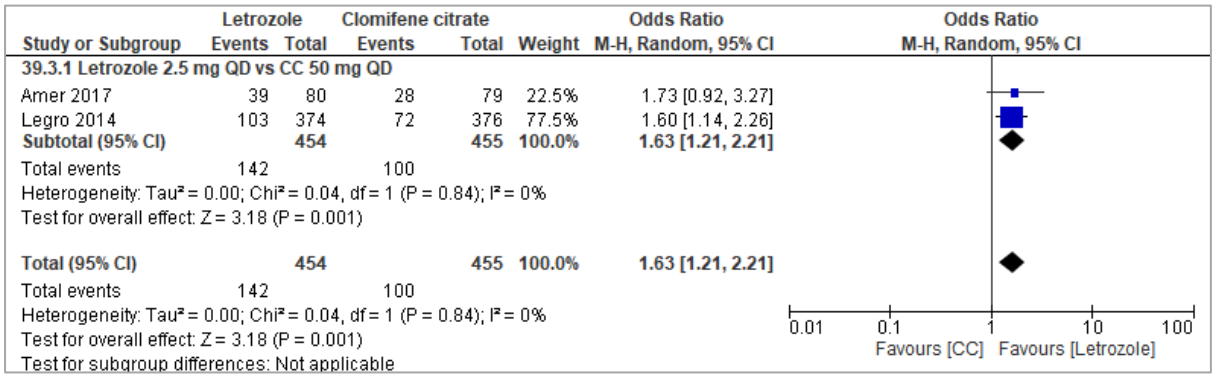


7.3.6 Live birth

7.3.6.1 Letrozole versus CC

In two RCTs (789, 804) compared letrozole 2.5 mg QD with CC 50 mg QD showed a significant increase in live birth rate (OR: 1.63; 95%CI: 1.21, 2.21, I^2 = 0%, *p* = 0.001) (Figure 7-9) (low grade evidence, table 12).

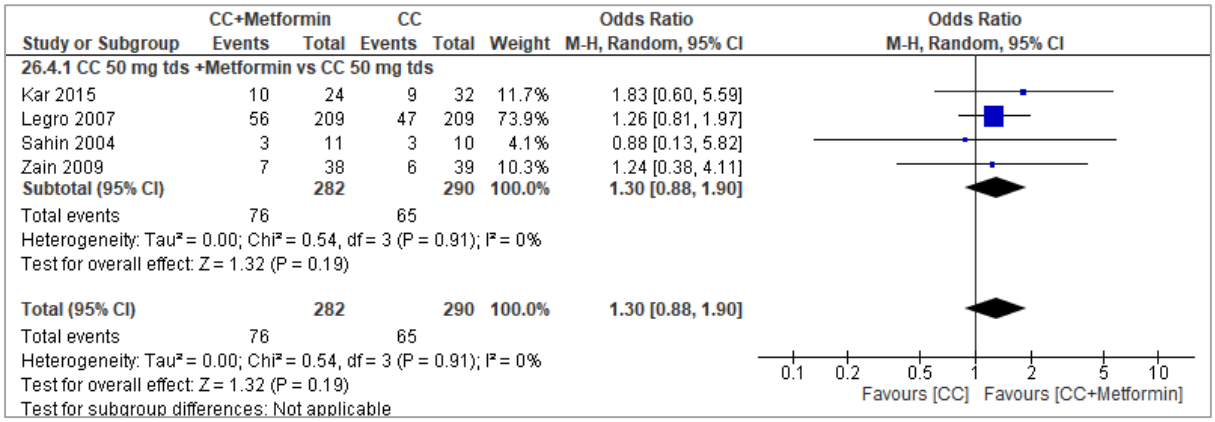
Figure 7-9: Forest plot of Letrozole versus CC on live birth rate



7.3.6.2 CC+ Metformin versus CC

In four RCTs (758, 798, 808, 809) CC 50 mg TDS added to metformin showed no effect on the live birth rate compared with CC 50 mg TDS alone (OR: 1.30; 95%CI: 0.88, 1.90, I^2 = 0%, p = 0.19) (Figure 7-10) (low grade evidence, table 12).

Figure 7-10: Forest plot of CC + Metformin versus CC on live birth rate



7.3.7 Sensitivity analysis and publication bias

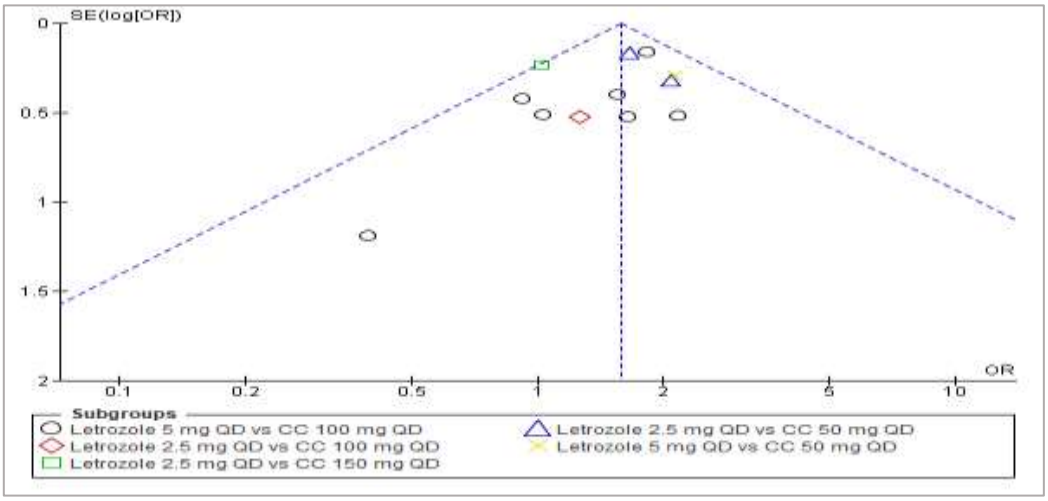
We performed subgroup analysis to reduce heterogeneity, and the sensitivity analysis did not significantly affect the outcomes as no study has been removed from the meta-analysis.

7.3.8 Publication bias

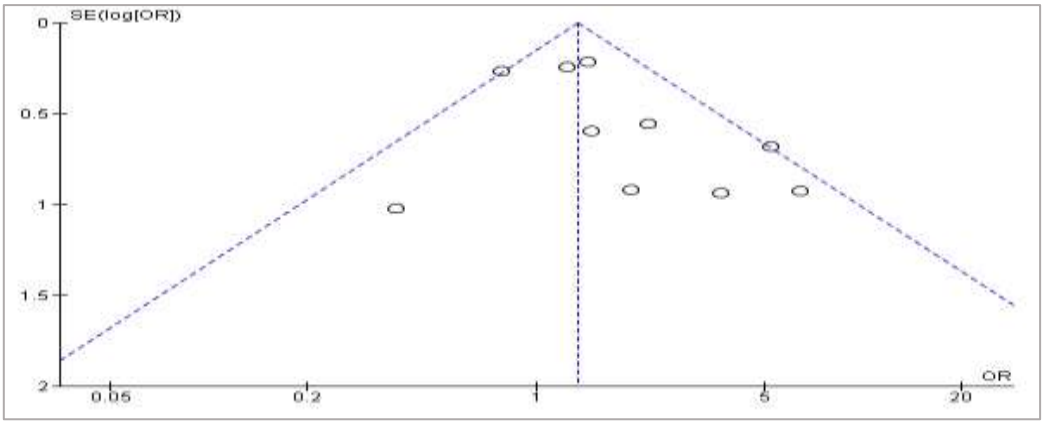
The funnel plot did revealed significant asymmetry (Figure 7-11, A & B), which indicates bias. Thus, the meta-analysis might have exaggerated the significant effect of letrozole as some RCTs with significant effects favouring CC were not reported. The Egger's test was statistically significant for publication bias (regression intercept = 0.456, *SE* = 0.084, *p* = 0.001).

Figure 7-11: Funnel plot of comparisons of pregnancy and ovulation rate

A) Letrozole versus CC



B) Metformin +CC versus CC



CC: clomiphene citrate, **SE:** standard error, **OR:** odds ratio

Table 12: Summary of findings

Outcome	№ of participants (studies)	Certainty of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects.	
				Assumed risk.	
				Risk with difference with intervention	Risk with comparison
Letrozole versus CC				**(first treatment minus second treatment)**	
Pregnancy rate	3480 (12 RCTs)	⊕⊕◯◯ LOW a,b	-	OR 1.58 higher (1.34 higher to 1.86 higher)	The mean pregnancy rate range 1-117
Ovulation rate	2733 (12 RCTs)	⊕⊕◯◯ LOW a,b	-	OR 1.76 higher (1.11 higher to 2.79 higher)	The mean ovulation rate range 21-318
Live birth rate	242 (2 RCTs)	⊕⊕◯◯ LOW a,b	-	OR 1.63 higher (1.21 higher to 2.21 higher)	The mean live birth rate range 28-103
Metformin versus placebo	169 (4RCTs)	⊕◯◯◯ VERY LOW a,c	-	**(first treatment minus second treatment)** OR 3.00 higher (1.95 higher to 4.59 higher)	The mean pregnancy rate range 1-97
Pregnancy rate	89 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	OR 2.57 higher (0.60 higher to 11.03 higher)	The mean ovulation rate range 4-11
Ovulation rate					
CC+ Metformin versus CC				**(first treatment minus second treatment)**	
Pregnancy rate	1261 (10RCTs)	⊕⊕◯◯ LOW a,b	-	OR 1.48 higher (1.02 higher to 2.16 higher)	The mean pregnancy rate range 4-65
Ovulation rate	2810 (10 RCTs)	⊕⊕◯◯ LOW a,b	-	OR 2.04 higher (1.35 higher to 3.08 higher)	The mean ovulation rate range 3-464
Live birth rate	572 (4RCTs)	⊕⊕◯◯ LOW a,b	-	OR 1.30 higher (0.88 higher to 1.90 higher)	The mean live birth rate range 3-56
Acarbose versus Metformin	152 (3RCTs)	⊕◯◯◯ VERY LOW a,c	-	**(first treatment minus second treatment)** OR 1.36 higher (0.40 higher to 4.62 higher)	The mean ovulation rate range 12-22
Ovulation rate					

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **OR:** odds ratio, **CI:** confidence interval**, RCTs:** randomised controlled trials, **CC:** clomiphene citrate, **FSH:** follicular stimulating hormone.

GRADE Working Group grades of evidence.
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.
Explanations
a. Some studies have a high risk of performance bias and an unclear risk of bias across 5 out of the 7 domains. Thus, we downgraded one level.
b. A considerable level of heterogeneity across the studies.
c. small sample size with a wide confidence interval. We downgraded one level.

7.4 Discussion

This systematic review found that treatment with CC and letrozole alone or combined with metformin was associated with a significant increase in pregnancy, ovulation and live birth rates. However, no data compared those rates with rates from untreated controls. A study of 22 patients with PCOS who were given three different dosages of FSH (75, 100 and 150 IU) indicated an increased pregnancy rate (810). Similarly, a systematic review and meta-analysis of 57 RCTs showed that both letrozole and the combination of clomiphene and metformin have significantly increased pregnancy and ovulation rates (811). However, a non-randomised controlled trial that compared the efficacy of metformin and CC on the ovulation induction found no significant difference in ovulation and pregnancy rates (812). When letrozole was compared with CC, pregnancy, ovulation, and live-birth rates were high. In a meta-analysis of RCTs that compared the efficacy of letrozole with CC, letrozole was associated with a high rate of live birth, pregnancy and ovulation compared with CC (813). However, the RCTs included in our review enrolled PCOS patients based on different diagnostic criteria, suggesting a degree of particular bias between the individual studies. Nevertheless, the pooled analysis showed favourable results for letrozole in pregnancy, ovulation and live birth rate.

Nonetheless, the current evidence was insufficient and of low grade to support either CC or letrozole's superiority concerning ovulation induction and pregnancy rate. However, the findings support the effectiveness and the favourable potential for ovulation induction in PCOS. Furthermore, the meta-analysis favoured letrozole to increase the pregnancy rate; this agrees with a previous review that reported similar results (814). Overall, the results of this

systematic review and meta-analysis are in line with those of previous comprehensive reviews on the efficacy of letrozole and CC for ovulation induction in women with PCOS (814, 815).

Limitations to this review include that most of these RCTs were conducted in Asia and the Middle East, which may not be applicable to other ethnic populations. Secondly, the quality of the included RCTs was generally low. The poor reporting of information regarding methods such as allocation concealment and blinding of participants led to the majority of the RCTs being graded as having an unclear risk of bias. There was also a significantly high level of heterogeneity among the included RCTs, which was due to the nature of the clinical trials; however, an attempt was made to address this issue by conducting a subgroup analysis, sensitivity analysis, using a random-effect model, and it was also addressed when we assessed the quality of evidence for the outcomes.

Finally, a relatively small number of trials assessed the efficacy of different therapeutic agents versus placebo. This precluded direct estimation of the effect size attributed to each treatment modality to improve different outcomes.

7.5 Conclusion

In conclusion, despite the significant limitations, this review has found that letrozole appeared to be more effective in inducing ovulation when compared with CC. Similar efficacy was also evident in increasing the rate of pregnancy and live birth. However, when metformin was added to CC, it showed a significant increase in the rate of both ovulation and pregnancy. In addition, the vast majority of women with PCOS are resistant to CC. Thus, the results of this review have suggested that letrozole could be an effective alternative for fertility treatment, particularly in CC-resistant women. Moreover, metformin enhances the efficacy of

medications used to improve fertility in women with PCOS. However, caution must be taken when interpreting these results until evidence is available from further high-quality RCTs.

8 Chapter 8: Impact of metformin in women with polycystic ovary syndrome: a systematic review and meta-analysis of randomised controlled trials

8.1 Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine condition that affects women of reproductive age, with a prevalence of up to 20% (816). PCOS is characterised by both biochemical and clinical features of excess androgen, menstrual irregularities and polycystic ovarian morphology (344). In PCOS, high insulin contributes to excess ovarian androgen production (55), and insulin enhances the steroid hormone release in the ovaries (785). High androgen levels drive hirsutism and reduced fertility levels in women with PCOS (817). Furthermore, women with PCOS have a significantly higher rate of impaired glucose tolerance and insulin resistance, risk factors for type 2 diabetes mellitus (T2DM)(818). In addition, nearly 70% of women with PCOS will develop metabolic syndrome (MS), characterised by the constellation of dyslipidaemia, central adiposity, hypertension and impaired glucose tolerance, all predisposing factors to coronary heart disease and diabetes (819, 820). A therapeutic approach targeting weight loss and improving insulin resistance is the cornerstone in managing PCOS and preventing its related complications (821); however, significant weight loss is still challenging in PCOS. Lifestyle intervention is the first-line therapy related to significant though minimal weight reduction (822). Pharmacological options for managing PCOS exist; however, their actual impact in clinical practice remains relatively unexplored (823). Metformin is a member of the biguanide family primarily used to manage T2DM (363). Metformin is also widely used in the management of women with PCOS, and it reduces androgen levels by improving insulin sensitivity and reducing the cardiometabolic

risks associated with hyperinsulinemia in PCOS (72,163). However, controversy exists on the effects of metformin on weight change, fertility and biochemical and hormonal changes (824, 825).

This review aimed to comprehensively assess and appraise the existing evidence and provide in-depth analyses of the impact of metformin on the clinical and biochemical parameters in women with PCOS.

8.2 Methods and materials

8.2.1 Protocol and registration

The protocol of this systematic review and meta-analysis is explained in chapter 2, section 2.1.1.1.

8.2.2 Eligibility criteria for the included studies

The eligibility criteria, including inclusion/exclusion criteria, is explained in chapter 2, section 2.1.1.2.

8.2.3 Literature search

The search strategy, including the search in the medical databases, is explained in chapter 2, section 2.1.1.3.

8.2.4 Study selection

The study selection, including screening titles and abstracts of the retrieved studies, is explained in chapter 2, section 2.1.1.4.

8.2.5 Data extraction

The method used to extract information from the included studies is explained in chapter 2, section 2.1.1.5.

8.2.6 Risk of bias assessment in the included studies

The RoB assessment for the included studies is explained in chapter 2, section 2.1.1.6.

8.2.7 GRADE scoring

The robustness of evidence for each chosen outcome is assessed using the GRADE scoring system and is explained in chapter 2, section 2.1.1.7.

8.2.8 Statistical analysis

The statistical method used to estimate the pooled effects of the various interventions is explained in chapter 2, section 2.1.1.12.

8.2.9 Assessment of heterogeneity

Heterogeneity for the outcomes across each RCT was evaluated and explained in chapter 2, section 2.1.1.8.

8.2.10 Subgroup analysis

Subgroup analysis was performed at different levels where data from at least 2 RCTs were available on the same outcome. Subgroup analysis was performed based on the administered dosage of the metformin (e.g. 500 mg, 750mg, 1,000 mg, 1,500 mg and 2,000 mg), frequency of administration (once a day-QD, twice a day-BID or three times a day-TDS), and duration of the intervention (weeks/months). Also, outcome-specific weighted effect estimates, regardless of the metformin dosage, frequency and duration of administration, were

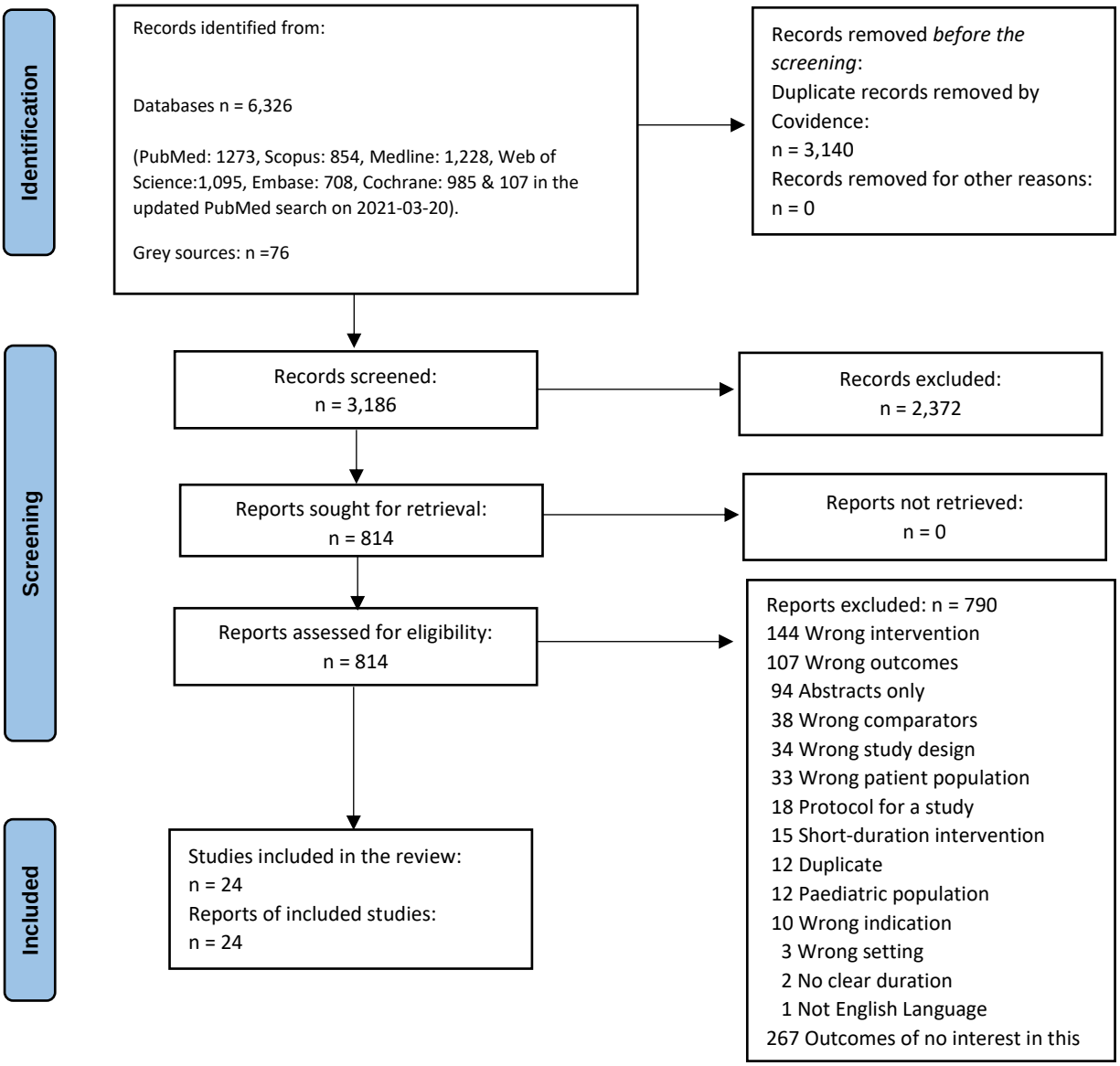
quantified and reported. The funnel plot of the RevMan with standard error (SE) was used to assess publication bias where more than 10 RCTs were meta-analysed.

8.3 Results

8.3.1 Search results

A total of 6,326 unique records were identified in the literature search in electronic databases and grey sources. 2,372 of those were excluded after the title and abstract screening against the pre-set inclusion and exclusion criteria. Of the 814 studies screened in full text, 24 RCTs involving 564 individuals met the eligibility criteria and were included in the systematic review and the meta-analysis. Figure 8-1.

Figure 8-1: PRISMA flow diagram



8.3.2 Characteristics of the included RCTs

The 24 RCTs included were published until 2020, of which 13 RCTs (607, 610, 616, 618, 663, 670, 671, 673, 689, 711, 714, 715, 758) diagnosed PCOS based on the Rotterdam criteria 2003 (30). Two RCTs (625,682) diagnosed PCOS based on the National Institute of Health (NIH/NICHD) criteria (626). No diagnostic criteria were specified for the remaining RCTs. The characteristics of the included RCTs are presented in Table 13.

Table 13: Characteristics of the studies included in the systematic review and meta-analysis

Author	*Country*	*PCOS diagnostic criteria*	*PCOS patient's Characteristics*		*Duration*	*Outcomes*
			Age (Mean±SD)	**BMI (Mean±SD)**		
Gambineri et al.(627)	Italy	N/A	27·1 ± 3·6	37·6± 4·1	Six months	FBG,FI, Wt., BMI, HOMA-IR
Trolle et al.(630)	Denmark	N/A	31±0	32±0	Six months	Wt,WHR,FBG,FI,HOMA-IR, LDL,HDL
Eisenhardt et al.(663)	Germany	Rotterdam	27.0±0	28.9	12 weeks	FBG.FI,HOMA-IR
Heidari et al.(610)	USA	Rotterdam	32.4±7.5	37.1±9.1	Three months	BMI,WC,WHR, wt
Vandermolen et al.(688)	USA	N/A	29 6 ±1.2	37.6 ± 4.3	Seven weeks	Wt,BMI, FBG,FI
Zain et al. (758)	Australia	Rotterdam	27.8 ±3.6	N/A	Six months	TG,TC,HOMA-IR, HOMA-B .BMI, FI,FBG
Lingaiah et al.(673)	Finland	Rotterdam	27.6 ±4.0	26.5 ±6.0	Three months	Wt, WC,BMI,WHR
Morin-Papunen et al.(711)	Finland	Rotterdam	28.4 ± 3.9	27.1 ±6.3	Three months	Wt,WC,WHR,BMI,FBG,FI
Sova et al.(616)	Finland	Rotterdam	27.7 ±4.0	27.5 ±6.2	N/A	Wt,BMI,WC,WHR
Underdal et al.(618)	Denmark	Rotterdam	29.5 ±3.9	28.7± 6.9	Six months	Wt, BMI, WC, WHR, LDL,HDL, TG,TC
Cheng et al.(721)	Australia	Rotterdam	26 ± 4	24.2±5.3	Two months	BMI, FI, FBG, WHR
Kocak et al.(671)	Turkey	Rotterdam	26.2 ±3.7	31.91± 5.38	Six weeks	WHR,FBG,FI, TG,TC,HOMA-IR, HOMA-B
Yarali et al.(687)	Turkey	N/A	29.7±5.6	28.6±4	Three months	BMI,WHR,FBG, FI, TG,TC,HDL, LDL
Chou et al.(689)	Brazil	N/A	24±5	35.6±4.9	12 weeks	BMI, FI,FBG,TC, TG, HDL,LDL
Kazerooni et al.(670)	Iran	Rotterdam	25.6± 4.32	28.52± 1.61	Three months	WC,BMI, FI,FBG,TC, TG, WHR, LDL,HDL
Lord et al.(628)	UK	N/A	27.76 ±4.89	33.74± 6.74	Three months	BMI,FBG,FI,TC,TG
Ng et al.(629)	China	N/A	30.5±0	N/A	Six months	BMI, WHR, WC, FBG,LDL,HDL, TG BMI,LDL

Amiri et al.(607)	Iran	Rotterdam	25.6±4.02	28.9±5	24 months	BMI,WHR,LDL,HDL,TC
Palomba et al.(722)	Italy	N/A	24.3 ± 3.1	22.2 ±2.0	Six months	BMI, DHEAS
Romualdi et al.(714)	Italy	Rotterdam	24.7 ±4.4	22.2 ±2.2	36 weeks	WC,BMI, FI,FBG,TC, TG, WHR, LDL,HDL
Vanky et al.(715)	Norway	Rotterdam	28.9 ±4.8	30.6 ± 7.3	Six months	TG,TC,HOMA-IR, HOMA-B
Naka et al.(631)	Greece	N/A	23.3± 4.9	28.7± 5.5	Six months	WHR,BMI,FBG,FI
Ladson et al.(682)	USA	NIH	29±4.5	38±7.8	Six months	WC,BMI, FI,FBG,TC, TG, WHR, LDL,HDL
Moghetti et al.(625)	Italy	NICHD	23.9 ±6 1.2	27.1 ±6 1.5	Six months	BMI,WHR,LDL,HDL,TC

RCT: randomised clinical trial, **N/A**: not available, **BMI**: body mass index, **Wt.:** weight, **WHR:** waist to hip ratio, **WC**: waist circumference, **FBG**: fasting blood glucose, **FI**: fasting insulin, **HDL**: high-density lipoprotein**,** **LDL:** Low-density lipoprotein, **TG:** triglycerides, **TC**: total cholesterol, **HOMA-IR:** the homeostatic model of insulin resistance**, NIH**: national institute for health, **NICHD**: national institute of child health and development.

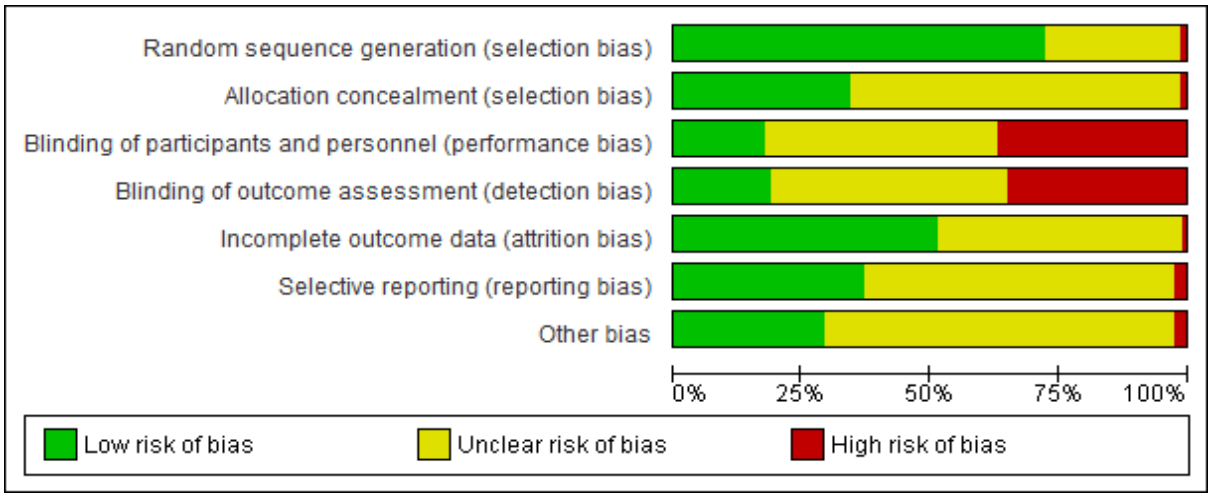
8.3.3 Sensitivity analysis

Small sample-sized RCTs and those with high RoB were eliminated from the analysis while monitoring their impact on the final results. One RCT (Vandermolen et al,) has high weight and showed significant increase in FSH when metformin was comapred with placebo. However, when this study removed from the meta-analysis no significant effect was found.

8.3.4 Assessment of risk of bias

Most of the RCTs were judged to have poor quality due to inadequate randomisation and blinding of assessors and participants. Moreover, the vast majority of the included RCTs were not sufficiently reported; therefore, they were judged to have an unclear RoB. The overall risk of bias for the included RCTs is shown. Figure 8-2.

Figure 8-2: the overall risk of bias of the included studies



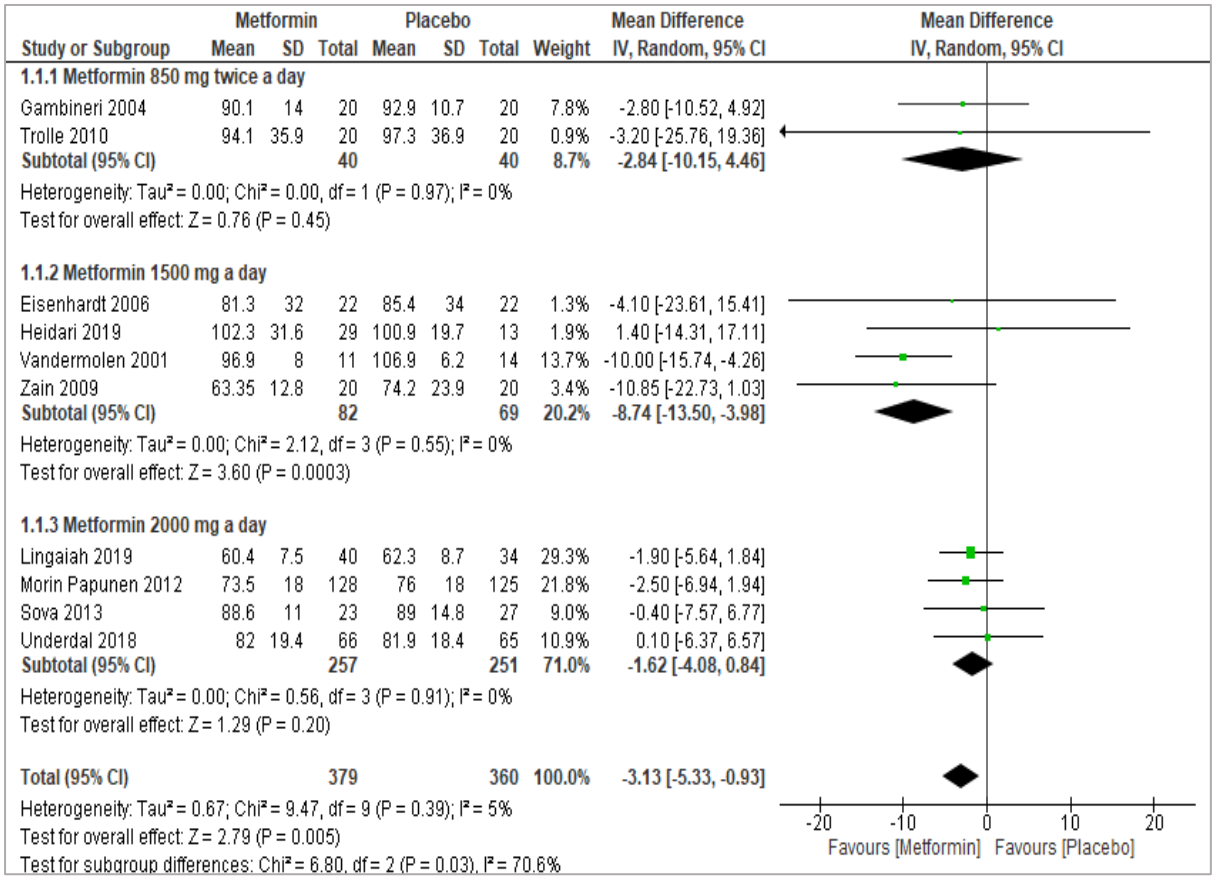
8.3.5 Effects of Metformin on anthropometric parameters

8.3.5.1 Body weight

Ten RCTs included 739 women with PCOS; 379 were assigned in the metformin group and 360 in the placebo group. In two RCTs, metformin 850 mg had no effect on body weight (MD: -2.84 Kgs; 95% CI: -10.15,4.46). In four RCTs, metformin 1500 mg had significantly reduced

body weight by 8.74 kgs (95% CI: -13.50, -3.98). In four RCTs, metformin 2000 mg had no effect on body weight compared with placebo (MD: -1.62 kg; 95% CI: -4.08, 0.84). Overall, regardless of the administered dosage and the duration, metformin significantly reduced body weight by 3.13 kgs (95% CI: -5.33, -0.93, 739 participants, $p < 0.005$) (Figure 8-3) (Moderate grade evidence, Table 14).

Figure 8-3: Forest plot of Metformin versus placebo on body weight (kg)

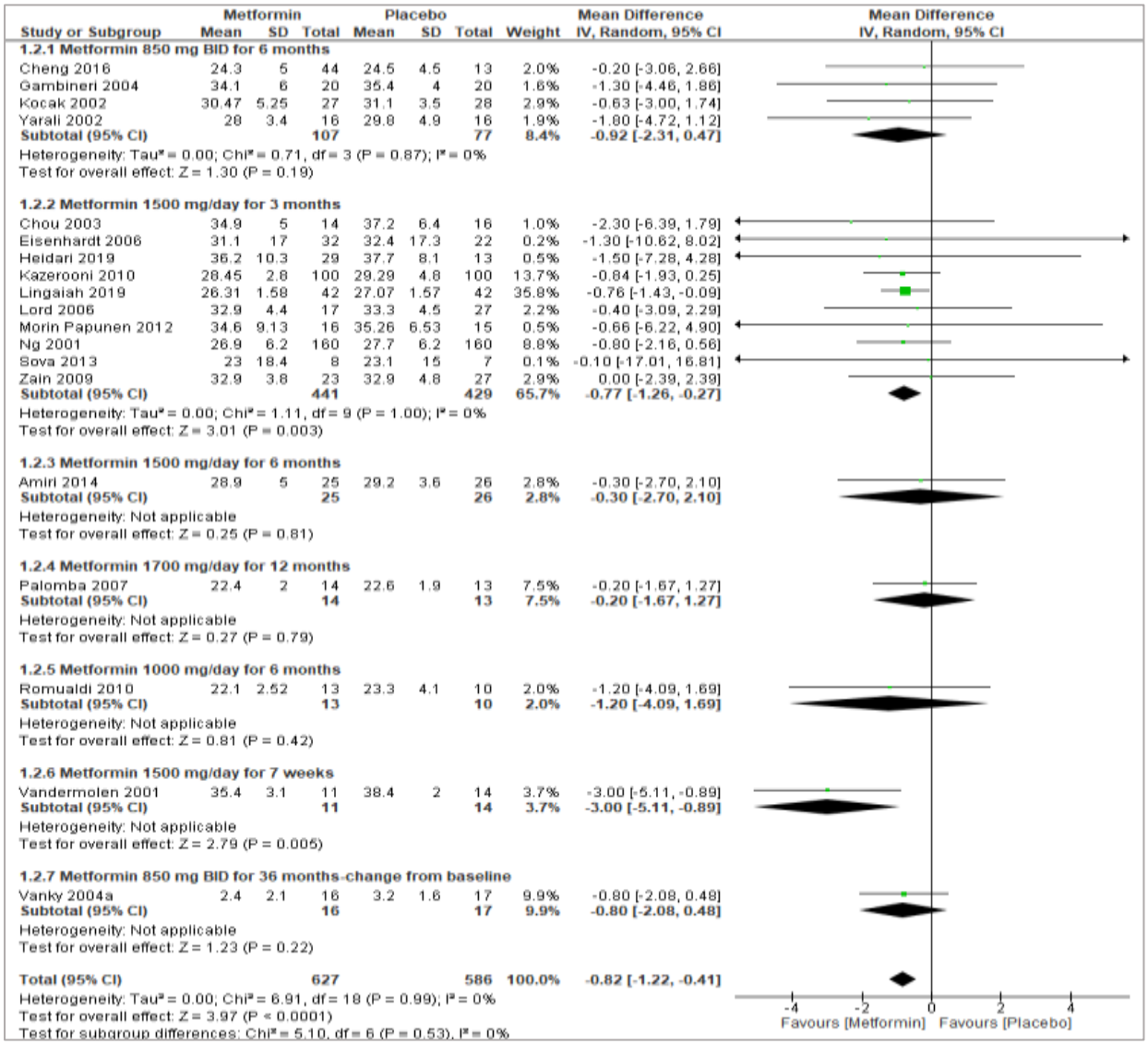


8.3.5.2 BMI

Nineteen RCTs reported on the effect of various dosages and duration of metformin compared to placebo on the BMI of women with PCOS. Four RCTs evaluated metformin 850 mg BID for 6 months and showed non-significant reduction in BMI (MD: -0.92 kg/m²; 95% CI: -2.31, 0.47). In ten RCTs, metformin 1500 mg QD for three months significantly reduced the

BMI by 0.77 kg/m² (95% CI: -1.26, -0.27) compared to placebo. In one RCT, metformin 1500 mg QD for six months had no effect on BMI (MD: -0.30 kg/m²; 95% CI: -2.70, 2.10). In one RCT, metformin 1700 mg QD had no effect on BMI (MD: -0.20 kg/m²; 95% CI: -1.67, 1.27). In one RCT, metformin 1000 mg QD for six months had no effect on BMI (MD: -1.20 kg/m²; 95% CI: -4.09, 1.69). One RCT of metformin 1500 mg QD for seven weeks had no effect on BMI (MD: -3.00 kg/m²; 95%CI: -5.11, -0.89). Overall, regardless of the administered dosages, frequency and durations, metformin significantly reduced the mean BMI by 0.82 kg/m² (95% CI: -1.22, -0.41, 1213 participants, $P < 0.0001$) (Figure 8-4) (Moderate grade evidence, Table 14).

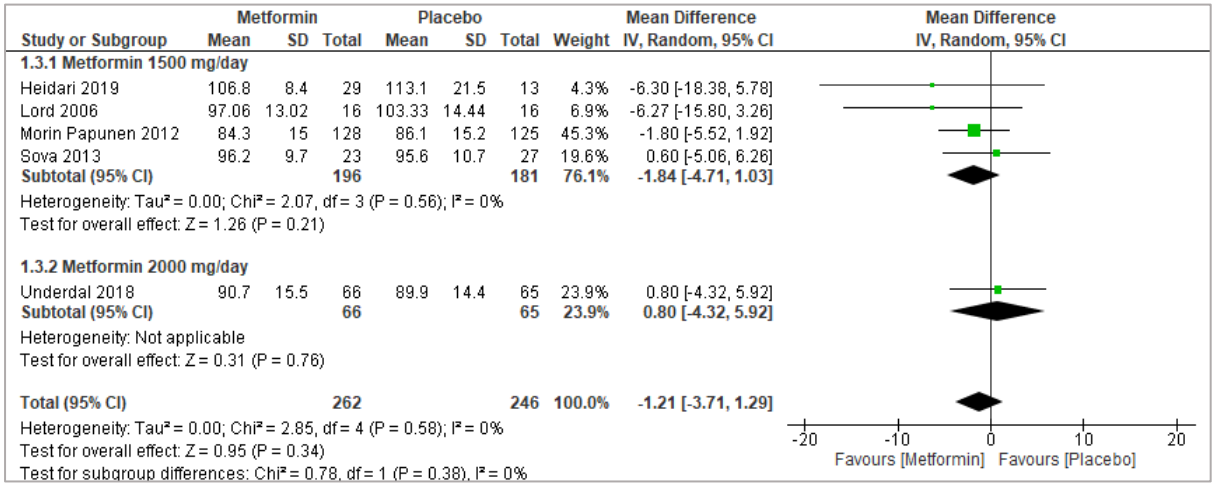
Figure 8-4: Forest plot of Metformin versus placebo on BMI (kg/m²)



8.3.5.3 Waist circumference (WC)

Four RCTs compared metformin 1500 mg QD with placebo showed no effect on the mean WC (MD: -1.84 cm; 95%CI: -4.71-1.03). Another RCT compared metformin 2000 mg QD with placebo, showed no effect on the mean WC (MD: 0.80 cm; 95%CI: -4.32-5.92). Overall, metformin of various dosage has no effect on the mean WC compared with placebo (MD: -1.21 cm; 95%CI: -3.71-1.29; 508 participants, *P* = 0.34) (Figure 8-5) (moderate grade evidence, table 14).

Figure 8-5: Forest plot of Metformin versus placebo on WC (cm)

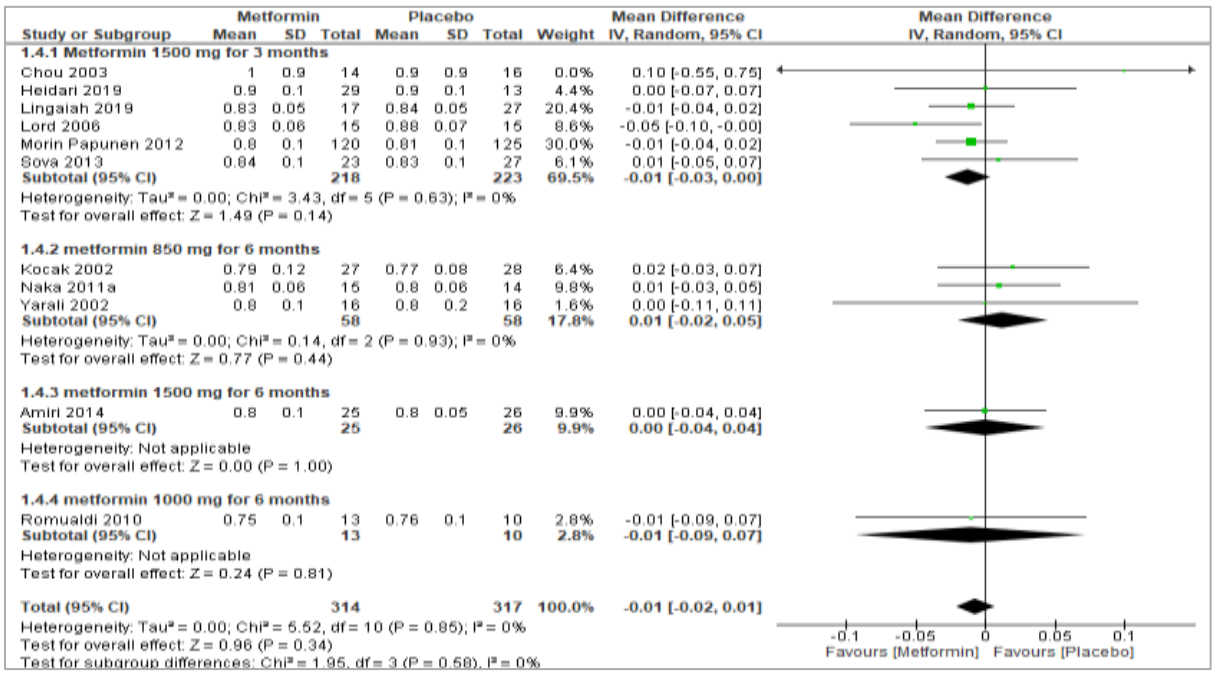


8.3.5.4 Waist to hip ratio (WHR)

In six RCTs compared metformin 1500 mg QD for three months with placebo showed no effect on the mean WHR (MD: -0.01; 95%CI: -0.03, 0.00). Three RCTs compared metformin 850 mg BID with placebo for six months showed no effect on the mean WHR (MD: -0.01; 95%CI: -0.02,0.05). One RCT compared metformin 1500 mg QD for six months with placebo showed no effect on the mean WHR (MD: 0.00;95%CI:-0.04,0.04). Similarly, one RCT compared metformin 1000 mg QD for six months showed no effect on the mean WHR (MD: -0.01;95%CI: -0.09,0.07). Overall, regardless of the dosage and the duration, metformin has no effect on

the mean WHR compared with placebo (MD: -0.01; 95%CI: -0.02-0.01; 632 participants, *p* =0.34) (Figure 8-6) (moderate grade evidence, table 14).

Figure 8-6: Forest plot of Metformin versus placebo on WHR



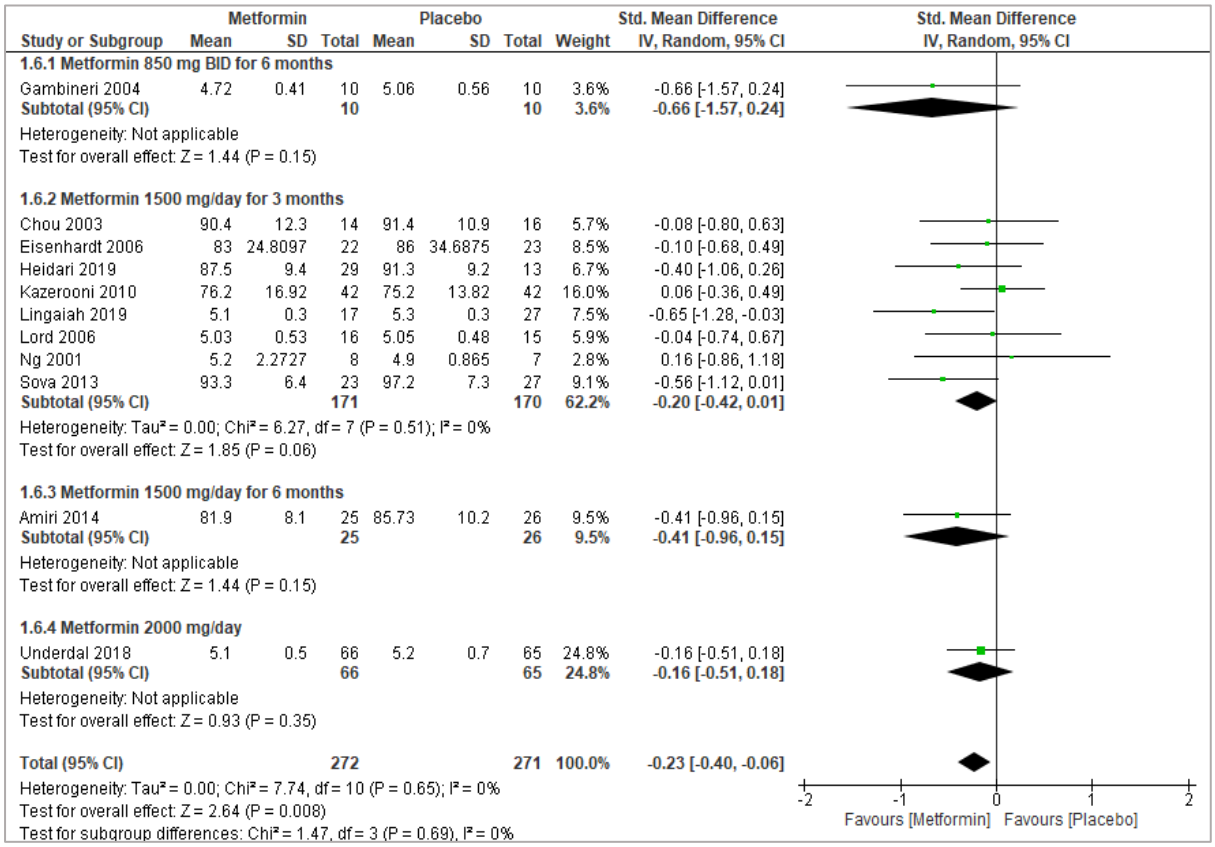
8.3.6 Effect of Metformin on insulin resistance

8.3.6.1 Fasting blood glucose (FBG)

Eleven RCTs investigated the effect of metformin on FBG in 272 women with PCOS compared to 271 in the placebo group. In one RCT, metformin 850 mg BID for six months did not affect fasting blood glucose compared with placebo (SMD: -0.66; 95% CI: -1.57, 0.24). In eight RCTs, metformin 1500 mg QD for three months significantly reduced the mean fasting blood glucose compared with placebo (SMD: -0.20; 95% CI: -0.42, 0.01). In one RCT, metformin, 1500 mg QD for six months, did not affect the mean fasting blood glucose (SMD: -0.41; 95% CI: -0.96, 0.15). In one RCT, metformin 2000 mg QD did not affect the mean blood glucose (SMD: -0.16; 95% CI: -0.51, 0.18). Overall, regardless of the duration and the administered dosages, metformin significantly reduced the mean fasting blood glucose compared with placebo

(SMD: -0.23; 95% CI: -0.40, -0.06; 543 participants, *P* = 0.008) (Figure 8-7) (Moderate grade evidence, Table 14).

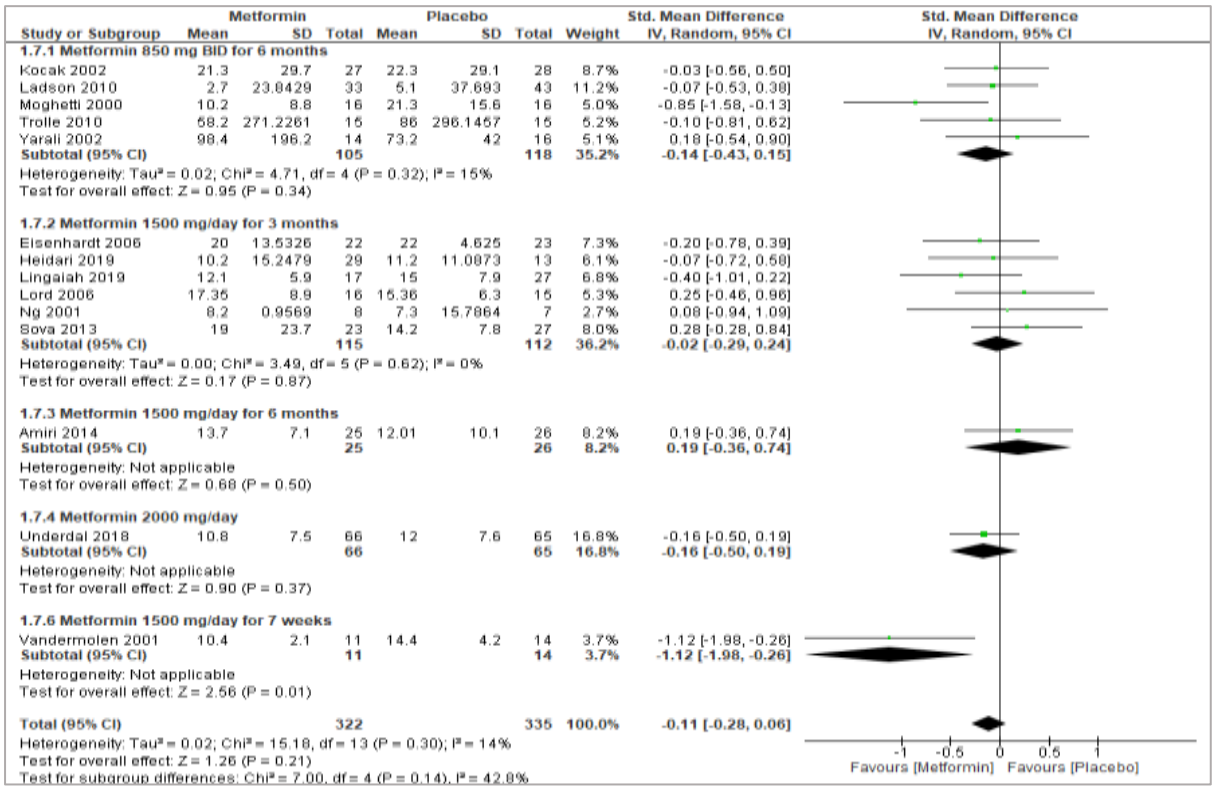
Figure 8-7: Forest plot of Metformin versus placebo on FBG



8.3.6.2 Fasting insulin

Fourteen RCTs investigated the effect of different dosages, frequency, and duration of metformin on FI in 657 (322 metformin group, 335 placebo group) women with PCOS. Only one study reported a significant reduction in FI (SMD: -1.12;95% CI: -1.98, -0.26). Overall, within each subgroup based on and regardless of the dosage, frequency, and duration of metformin, metformin was associated with a non-significant reduction in FI (SMD: -0.11; 95% CI: -0.28, 0.06, *p* = 0.21) (Figure 8-8) (Moderate grade evidence, table 14).

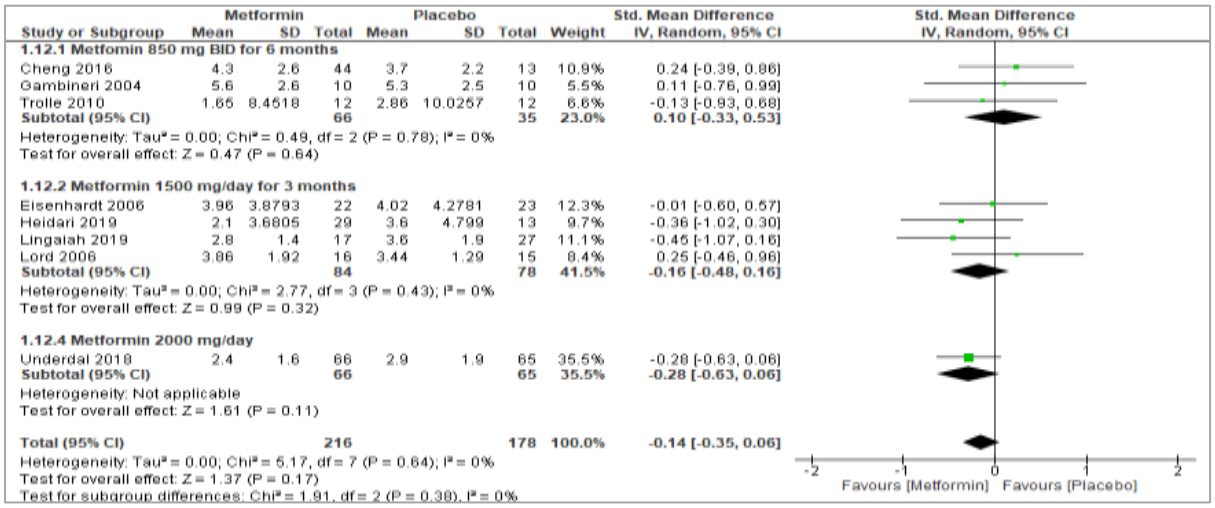
Figure 8-8: Forest plot of Metformin versus placebo on FI



8.3.6.3 Homeostatic model assessment of insulin resistance (HOMA-IR)

Eight RCTs (394 participants) compared metformin of various dosage, frequencies and duration with placebo showed no effect on the mean HOMA-IR (SMD: -0.14; 95%CI: -0.35,0.06, *p* = 0.17) (Figure 8-9) (moderate grade evidence, table 14).

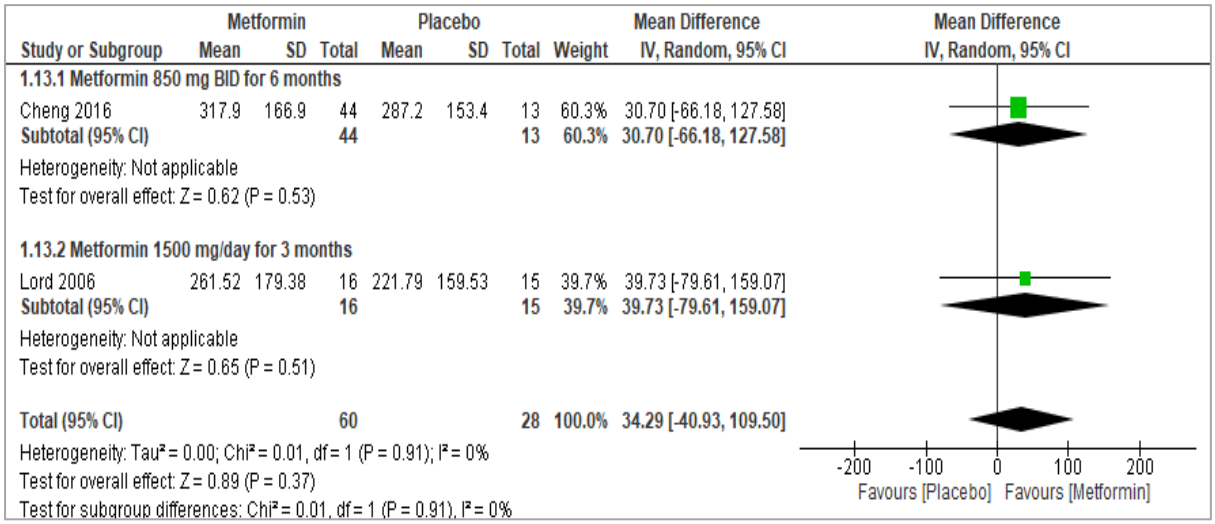
Figure 8-9: Forest plot of Metformin versus placebo on HOMA-IR



8.3.6.4 Homeostatic model assessment of B-cell (HOMA-B)

Two RCTs compared metformin of various dosages and for the various duration with placebo showed no effect on the mean HOMA-B (MD: 34.29; 95%CI: -40.93, 109.50) (Figure 8-10) (low-grade evidence, table 14).

Figure 8-10: Forest plot of Metformin versus placebo on HOMA-B

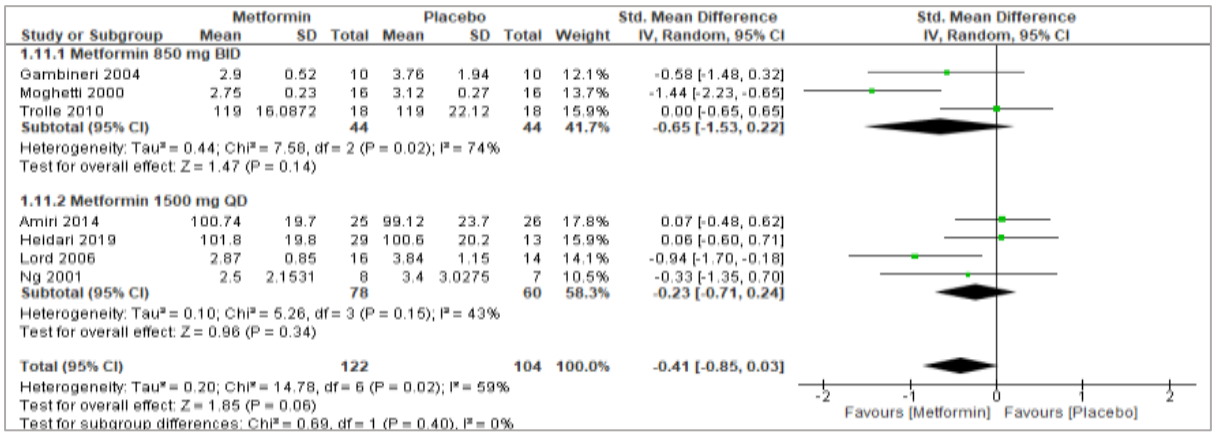


8.3.7 Effects of metformin on the lipid profiles and CRP

8.3.7.1 Low density lipoprotein cholesterol (LDL-C)

In three RCTs, metformin 850 mg BID had no effect on the mean LDL-C (SMD: -0.65; 95% CI: -1.53, 0.22). In four RCTs, metformin 1500 mg QD had no effect on the mean LDL-C (SMD: -0.23; 95% CI: -0.71, 0.24). Overall, regardless of the administered dosages metformin significantly reduced the mean LDL-C compared with placebo (SMD: -0.41; 95% CI: -0.85, 0.03, 226 participants, *P = 0.06*) (Figure 8-11) (Moderate grade evidence, Table 14).

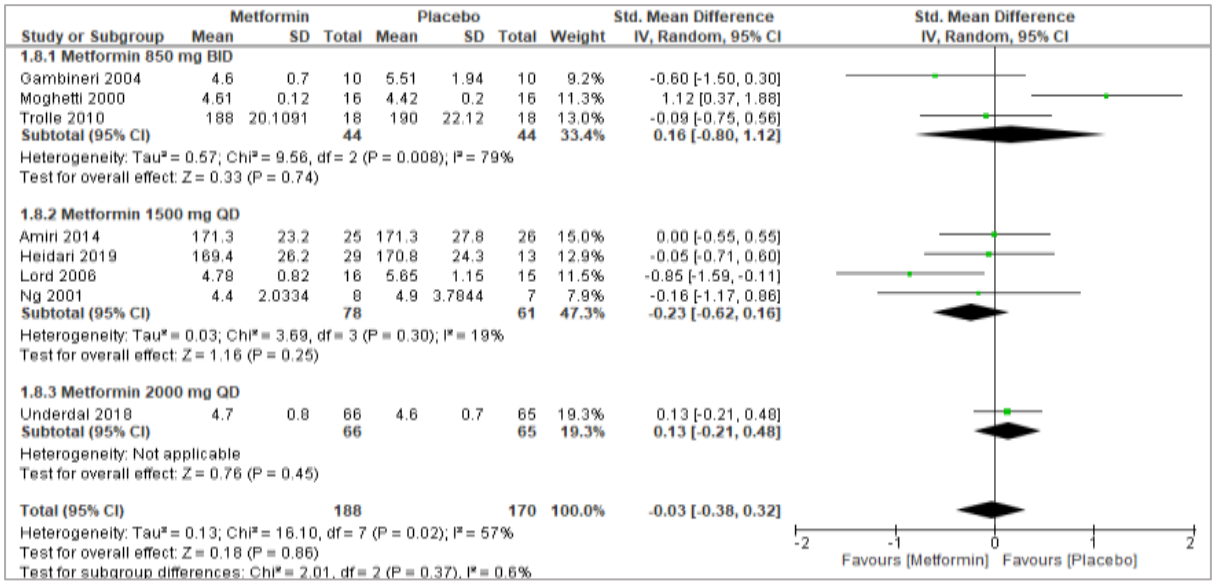
Figure 8-11: Forest plot of Metformin versus placebo on LDL-C



8.3.7.2 Total cholesterol (TC)

Three RCTs compared metformin 850 mg BID with placebo showed no effect on the mean TC (SMD: 0.16; 95%CI: -0.80, 1.12). Four RCTs compared metformin 1500 mg QD with placebo showed no effect on the mean TC (SMD: -0.23; 95%CI: -0.62, 0.16). One RCT compared metformin 2000 mg QD with placebo showed no effect on the mean TC (SMD: 0.13;95%CI: -0.21, 0.48). Overall, regardless of the dosage and frequency, metformin has no effect on the mean TC compared with placebo (SMD: -0.03; 95%CI: -0.38, 0.32, p= 0.66) (Figure 8-12) (moderate grade evidence, table 14).

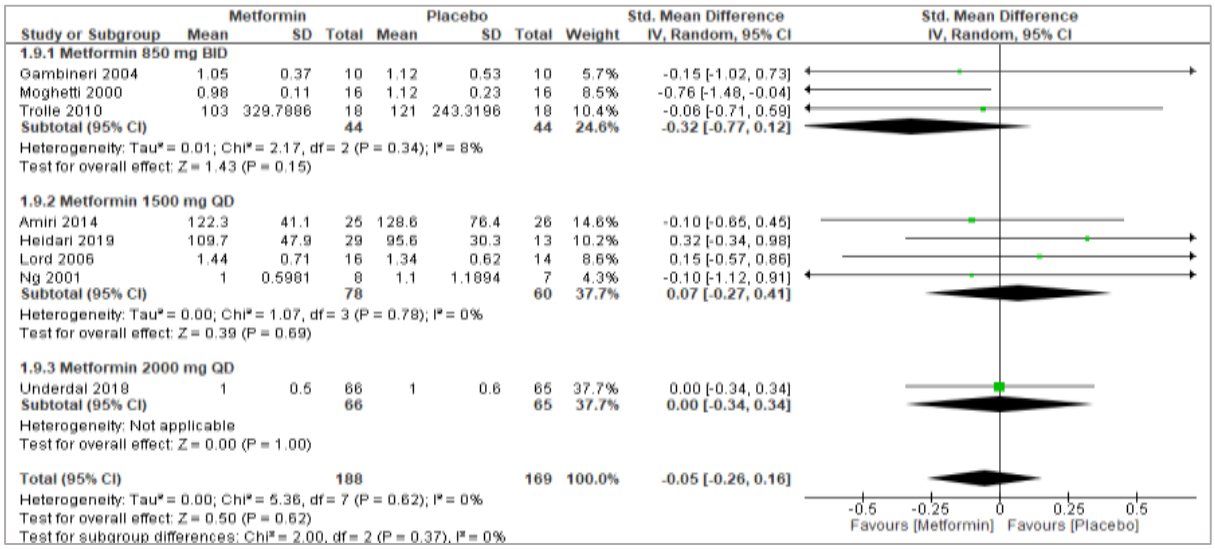
Figure 8-12: Forest plot of Metformin versus placebo on TC



8.3.7.3 Triglycerides (TGs)

Three RCTs comparing metformin 850 mg BID with placebo showed no effect on the mean TGs (SMD: -0.32; 95%CI: -0.77, 0.12). Four RCTs comparing metformin 1500 mg QD with placebo showed no effect on the mean TGs (SMD: 0.07; 95%CI: -0.27, 0.41). One RCT compared metformin 2000 mg QD with placebo showed no effect on the mean TGs (SMD: 0.00; 95%CI: -0.34, 0.34). Overall, metformin of various dosage compared with placebo has no effect on the mean TGs (SMD: -0.05; 95%CI: -0.26, 0.16, *p*=0.62) (Figure 8-13) (moderate grade evidence, table 14).

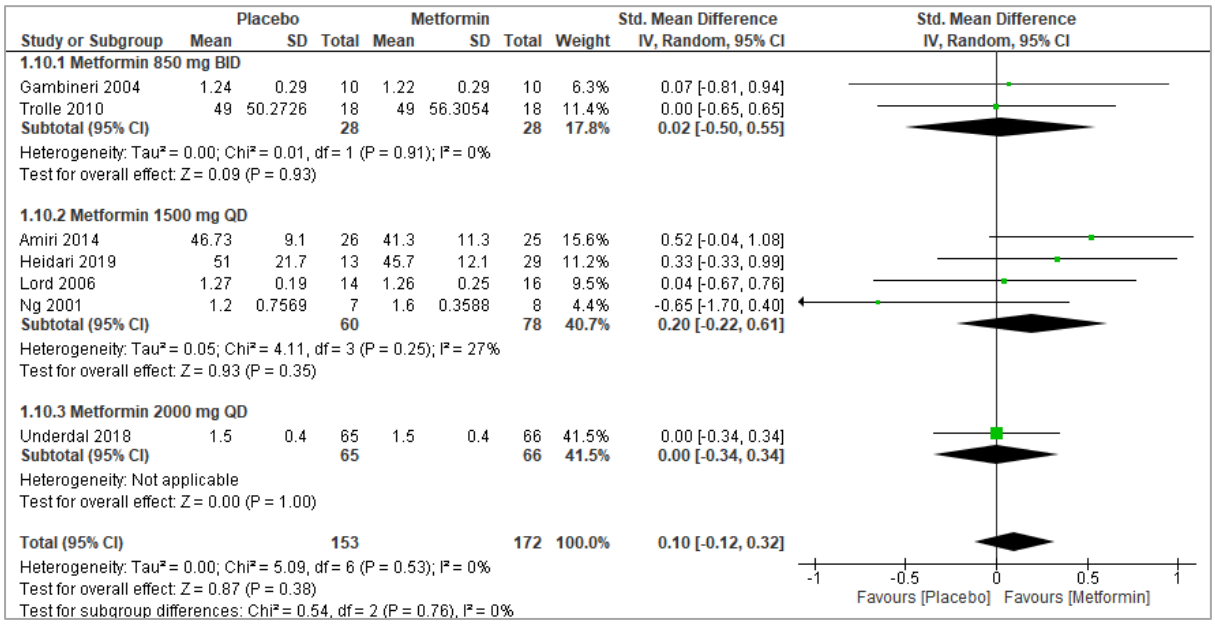
Figure 8-13: Forest plot of Metformin versus placebo on TGs



8.3.7.4 High density lipoprotein cholesterol (HDL-C)

Seven RCTs compared metformin of various dosage and frequencies with placebo showed no effect on the mean HDL-C (SMD: 0.10; 95%CI: -0.12, 0.32 , *p* =0.38) (Figure 8-14) (moderate grade evidence, table 14).

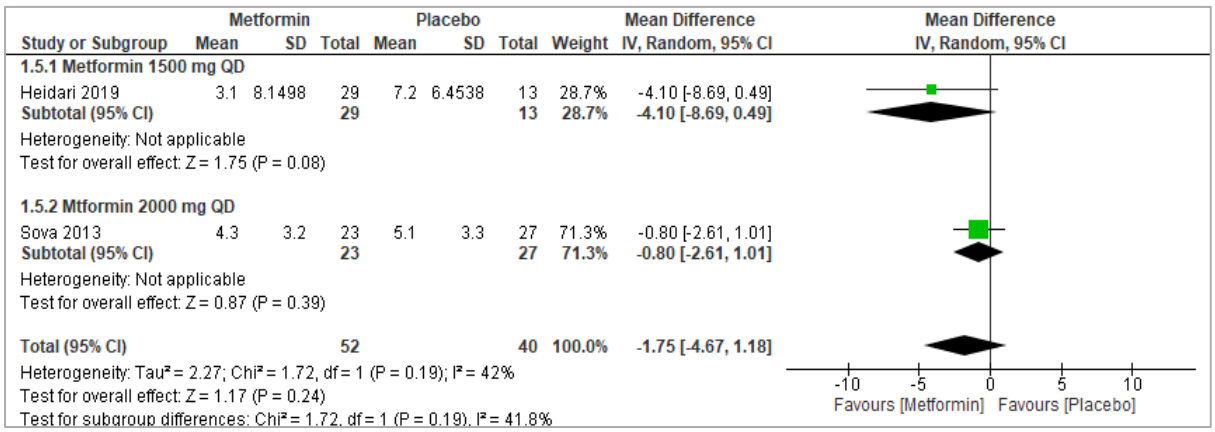
Figure 8-14: Forest plot of Metformin versus placebo on HDL-C



8.3.7.5 C-reactive protein (CRP)

One RCT comparing metformin 1500 mg QD with placebo showed a significant reduction in the mean CRP (MD: -4.10 mg/L; 95%CI: -8.69, 0.49) while in another RCT comparing metformin 2000 mg QD with placebo showed no effect on the mean CRP (MD: -0.80 mg/L ;95%CI: -2.61, 1.01). Overall, metformin of various dosage and frequencies has no effect on the mean CRP comparing with placebo (MD: -1.75 mg/L; 95%CI: -4.67, 1.18, *p* = 0.24) (Figure 8-15) (low grade evidence, table 14).

Figure 8-15: Forest plot of Metformin versus placebo on CRP(mg/dL)

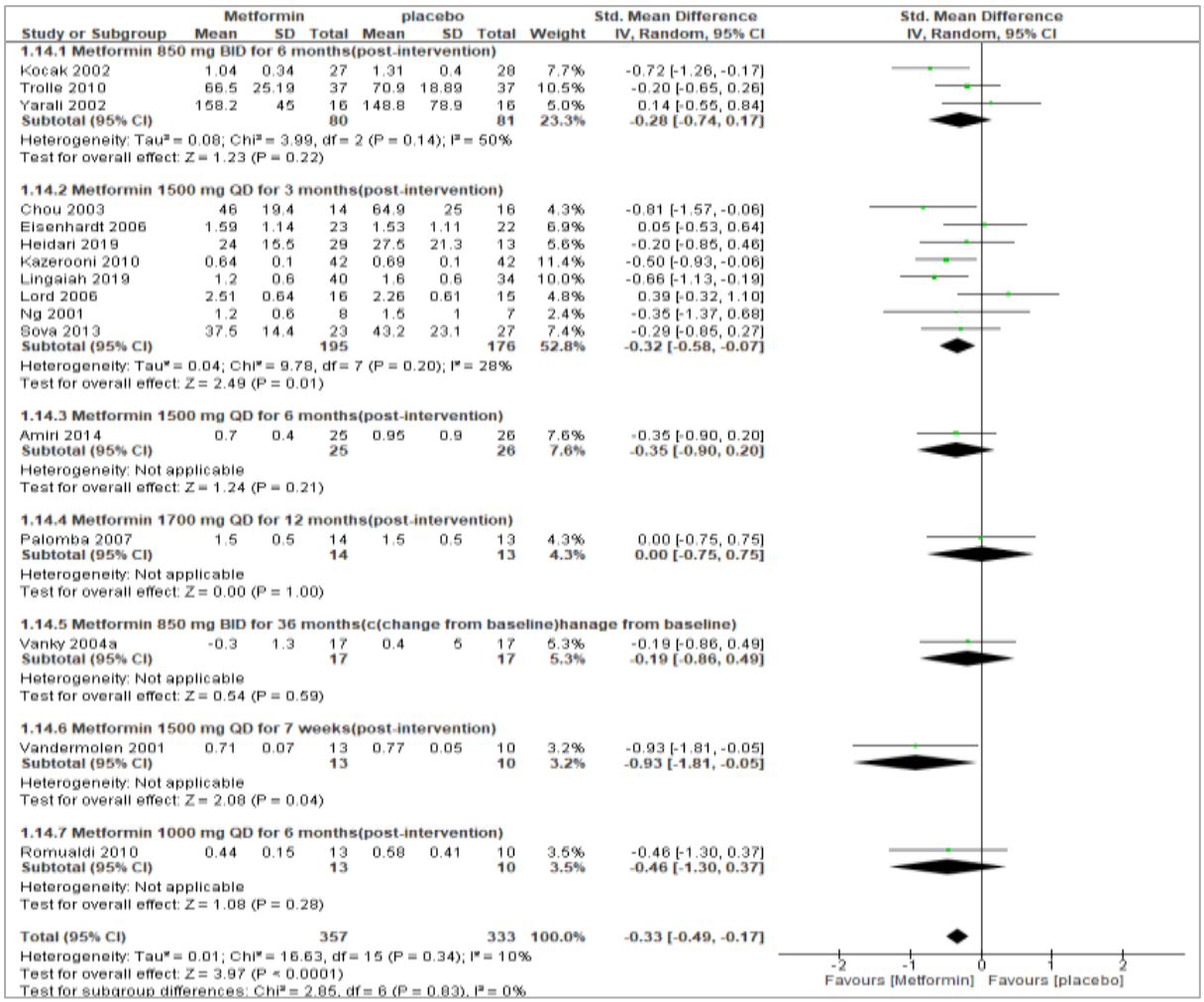


8.3.8 Effects of Metformin on the androgen hormones

8.3.8.1 Total testosterone

In three RCTs, metformin 850 mg BID for six months had no significant effect on the mean total testosterone (SMD: -0.28; 95% CI: -0.74, 0.17). In eight RCTs, metformin 1500 mg QD for three months significantly reduced the mean total testosterone compared with placebo (SMD: -0.32; 95% CI: -0.58, -0.07). One RCT of metformin 1500 mg QD for six months showed no effect on total testosterone compared with placebo (SMD: -0.35; 95% CI: -0.90, 0.20). One RCT compared metformin 1700 mg QD with placebo showed no effect on the mean total testosterone (SMD: 0.00; 95% CI: -0.75, 0.75). One RCT compared metformin 850 mg BID for 36 months showed no effect on total testosterone (SMD: -0.19; 95% CI: -0.86, 0.49). One RCT compared metformin 1500 mg QD for seven weeks showed a significant reduction in the mean total testosterone (SMD: -0.93; 95% CI: -1.81, -0.05). One RCT compared metformin 1000 mg QD with placebo for six months showed a significant reduction in the total testosterone (SMD: -0.46; 95% CI: -1.30, 0.37). Overall, regardless of the administered dosages and the duration, metformin significantly reduced the mean total testosterone compared with placebo (SMD: -0.33; 95% CI: -0.49, -0.17, 690 participants, $P < 0.0001$) (Figure 8-16) (Moderate grade evidence, Table 14).

Figure 8-16: Forest plot of Metformin versus placebo on total testosterone

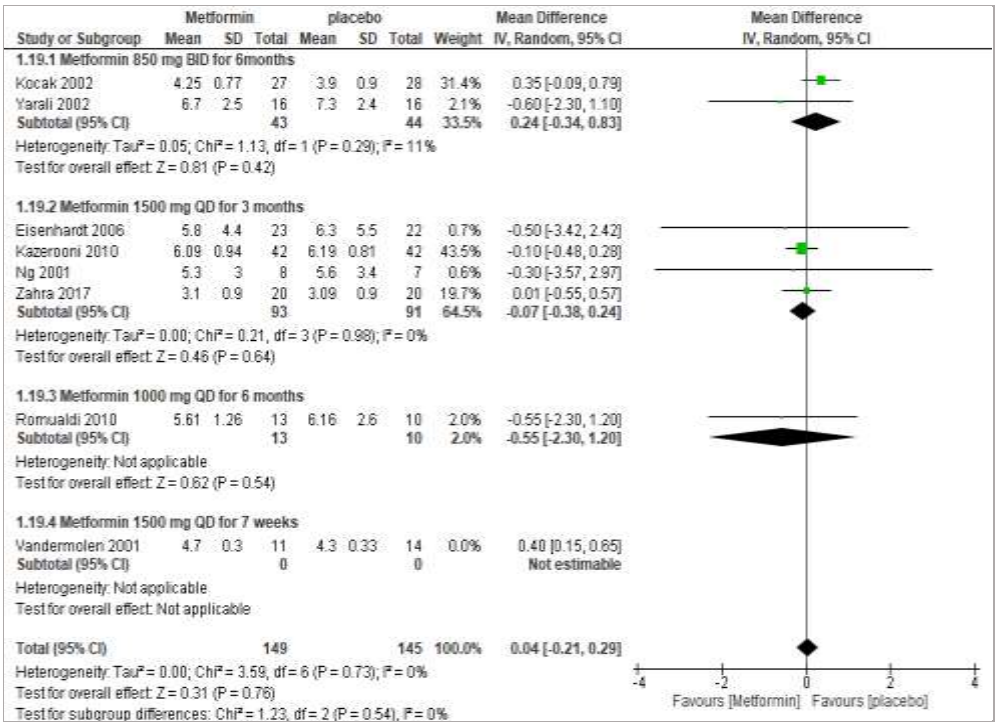


8.3.8.2 FSH

In two RCTs, metformin 850 mg BID for six months had no effect on the mean FSH compared with placebo (MD: 0.24 IU/L; 95% CI: -0.34, 0.83). In four RCTs, metformin 1500 mg QD for three months had no effect on the mean FSH compared with placebo (MD: -0.07 IU/L; 95% CI: -0.38, 0.24). In one RCT, metformin 1000 mg QD for six months had no effect on the mean FSH compared with placebo (MD: -0.55 IU/L; 95% CI: -2.30, 1.20). However, in one RCT, metformin 1500 mg QD for seven weeks significantly increased the mean FSH compared with placebo (MD: 0.40 IU/L; 95% CI: 0.15, 0.65). Overall, regardless of the administered dosages and the duration, metformin has no effect on the mean level of FSH compared with placebo

(MD: 0.04 IU/L; 95% CI: -0.21, 0.29, 294 participants, *P = 0.76*) (Figure 8-17)(Moderate grade evidence, Table 14).

Figure 8-17: Forest plot of Metformin versus placebo on FSH (IU)

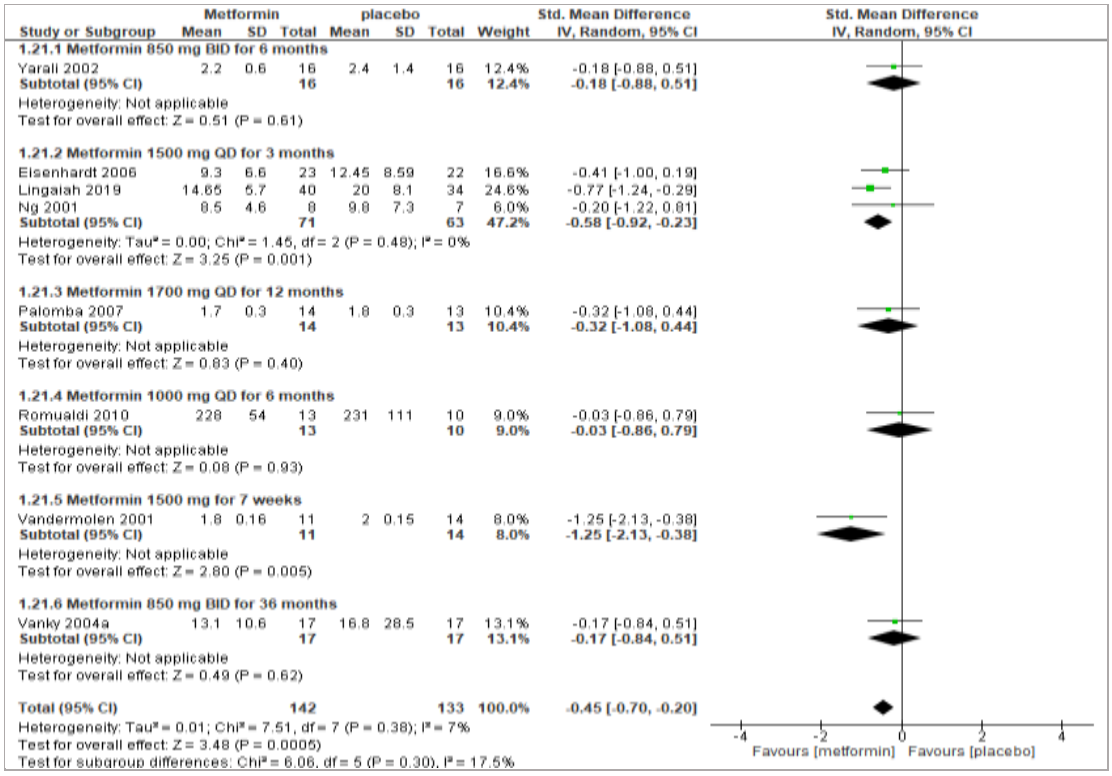


8.3.8.3 Androstenedione

In one RCT, metformin 850 mg BID for six months had no effect on androstenedione (SMD: -0.18; 95% CI: -0.88, 0.51). Three RCTs compared metformin 1500 mg QD for three months showed a significant reduction in androstenedione (SMD: -0.58; 95% CI: -0.92, -0.23). One RCT compared metformin 1700 mg QD for 12 months showed no effect on androstenedione (SMD: -0.32; 95% CI: -1.08, 0.44). One RCT compared metformin 1000 mg QD for six months showed no effect on androstenedione. However, another RCT that compared metformin 1500 mg QD for seven weeks showed a significant reduction in the androstenedione (SMD: -1.25; 95%CI: -2.13, -0.38). One RCT compared metformin 850 mg BID for 36 months showed no reduction in the level of androstenedione (SMD: -0.17; 95%CI: -0.84, 0.51). Overall, metformin at various dosages significantly reduced the level of androstenedione when

compared with placebo (SMD: -0.45; 95% CI: -0.70, -0.20; 275 participants, *p* = 0.0005) (Figure 8-18) (Very low-grade evidence, Table 14).

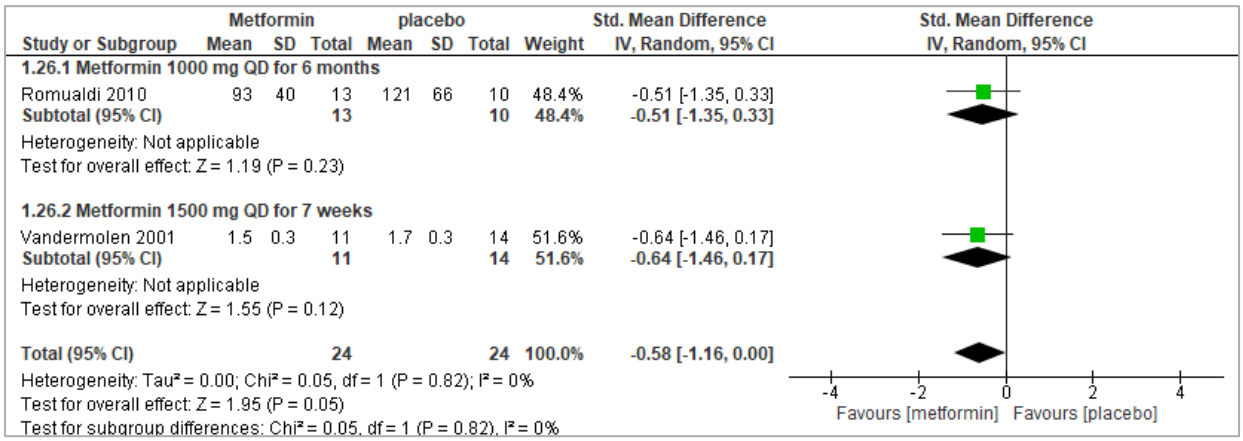
Figure 8-18: Forest plot of Metformin versus placebo on androstenedione



8.3.8.4 17-hydroxyprogesterone (17-OHP)

In one RCT, metformin 1000 mg QD for six months did not affect 17-OHP compared with placebo (SMD: -0.51; 95%CI: -1.35, 0.33). In another RCT, metformin 1500 mg QD for seven weeks did not affected 17-OHP (SMD: -0.64; 95%CI: -1.46, 0.17). However, the pooled estimate showed that metformin significantly reduced 17-OHP when compared with placebo (SMD: -0.58; 95%CI: -1.16, 0.00; 48 participants, *P* = 0.05) (Figure 8-19) (very low-grade evidence, Table 14).

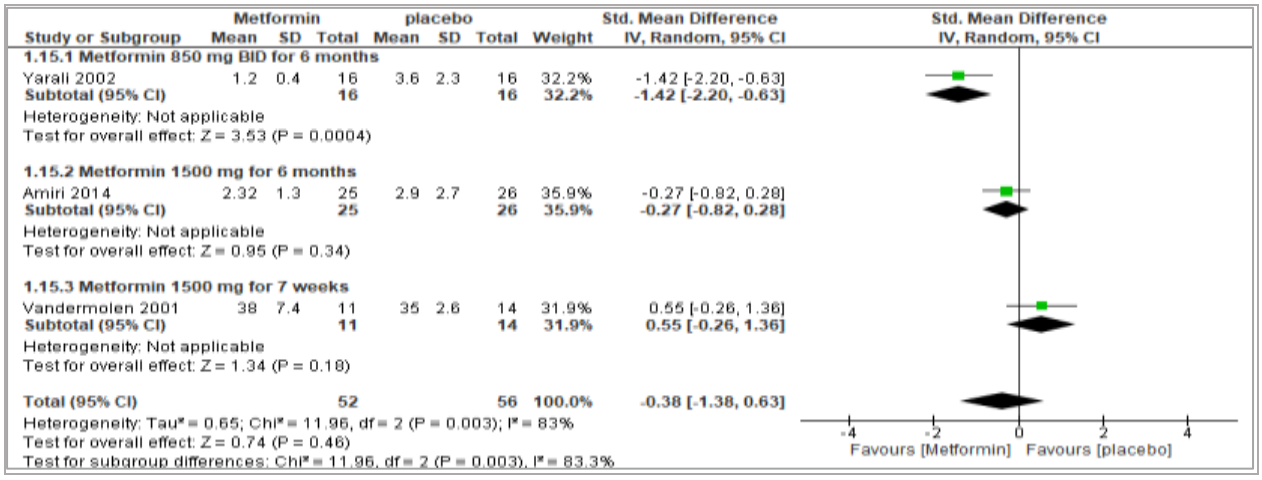
Figure 8-19: Forest plot of Metformin versus placebo on 17-OHP



8.3.8.5 Free testosterone (FT)

In one RCT compared metformin 850 mg BID for six months with placebo showed significant reduction in the mean FT (SMD: -1.42; 95%CI: -2.20, -0.63). Two RCTs compared metformin 1500 mg QD for six months and for seven weeks showed no effect on the mean FT (SMD: -0.27; 95%CI: -0.82-0.28) and (SMD: 0.55; 95%CI: -0.26, 1.36), respectively. Overall, metformin at various dosage and for various duration have no effect on the mean FT compared with placebo (SMD: -0.38; 95%CI: -1.38, 0.63, *p* = 0.46) (Figure 8-20) (very low-grade evidence, table 14).

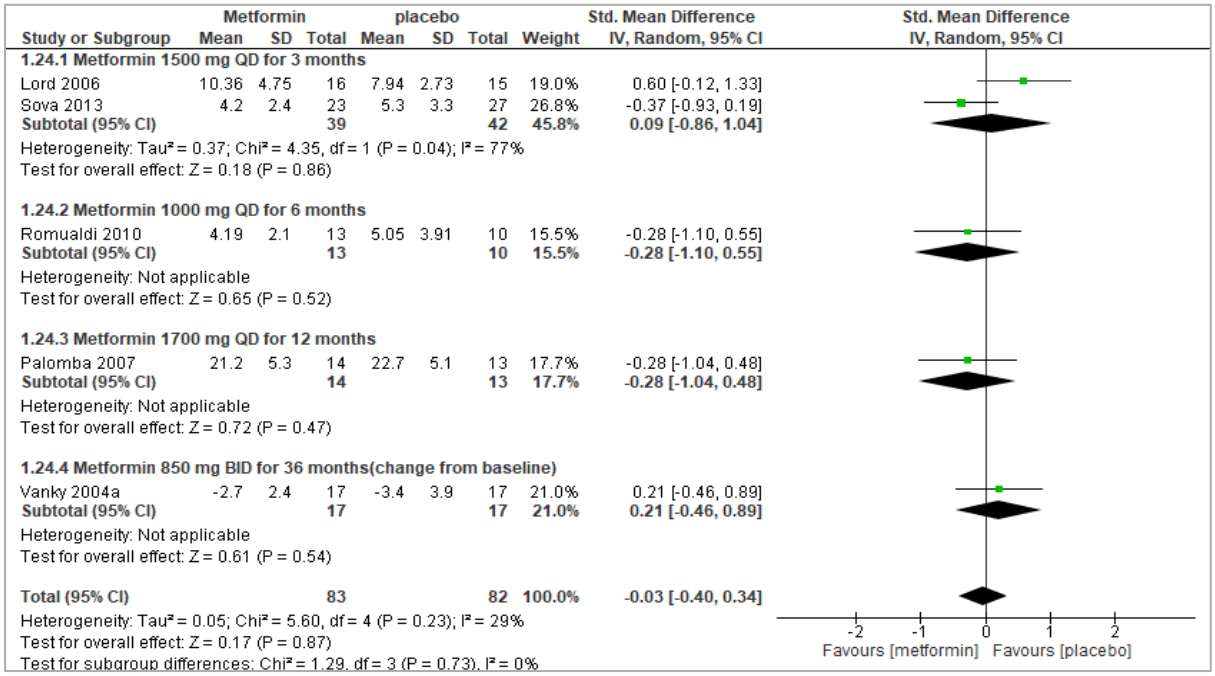
Figure 8-20: Forest plot of Metformin versus placebo on FT



8.3.8.6 Free androgen index (FAI)

Four RCTs compared metformin of various dosage, frequencies and duration with placebo showed no effect on the mean FAI (SMD: -0.03; 95%CI: -0.40, 0.34, *p* = 0.87) (Figure 8-21) (very low-grade evidence, table 14).

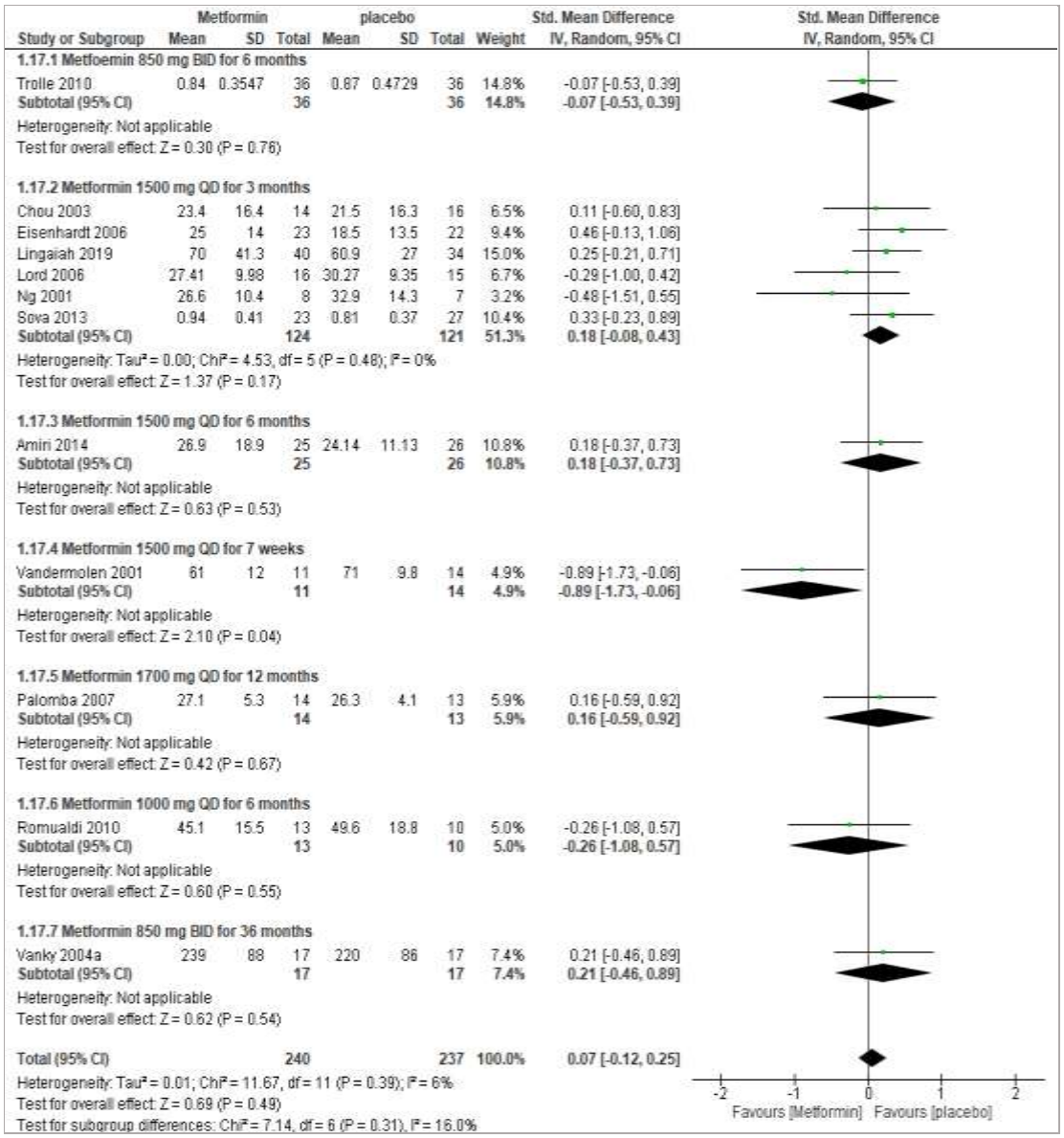
Figure 8-21: Forest plot of Metformin versus placebo on FAI



8.3.8.7 SHBG

In 12 RCTs compared various dosages and frequencies of metformin with placebo, only one RCT that compared metformin 1500 mg QD for seven weeks showed a significant reduction in the mean SHBG (SMD: -0.89; 95%CI: -1.73, -0.06). However, overall, metformin of various dosages showed no effect on the mean SHBG when compared with placebo (SMD: 0.07; 95%CI: -0.12, 0.25, *p* = 0.49) (Figure 8-22) (moderate grade evidence, table 14).

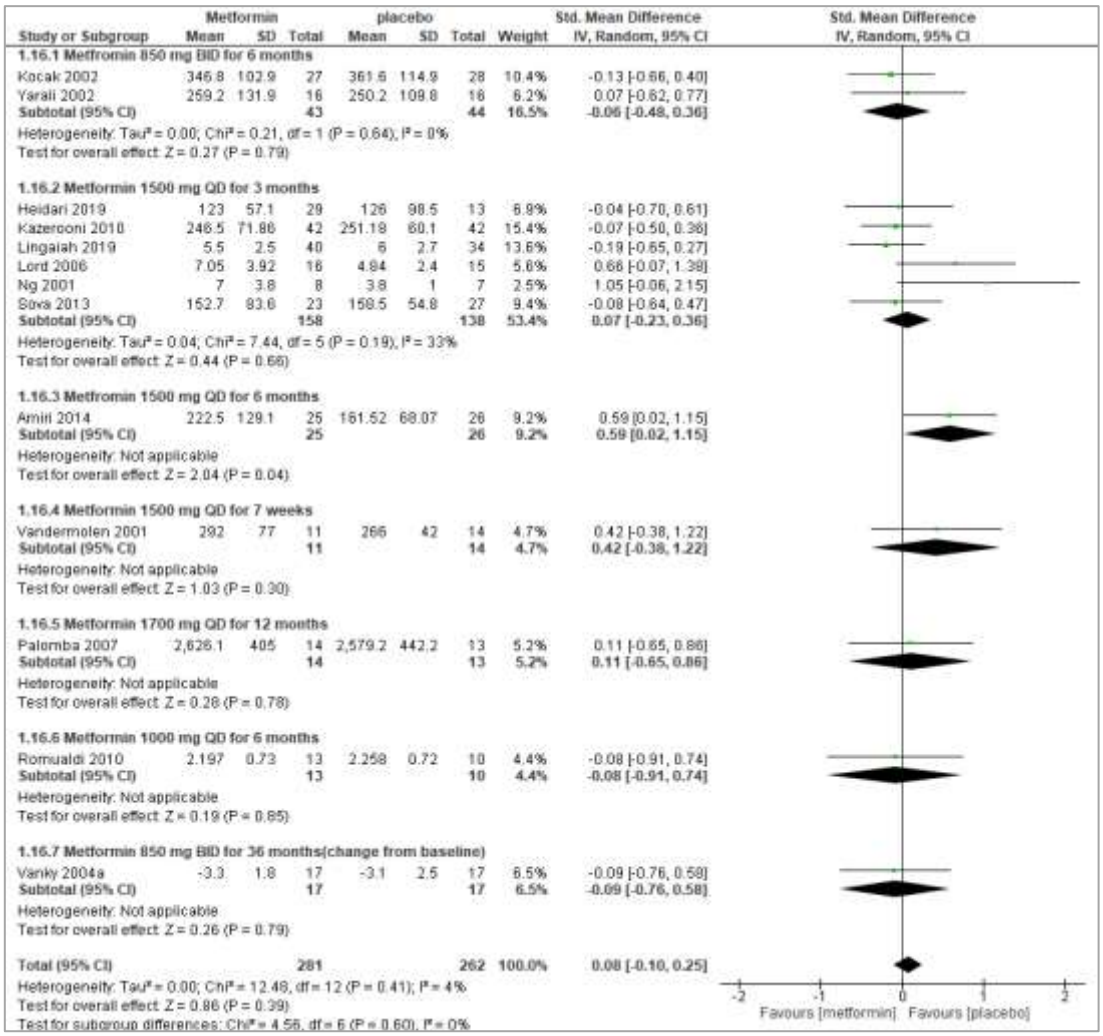
Figure 8-22: Forest plot of Metformin versus placebo on SHBG



8.3.8.8 DHEAS

In 13 RCTs compared metformin of various dosage, frequencies and duration, only one RCT compared metformin 1500 mg QD for six months with placebo showed a significant increase in the mean DHEAS (SMD: 0.59; 95%CI: 0.02, 1.15). However, overall, metformin of various dosages and duration has no effect on the mean DHEAS when compared with placebo (SMD: 0.08; 95%CI: -0.10, 0.25, *p* = 0.39) (Figure 8-23) (moderate grade evidence, table 14).

Figure 8-23: Forest plot of Metformin versus placebo on DHEAS

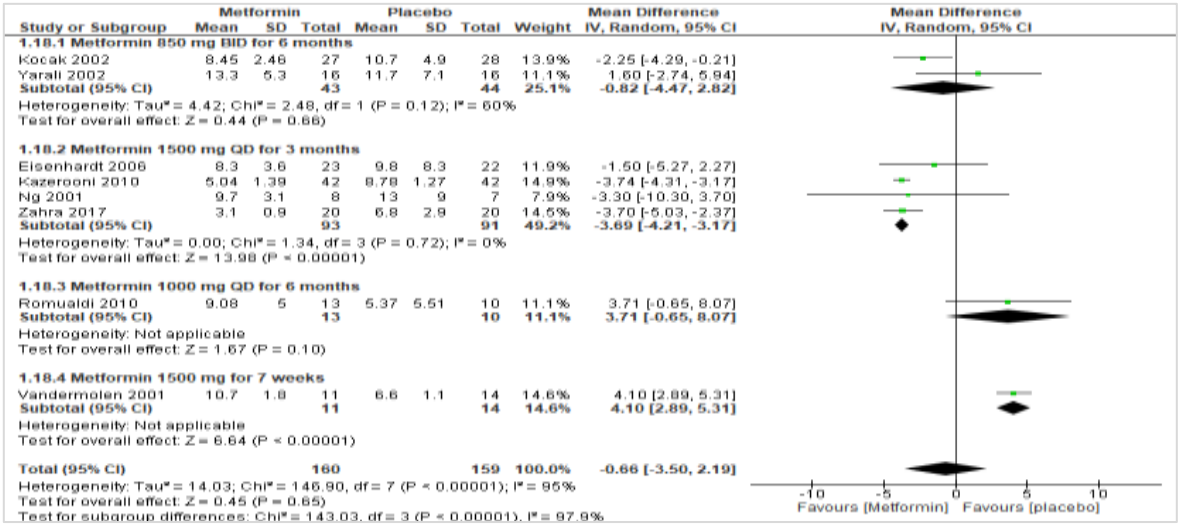


8.3.8.9 LH

Two RCTs compared metformin 850 mg BID with placebo for six months showed no effect on the mean LH (MD: -0.82 IU/L; 95%CI: -4.47,2.82). Four RCTs compared metformin 1500 mg QD for three months with placebo showed a significant reduction in the mean LH (MD: -3.69 IU/L; 95%CI: -4.21, -3.17). One RCT compared metformin 1000 mg QD for six months showed no effect on the mean LH (MD: 3.71 IU/L; 95%CI: -0.65, 8.07). However, one RCT compared metformin 1500 mg QD with placebo for seven weeks showed a significant increase in the mean LH (MD: 4.10 IU/L; 95%CI: 2.89, 5.31). The RCTs compared metformin of various dosage, duration and frequencies reported inconsistency in the effect of metformin on the LH. Overall,

metformin of various dosage has no effect on the mean LH (MD: -0.66 IU/L; 95% CI: -3.50, 2.19, *p* = 0.65) (Figure 8-24) (moderate grade evidence, table 14).

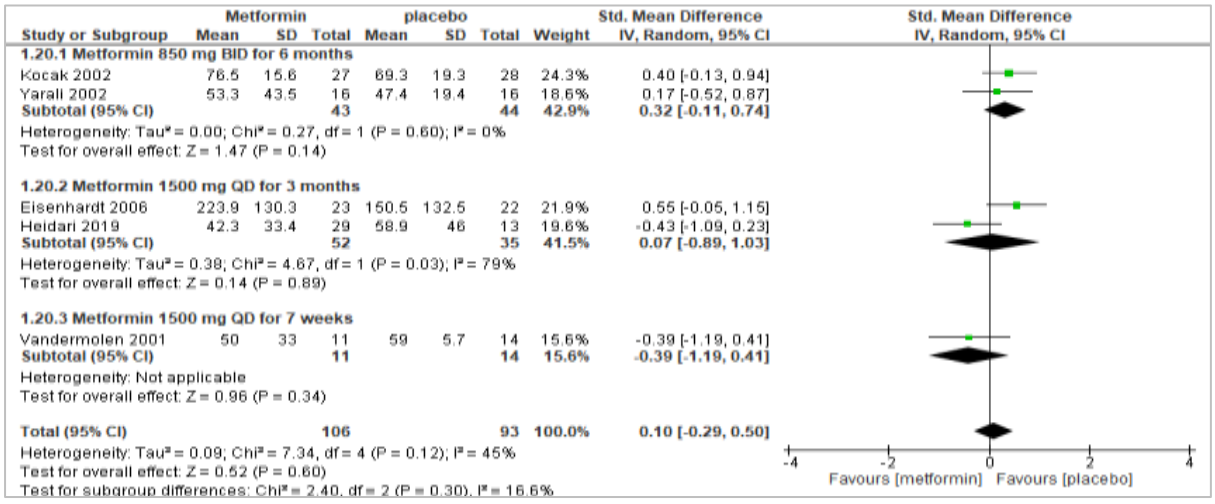
Figure 8-24: Forest plot of Metformin versus placebo on LH (IU/L)



8.3.8.10 Oestradiol

In five RCTs compared metformin of various dosage, frequencies and duration with placebo showed no effect on the mean oestradiol (SMD: 0.10; 95%CI: -0.29, 0.50, *p* = 0.60) (Figure 8-25) (very low-grade evidence, table 14).

Figure 8-25: Forest plot of Metformin versus placebo on oestradiol

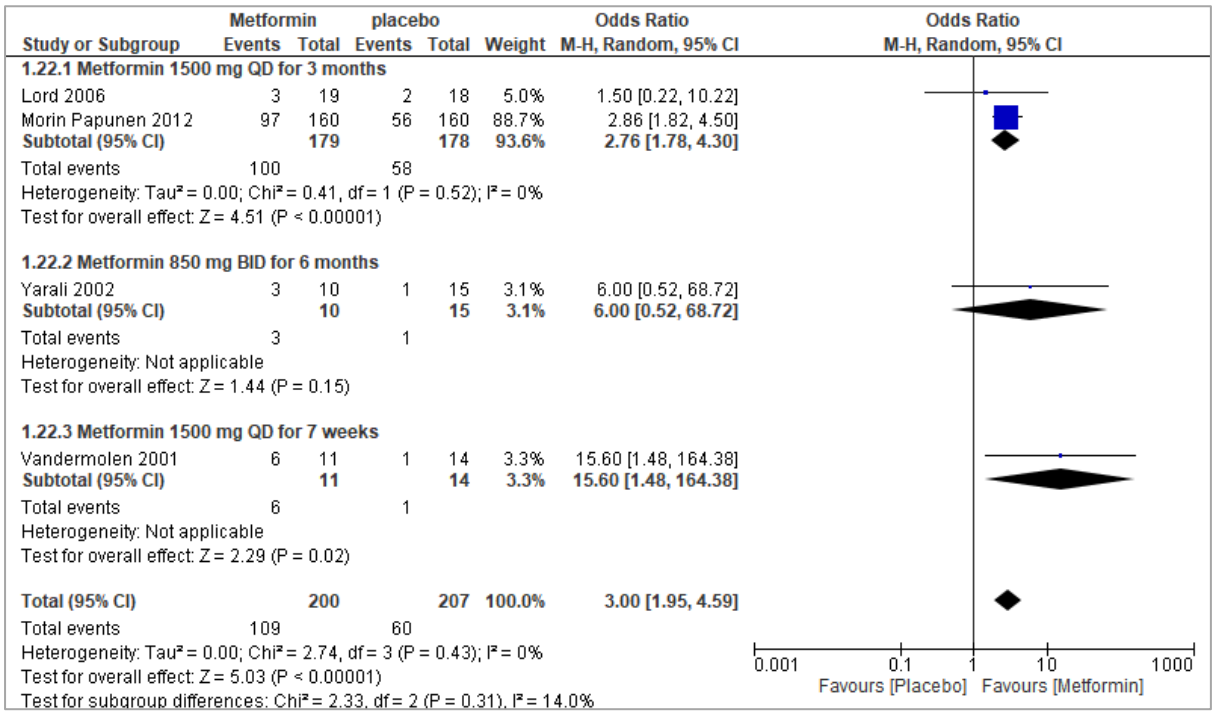


8.3.9 Metformin effect on the fertility outcomes

8.3.9.1 Pregnancy rate

In two RCTs, metformin 1500 mg QD for three months significantly increased the pregnancy rate (OR: 2.76; 95%CI: 1.78, 4.30). One RCT metformin 850 mg BID for six months did not affect the pregnancy rate (OR: 6.0; 95%CI: 0.52, 68.72). In one RCT, metformin 1500 mg QD for seven weeks significantly increased the pregnancy rate (OR: 15.60; 95%CI: 1.48,164.38). Overall, regardless of the administered dosage or the duration, metformin significantly increased the rate of pregnancy (OR: 3.00; 95%CI: 1.95, 4.59, I^2= 0%, $p < 0.00001$) (Figure 8-26) (very low-grade evidence, Table 14).

Figure 8-26: Forest plot of Metformin versus placebo on pregnancy rate



8.3.9.2 Ovulation rate

The pooled estimate effect of metformin of various dosage, duration and frequencies showed no effect on the ovulation rate compared with placebo (OR: 2.57; 95%CI: 0.60, 11.03, p = 0.20) (Figure 8-27) (very low-grade evidence, table 14). However, one RCT showed a significant

increase in the ovulation rate when metformin 1500 mg QD for seven weeks was compared with placebo.

Figure 8-27: Forest plot of Metformin versus placebo on ovulation rate

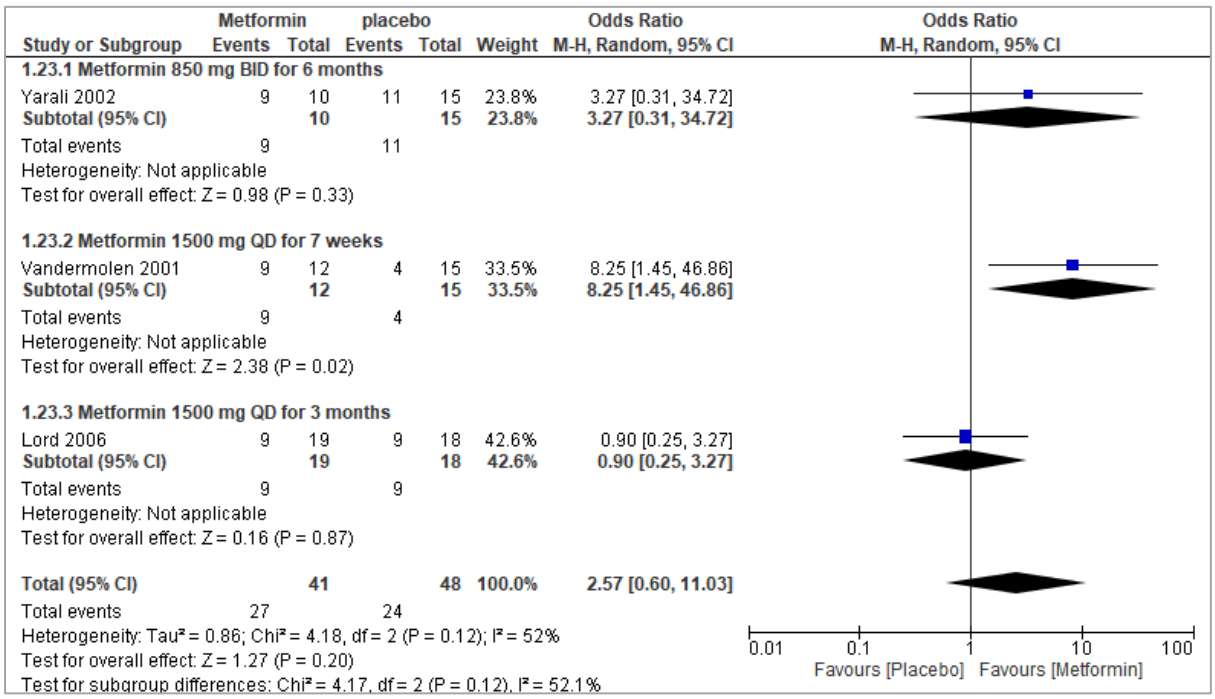


Table 14: 'Summary of findings'

No. of participants (studies)	Design	Limitations (RoB)	Indirectness of patients, intervention and comparator	Inconsistency	Imprecision	Other considerations	Quality of evidence
Bodyweight							
739 patients (10 RCTs)	RCT	Some concerns [a]	None	None	None	None	⊕⊕⊕◯Moderate
BMI							
1314 (22 RCTs)	RCT	Some concerns [a]	None	None	None	None	⊕⊕⊕◯ Moderate
Waist circumference							
508 (5 RCTs)	RCT	Some concerns [a]	None	None	None	None	⊕⊕⊕◯ Moderate
Waist hip ratio							
639 (11 RCTs)	RCT	None	None	None	Some concerns [b]	None	⊕⊕⊕◯ Moderate
Fasting blood glucose							
543 (11 RCTs)	RCT	None	None	None	Some concerns [b]	None	⊕⊕⊕◯ Moderate
Fasting insulin							
657 (14 RCTs)	RCT	Some concerns [a]	None	None	None	None	⊕⊕⊕◯ Moderate
HOMA-IR							
394 (8 RCTs)	RCT	None	None	None	Some concerns [b]	None	⊕⊕⊕◯ Moderate
HOMA-B							
88 (2 RCTs)	RCT	None	None	None	Some concerns [d]	Some concerns [e]	⊕⊕◯◯ Low
Total cholesterol							
581 (10 RCTs)	RCT	None	None	Some concerns [c]	None	None	⊕⊕⊕◯ Moderate
Triglycerides							
610 (11 RCTs)	RCT	None	None	None	Some concerns [b]	None	⊕⊕⊕◯ Moderate
HDL-C							

492 (11 RCTs)	RCT	None	None	None	Some concerns [b]	None	⊕⊕⊕◯ Moderate
LDL-C							
506 (11 RCTs)	RCT	None	None	None	Some concerns [b]	None	⊕⊕⊕◯ Moderate
CRP							
92 (2 RCTs)	RCT	None	None	Some concerns [b]	Some concerns [c, d]	None	⊕⊕◯◯ Low
Total testosterone							
690 (16 RCTs)	RCT	None	None	None	Some concerns [a]	None	⊕⊕⊕◯ Moderate
Free testosterone							
108 (3 RCTs)	RCT	Some concerns [a]	None	None	Some concerns [c]	None	⊕◯◯◯ Very low
Free androgen index							
165 (5 RCTs)	RCT	Some concerns [a]	None	None	Some concerns [c]	None	⊕◯◯◯ Very low
SHBG							
477 (12RCTs)	RCT	None	None	None	Some concerns [c]	None	⊕⊕⊕◯ Moderate
DHEAS							
534 (13 RCTs)	RCT	None	None	None	Some concerns [c]	None	⊕⊕⊕◯ Moderate
LH							
319 (8 RCTs	RCT	None	None	None	Some concerns [c]	None	⊕⊕⊕◯ Moderate
FSH							
319 (8RCTs)	RCT	None	None	None	Some concerns [c]	None	⊕⊕⊕◯ Moderate
Oestradiol							
199 (5RCTs)	RCT	Some concerns [a]	None	None	Some concerns [c]	None	⊕◯◯◯ Very low
Androstenedione							
175 (6 RCTs)	RCT	Some concerns [a]	None	None	Some concerns [c]	None	⊕◯◯◯ Very low

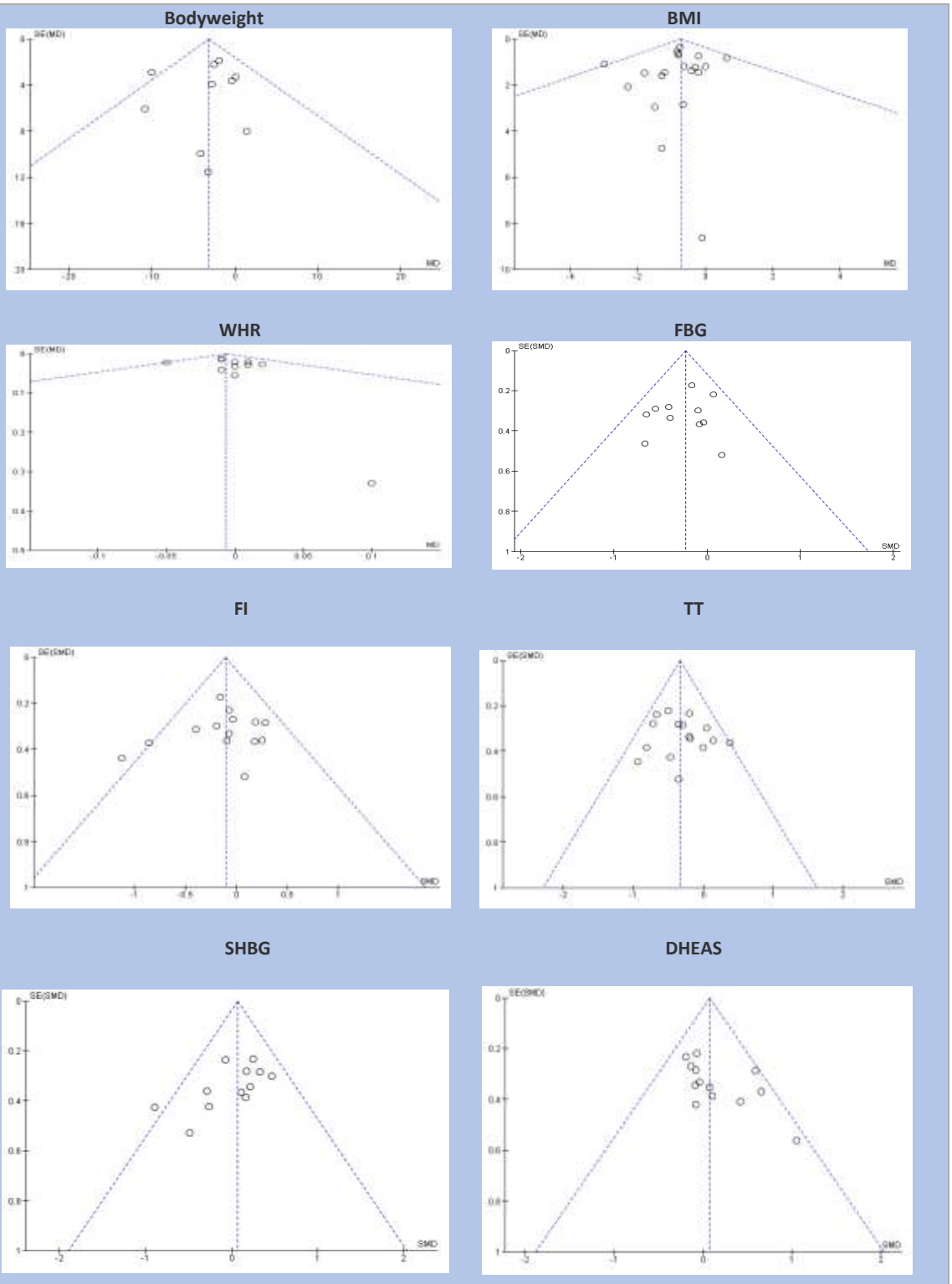
17-OHP							
48 (2RCTs)	RCT	Some concerns [a]	None	None	Some concerns [c]	None	⊕◯◯◯ Very low
Pregnancy rate							
169 (4RCTs)	RCT	Some concerns [a]	None	None	Some concerns [c]	None	⊕◯◯◯ Very low
Ovulation rate							
89 (3RCTs)	RCT	Some concerns [a]	None	None	Some concerns [c]	None	⊕◯◯◯ Very low

GRADE Working Group grades of evidence.
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.
Explanations
a. The majority of the studies have an unclear risk of selection bias, detection bias, allocation concealment, and one study has a high risk of performance bias. Thus, we downgraded it by one level.
b. a Small number of participants, wide confidence intervals and small or negligible effect, appreciable benefit included in the confidence interval for the mean difference. Thus, we downgraded it by one level.
c. considerably high level of heterogeneity among the studies. Thus, we downgraded one level.
d. Very small sample cohort size with a significant effect on the CI of the MD/SMD. Therefore, we downgraded one level.
e. There is a high probability of publication bias for small unreported studies, and there was no overlapping in the CI. Thus, we downgraded one level.

8.3.10 Assessment of publication bias

The funnel plot of the RevMan with standard error (SE) was used to assess publication bias where more than 10 RCTs were meta-analysed. Thus, there was no significant asymmetry of the treatment effect for the assessed outcomes, which implied a low chance of publication bias (Figure 8-28).

Figure 8-28: Funnel plots



BMI: body mass index, **WHR**: waist-hip ratio, **FBG:** fasting blood glucose, **FI:** fasting insulin**, TT:** total testosterone, **SHBG:** sex hormone binding globulin, **DHEAS:** dehdroepianderostendione sulphate**. SE:** standard error (X-axis), **SMD**: standardised mean difference (Y-axis).

8.4 Discussion

This systematic review has outlined the up-to-date evidence supporting the effectiveness of metformin in the management of PCOS. To our knowledge, this is the first comprehensive systematic review to report the effects of metformin on the anthropometric outcomes, insulin resistance indices, lipid profiles and CRP, androgen hormones and fertility outcomes of women with PCOS. When metformin was administered at various therapeutic doses and compared with placebo, there were statistically significant reductions in the mean body weight, BMI, WC, fasting blood glucose, total testosterone, 17-OHP, and LDL-C, and an increase in the pregnancy rate in women with PCOS. However, we should acknowledge that metformin is unlike CC; it indirectly induces ovulation by reducing insulin and is less effective in ovulation induction. On the other hand, CC acts directly by inhibiting the negative feedback on HPO-axis and inducing ovulation (826). However, using metformin as an add-on therapy to CC significantly increases ovulation and the pregnancy rate.

Moreover, pre-treatment with metformin prior to ovulation induction with CC enhanced the ovulation and the pregnancy rate (827). These findings are in line with the findings of previous studies. In an RCT of 626 infertile women with PCOS, they were randomised to receive metformin plus placebo, CC plus placebo or the combination for six months. There was a higher pregnancy rate and live birth rate with CC than with metformin (437). In an RCT of obese women with PCOS evaluating the effect of metformin on body weight, a significant decrease in BMI independent of lifestyle changes was reported (373). Women with PCOS are also at a higher risk of developing CVD due to hyperinsulinaemia, high androgen levels, obesity and dyslipidaemia (376). There is evidence that both obesity and PCOS independently affect vascular endothelial function (377); however, the associations between

hyperinsulinemia and CVD are independent of body weight (378,379). Women with PCOS also have dyslipidaemia (380), manifested as low HDL and high triglyceride levels, a strong CVD predictor (381). Thus, the management of dyslipidaemia is crucial in PCOS. Metformin improves dyslipidaemia by directly affecting the hepatic metabolism of free fatty acids or indirectly by reducing hyperinsulinemia by enhancing insulin sensitivity (382); however, there was no beneficial effect of metformin on total cholesterol levels (385).

This study followed a comprehensive and systematic method to search for relevant databases and grey sources and only included RCTs. Steps were taken to minimise the risk of bias, and we excluded observational studies and non-randomised clinical trials. To the authors' knowledge, this review is the most comprehensive and up-to-date systematic review and meta-analysis on the effect of metformin in women with PCOS.

The limitations of this study include that the majority of the RCTs were small, and the statistical power used to calculate sample size was not fully reported. Moreover, all the trials were of short duration; therefore, the long-term effects of metformin in women with PCOS is not apparent.

8.5 Conclusion

Metformin, alone and irrespective of the dosage and duration of therapy, significantly reduces the mean body weight, BMI, LDL-C, total testosterone, androstenedione, 17-OHP, fasting blood glucose and increases the pregnancy rate in women with PCOS compared to placebo.

9 Chapter 9: The effect of thiazolidinediones in polycystic ovary syndrome: a systematic review and meta-analysis of randomised controlled trials

9.1 Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder that affects women of reproductive age, with a prevalence of up to 20% (816, 828). PCOS is reflected by biochemical and clinical features of high androgen levels, menstrual irregularities and polycystic ovarian morphology (344). The pathophysiology of PCOS remains elusive; however, insulin resistance plays a significant role in its aetiology. In PCOS, high insulin contributes to excess ovarian androgen release and positively correlated with insulin levels (55). On the other hand, increased body weight, a known feature of PCOS, can also increase the androgen and insulin levels that exacerbate its clinical features (737). High insulin stimulates the release of the androgen hormone by both ovaries and adrenal glands (785). High androgen levels lead to hirsutism and reduced fertility in women with PCOS (829). Women with PCOS have a significantly higher rate of impaired glucose tolerance and insulin resistance, which are features for type 2 diabetes mellitus (T2DM)(818). Nearly 70 % of women with PCOS will develop the metabolic syndrome (MS) characterised by dyslipidaemia, central adiposity, hypertension and impaired glucose tolerance, and all are predisposing factors for cardiovascular disease (CVD) (819, 820). A therapeutic approach focusing on weight loss and improving insulin resistance is the primary strategy in managing PCOS (506, 821).

Thiazolidinediones (TZDs) are insulin sensitisers primarily used to manage T2DM. They exert their action by activating the gamma isoform of the nuclear receptor peroxisome proliferator-activated receptors (PPRA-gamma)(657). TZDs reduce insulin resistance in adipose tissue,

muscle and the liver by increasing the transcription of several insulin-sensitive genes. Pioglitazone is the main TZD currently used in clinical practice to manage T2DM. Although Rosiglitazone is a less common TZD, it was withdrawn from the market in many countries worldwide due to concern over its cardiovascular safety (830, 831).

The TZD troglitazone has been formally withdrawn from the market due to its significant hepatotoxicity (832). Nevertheless, TZDs have been used in PCOS, with many beneficial effects have been reported. Furthermore, small clinical trials have compared the effectiveness of TZDs as monotherapy or add-on therapy to metformin in women with PCOS and reported variable metabolic benefits (833). Therefore, this systematic review aimed to thoroughly evaluate the effectiveness of pioglitazone and rosiglitazone as an add-on therapy to metformin in metformin-resistant women with PCOS or as monotherapy.

9.2 Methods and materials

9.2.1 Protocol and registration

The protocol of this systematic review and meta-analysis is explained in chapter 2, section 2.1.1.1.

9.2.2 Eligibility criteria for the included studies

The eligibility criteria, including inclusion/exclusion criteria, is explained in chapter 2, section 2.1.1.2.

9.2.3 Literature search

The search strategy, including the search in the medical databases, is explained in chapter 2, section 2.1.1.3.

9.2.4 Study selection

The study selection, including screening titles and abstracts of the retrieved studies, is explained in chapter 2, section 2.1.1.4.

9.2.5 Data extraction

The method used to extract information from the included studies is explained in chapter 2, section 2.1.1.5.

9.2.6 Risk of bias assessment in the included studies

The RoB assessment for the included studies is explained in chapter 2, section 2.1.1.6.

9.2.7 GRADE scoring

The robustness of evidence for each chosen outcome is assessed using the GRADE scoring system and is explained in chapter 2, section 2.1.1.7.

9.2.8 Statistical analysis

The statistical method used to estimate the pooled effects of the various interventions is explained in chapter 2, section 2.1.1.12.

9.2.9 Assessment of heterogeneity

Heterogeneity for the outcomes across each RCT was evaluated and explained in chapter 2, section 2.1.1.8.

9.2.10 Subgroup analysis

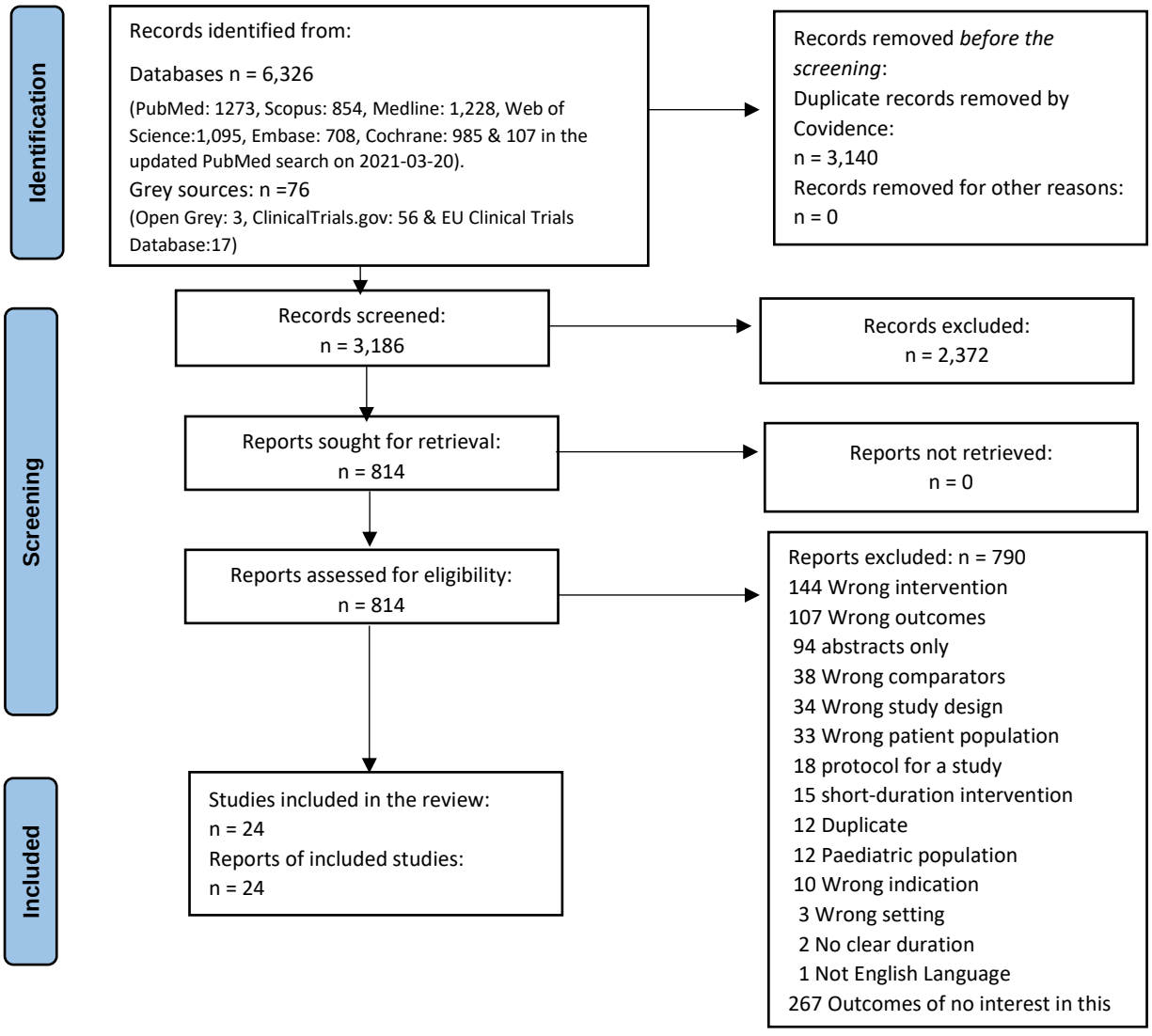
Subgroup analysis is explained in chapter 2, section 2.1.1.9.

9.3 Results

9.3.1 Search results

After deduplication, a total of 3,326 unique records were identified in the literature search, of which 2,372 were excluded after the title and abstract screening against the pre-set inclusion and exclusion criteria. Of the 814 records screened in full text, 24 RCTs met the eligibility criteria and were included in the systematic review and the meta-analysis. Figure 9-1.

Figure 9-1: PRISMA flow diagram



9.3.2 Characteristics of the included RCTs

The 24 RCTs (976 participants) were published until 2020, of which 11 RCTs (608, 612, 614, 615, 620, 659, 672, 678, 686, 691, 710) diagnosed PCOS based on the Rotterdam criteria-2003 (30), while six RCTs (622, 679, 681, 685, 834, 835) diagnosed PCOS using the National Institute of Health (NIH/NICHD) criteria (626). No diagnostic criteria were specified for the remaining seven RCTs. The characteristics of the included RCTs are presented in Table 15.

Table 15: Characteristics of the studies included in the systematic review and meta-analysis

Author	Year of publication	Country of the trial	PCOS diagnostic criteria	Duration of the trial	Measured outcome(s)
Naka et al.(690)	2011a	Greece	N/A	Six months	Body weight, BMI, WC and WHR, FI
Ortega Gonzlez et al.(632)	2005	Mexico	N/A	Six months	Body weight, BMI,WHR
Shahebrahimi et al.(614)	2016	Iran	Rotterdam	Three months	Body weight ,BMI,WC, FBG,LDL,HDL,TG
Sohrevardi et al.(615)	2016	Iran	Rotterdam	Three months	BMI,WHR, HOMA-IR, FBG, FI
Batista et al.(720)	2012	Brazil	AES-2006	12 weeks	FBG.FI,HOMA-IR
Cataldo et al.(834)	2006	USA	NICHD	12 weeks	BMI, WHR
Lam et al.(710)	2011	China	Rotterdam	12 months	BMI, FI,FBG,TC, TG
Cetinkalp et al.(659)	2009	Turkey	Rotterdam	Four months	TG,HDL,LDL, BMI, HOMA-IR, TC,
Kilicdag et al.(691)	2005	Turkey	Rotterdam	Three months	BMI, FI,FBG,TC, TG, HOMA-IR
Li et al.(61)	2020	China	Rotterdam	Six months	WC,BMI, FI,FBG,TC, TG, WHR, LDL,HDL
Cho et al.(686)	2009	UK	Rotterdam	12 months	BMI, HOMA-IR
Ziaee et al.(620)	2012	Iran	Rotterdam	12 weeks	BMI,HOMA-IR,HDL,LDL,TG,TC
Aroda et al.(679)	2009	USA	NIH	Six months	Bodyweight, BMI, WHR, WC, FBG,FI
Brettenthaler et al.(608)	2004	Switzerland	Rotterdam	Three months	BMI,WHR,FBG, FI, HOMA-IR
Glintborg et al.(637)	2005	Denmark	N/A	16 weeks	BMI,WHR, WC, FI
Glintborg et al.(633)	2006	USA	N/A	16 weeks	BMI, CRP, LDL
Glintborg et al.(836)	2008	USA	N/A	16 weeks	FI, HOMA-IR
Dereli et al.(729)	2005	Turkey	NICHD	Eight months	BMI, WHR
Rautio et al.(766)	2006	Finland	N/A	Four months	BMI, WHR
Mohiyiddeen et al.(612)	2013	UK	Rotterdam	Three months	WC,BMI, FI,FBG,TC, TG, WHR, LDL,HDL
Steiner et al.(685)	2007	Germany	NIH	Six months	BMI,HOMA-IR, FBG,FI
Yilmaz et al.(678)	2005	Turkey	Rotterdam	24 weeks	FBG,FI,BMI,WHR
Jensterle et al.(681)	2008a	Slovenia	NIH	Six months	FBG,FI,BMI, HOMA-IR
Jensterle et al.(622)	2008b	Slovenia	NIH	Six months	LDL,HDL,HOMA-IR,WC,BMI, FI,FBG,TC

NIH: national institute for health, **NICHD**: national institute of child health and development. **USA:** the United States of America, **UK**: United Kingdom, **PCOS:** polycystic ovary syndrome, **BMI:** body mass index, **WC:** waist circumference, **FI:** fasting insulin; **FBG**: fasting blood glucose, **LDL:** low-density lipoprotein; **HDL:** high density-lipoprotein; **TG:** triglycerides**, HOMA-IR**: the homeostatic model of insulin-resistance, **TC**: Total Cholesterol, **WHR:** waist to hip ratio, **CRP:** c-reactive protein.

9.3.3 Sensitivity analysis

Small sample-sized RCTs (< 10 patients) and those with high RoB were eliminated from the analysis while monitoring their impact on the final results. No significant effect was found, and hence none of the 24 RCTs was removed from the meta-analysis. There were less than 10 RCTs in each comparison. Therefore, no assessment of publication bias was performed.

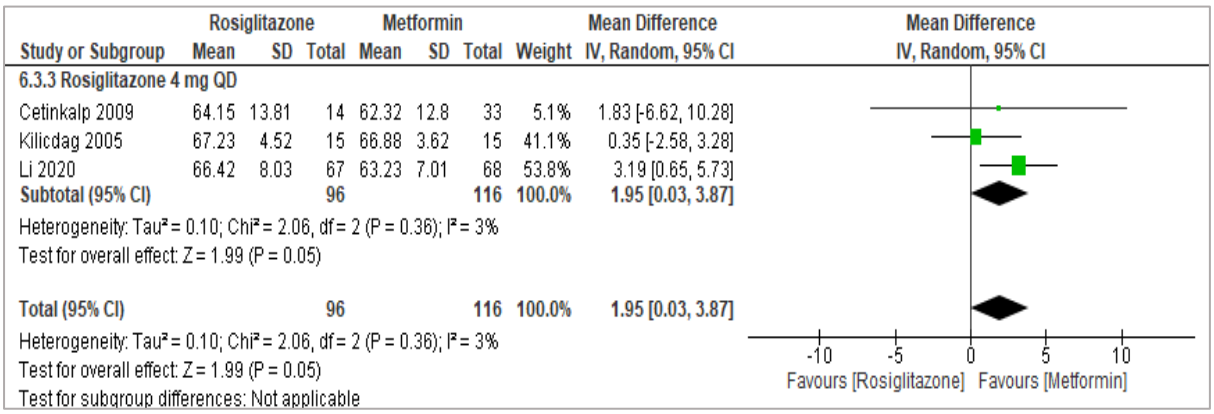
9.3.4 Effect of glitazones on the anthropometric outcomes

9.3.4.1 Body weight

9.3.4.1.1 Rosiglitazone versus Metformin

In three RCTs, rosiglitazone 4 mg QD compared with metformin significantly increased the mean body weight by 1.95 kg (95% CI: 0.03, 3.87; I^2 = 3 %; *p* = 0.05) (Figure 9-2) (very low-grade evidence, table 16).

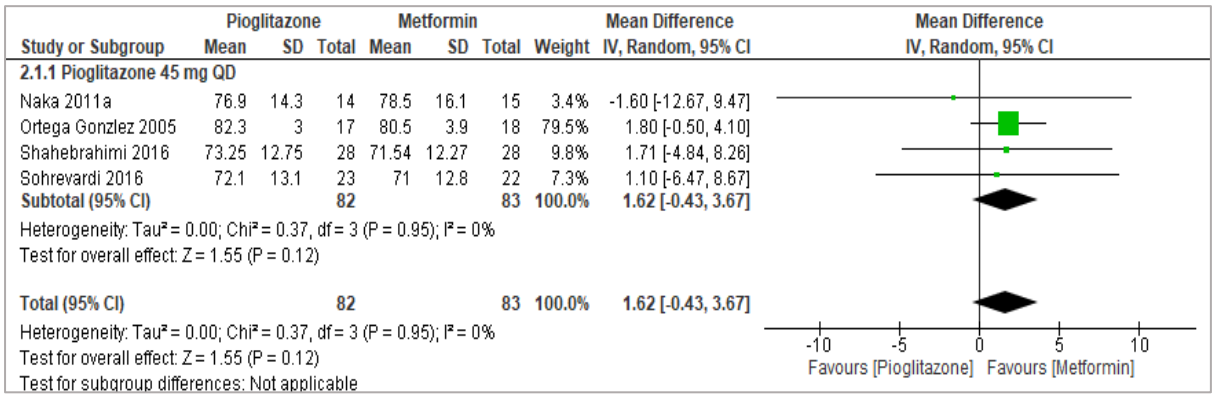
Figure 9-2: Forest plot of Rosiglitazone versus Metformin on body weight (kg)



9.3.4.1.2 Pioglitazone versus Metformin

Four RCTs compared pioglitazone 45 mg QD with metformin showed non-significant increase in the mean body weight (MD: 1.62 kgs; 95%CI: -0.43,3.67, I^2 = 0 %; *p* = 0.12) (Figure 9-3) (low grade evidence, table 16).

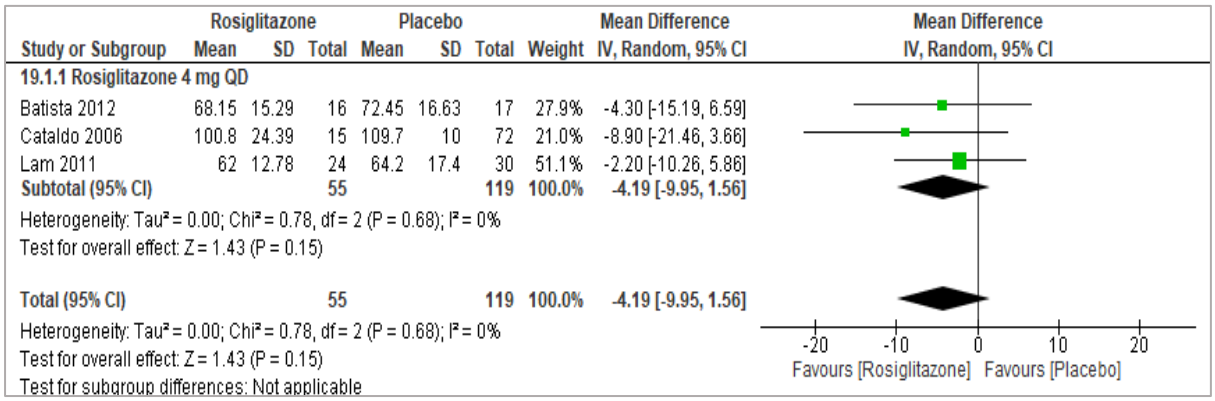
Figure 9-3: Forest plot of Pioglitazone versus Metformin on body weight (kg)



9.3.4.1.3 Rosiglitazone versus placebo

In three RCTs compared rosiglitazone 4 mg QD with placebo showed no significant effect in the mean body weight (MD: -4.19 kgs; 95%CI: -9.95,1.56, 0%; *p* = 0.15) (Figure 9-4) (very low grade evidence, table 16).

Figure 9-4: Forest plot of Rosiglitazone versus placebo on body weight (kg)



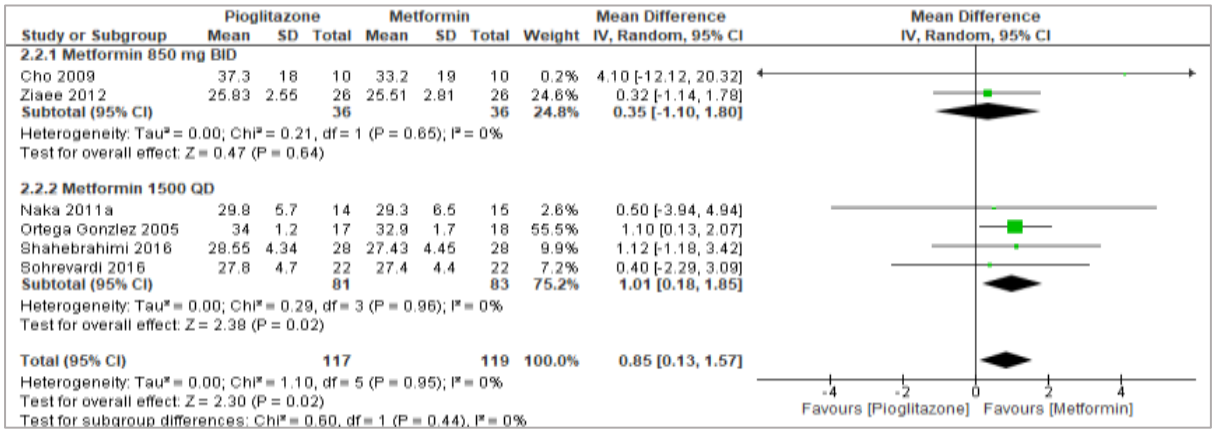
9.3.4.2 Body mass index (BMI)

9.3.4.2.1 Pioglitazone versus Metformin

Two RCTs comparing pioglitazone 45 mg QD with metformin 850 mg BID showed no effect on the mean BMI (MD: 0.35 kg/m^2; 95% CI: -1.10, 1.80). In four RCTs, pioglitazone 45 mg QD compared with metformin 1500 mg QD significantly increased the BMI by 1.01 kg/m^2 (95% CI: 0.18,1.85). Overall, pioglitazone compared with metformin significantly increased the

mean BMI by 0.85 kg/m^2 (95%CI: 0.13,1.57; I^2= 0%; p = 0.02) (Figure 9-5) (very low-grade evidence, table 16).

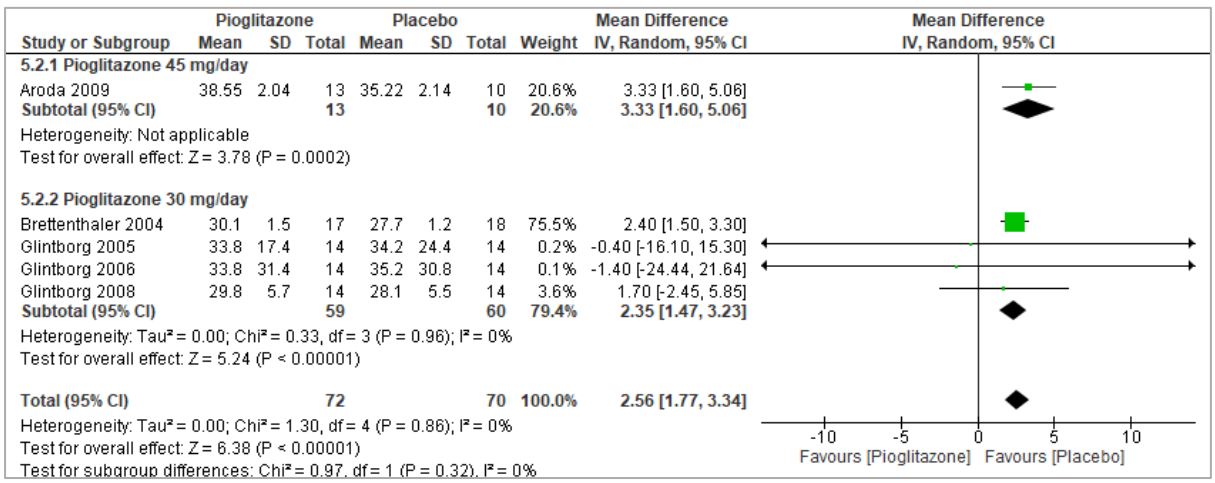
Figure 9-5: Forest plot of Pioglitazone versus Metformin on the BMI (kg/m^2)



9.3.4.2.2 Pioglitazone versus placebo

In one RCT, pioglitazone 45 mg QD significantly increased the mean BMI by 3.33 kg/m^2 (95% CI: 1.60,5.06). In four RCTs pioglitazone 30 mg QD compared with placebo significantly increased the mean BMI by 2.35 kg/m^2 (95% CI: 1.47,3.23). Overall, pioglitazone at various dosage compared with placebo significantly increased the mean BMI by 2.56 kg/m^2 (95% CI: 1.77,3.34; I^2= 0 %, p < 0.00001) (Figure 9-6) (low-grade evidence, table 16).

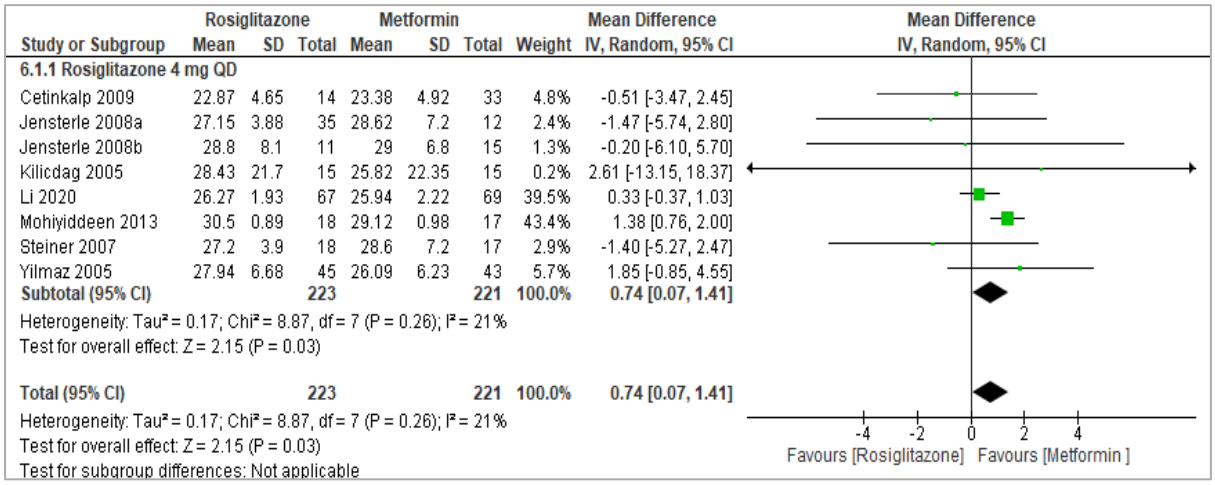
Figure 9-6: Forest plot of Pioglitazone versus placebo on the BMI (kg/m^2)



9.3.4.2.3 Rosiglitazone versus Metformin

Eight RCTs comparing rosiglitazone 4 mg QD with metformin showed a significant increase in the mean BMI by 0.74 kg/m^2 (95% CI: 0.07,1.41; I^2= 21 %, *p = 0.03)* (Figure 9-7) (moderate grade evidence, table 16).

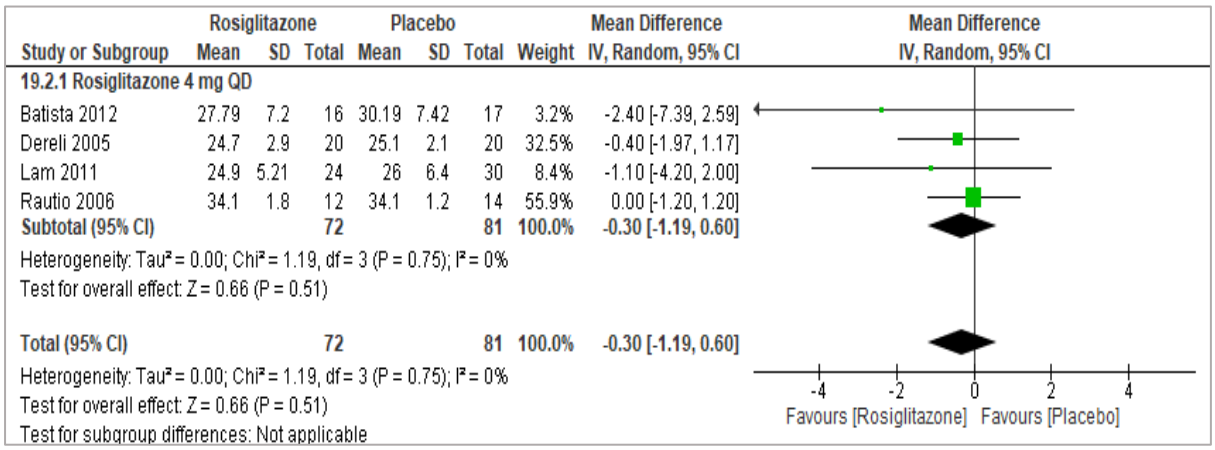
Figure 9-7: Forest plot of Rosiglitazone versus Metformin on the BMI(kg/m^2)



9.3.4.2.4 Rosiglitazone versus placebo

Four RCTs compared rosiglitazone 4 mg QD with placebo showed no effect on the mean BMI (MD: -0.30 kg/m^2; 95%CI: -1.19, 0.60, I^2= 0 %, *p = 0.51)* (Figure 9-8) (very low-grade evidence, table 16).

Figure 9-8: Forest plot of Rosiglitazone versus placebo on the BMI (kg/m^2)

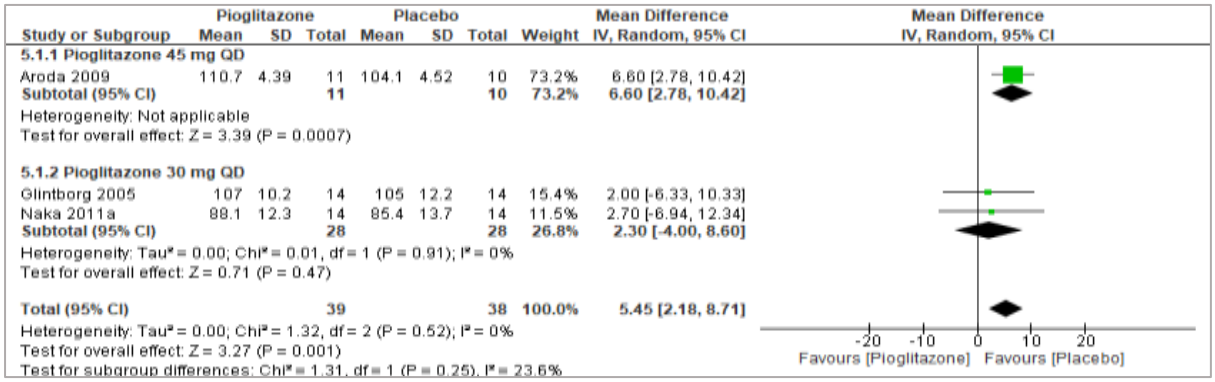


9.3.4.3 Waist circumference (WC)

9.3.4.3.1 Pioglitazone versus placebo

One RCT compared pioglitazone 45 mg QD with placebo showed a significant increase in the mean WC by 6.60 cm (95% CI: 2.78,10.42). Two RCTs compared pioglitazone 30 mg QD with placebo showed a significant increase in the mean WC by 2.30 cm (95% CI: -4.0, 8.60). Overall, pioglitazone at various doses compared with placebo significantly increased the mean WC by 5.45 cm (95%CI: 2.18, 8.71; I^2= 0 %, *p = 0.001*)(Figure 9-9) (very-low grade evidence, table 16).

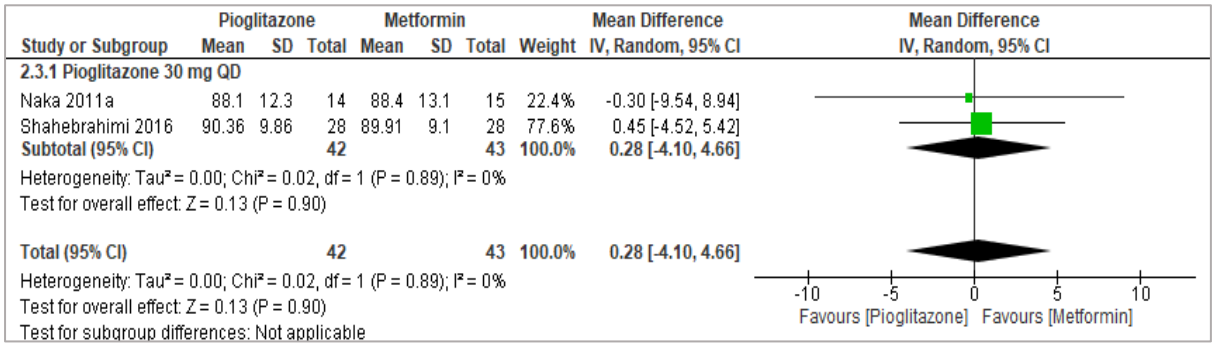
Figure 9-9: Forest plot of Pioglitazone versus placebo on the WC (cm)



9.3.4.3.2 Pioglitazone versus Metformin

Two RCTs compared pioglitazone 30 mg QD with metformin showed no effect on the mean WC (MD: 0.28 cm; 95%CI: -4.10, 4.66, I^2= 0 %, *p = 0.90*) (Figure 9-10) (very-low grade evidence, table 16).

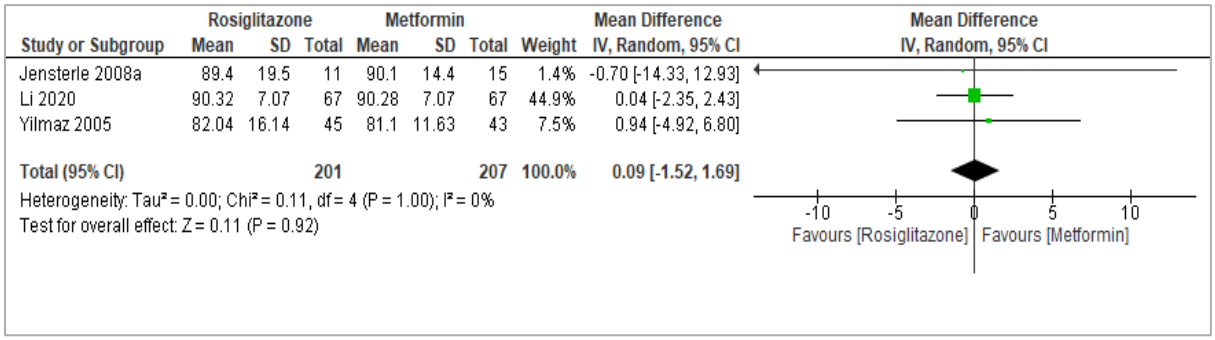
Figure 9-10: Forest plot of Pioglitazone versus Metformin on the WC (cm)



9.3.4.3.3 Rosiglitazone versus Metformin

Three RCTs compared rosiglitazone 4 mg QD with metformin showed no effect on the mean WC (MD: 0.09 cm; 95%CI: -1.52,1.69; I^2= 0 %, *p = 0.92*) (Figure 9-11) (low grade evidence, table 16).

Figure 9-11: Forest plot of Rosiglitazone versus Metformin on the WC (cm)

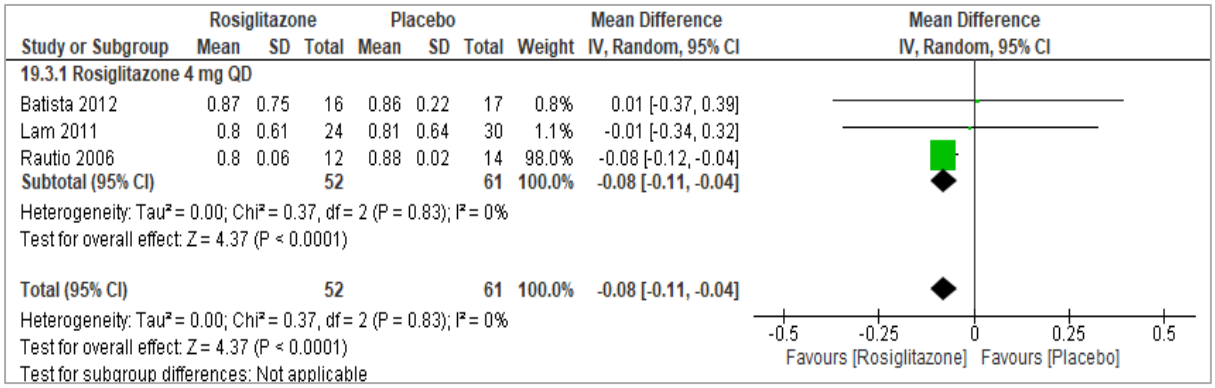


9.3.4.4 Waist to hip ratio (WHR)

9.3.4.4.1 Rosiglitazone versus placebo

Three RCTs comparing rosiglitazone 4 mg QD with placebo showed a significant reduction on the mean WHR by 0.08 (95 % CI: -0.11,-0.04; I^2= 0 %, *p < 0.0001)* (Figure 9-12) (very-low grade evidence, table 16).

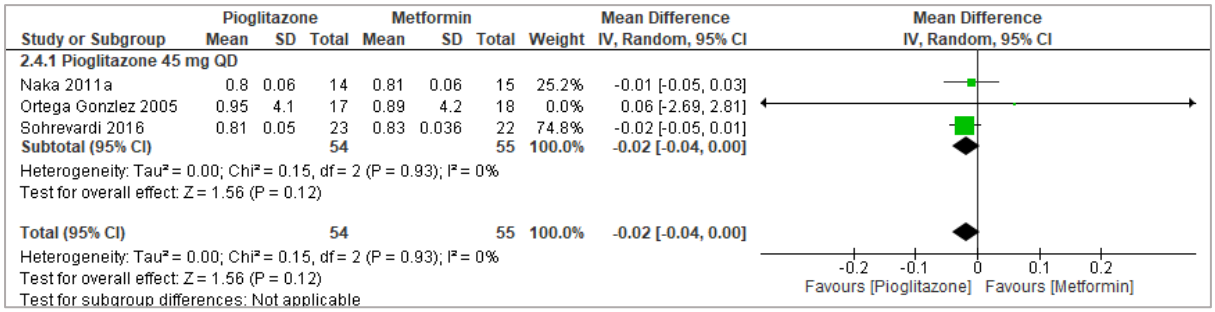
Figure 9-12: Forest plot of Rosiglitazone versus placebo on the WHR



9.3.4.4.2 Pioglitazone versus Metformin

Three RCTs compared pioglitazone 45 mg QD with metformin showed nonsignificant reduction in the mean WHR (MD: -0.02; 95%CI: -0.04, 0.00; I^2= 0 %, *p = 0.12)* (Figure 9-13) (very-low grade evidence, table 16).

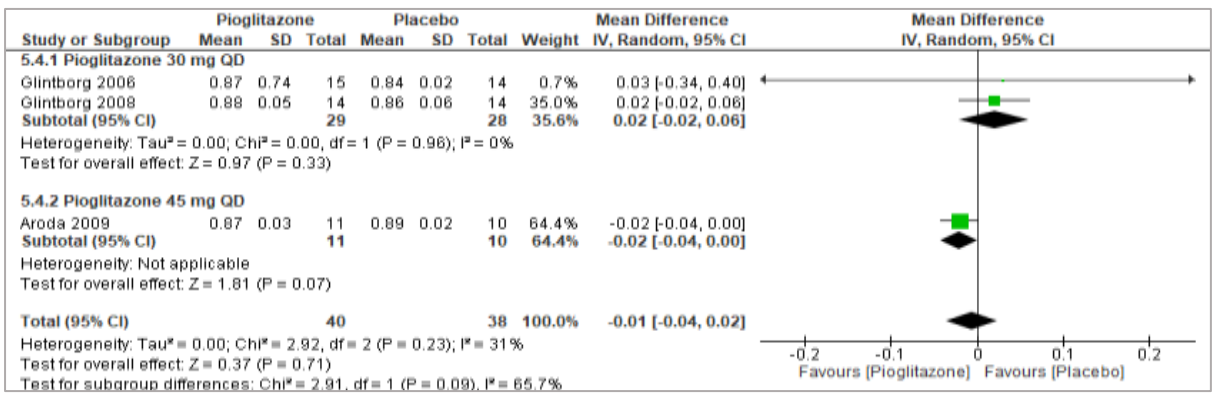
Figure 9-13: Forest plot of Pioglitazone versus Metformin on the WHR



9.3.4.4.3 Pioglitazone versus placebo

Two RCTs compared pioglitazone 30 mg QD with placebo showed no effect on the mean WHR (MD: 0.02; 95%CI: -0.02, 0.06). One RCT compared pioglitazone 45 mg QD with placebo showed a significant reduction in the mean WHR (MD: -0.02; 95%CI: -0.04, 0.00). Overall, regardless to the administered dosage pioglitazone has no effect on the mean WHR compared with placebo (MD: -0.01; -0.04, 0.02; I^2= 65.7%, *p = 0.71)* (Figure 9-14) (low grade evidence, table 16).

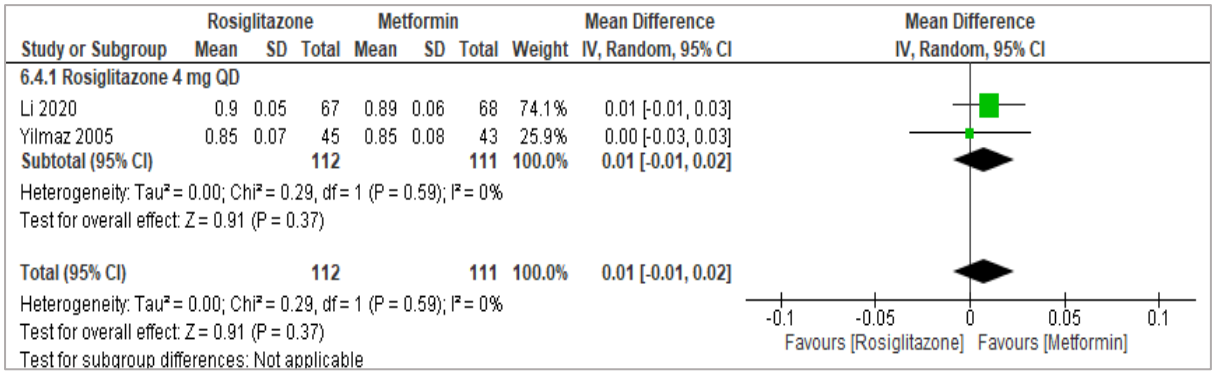
Figure 9-14: Forest plot of Pioglitazone versus placebo on the WHR



9.3.4.4.4 Rosiglitazone versus Metformin

Two RCTs compared rosiglitazone 4 mg QD with metformin showed nonsignificant effect on the mean WHR (MD: 0.01; 95%CI: -0.01, 0.02; I^2= 0%, *p = 0.37)* (Figure 9-15) (low-grade evidence, table 16).

Figure 9-15: Forest plot of Rosiglitazone versus Metformin on the WHR



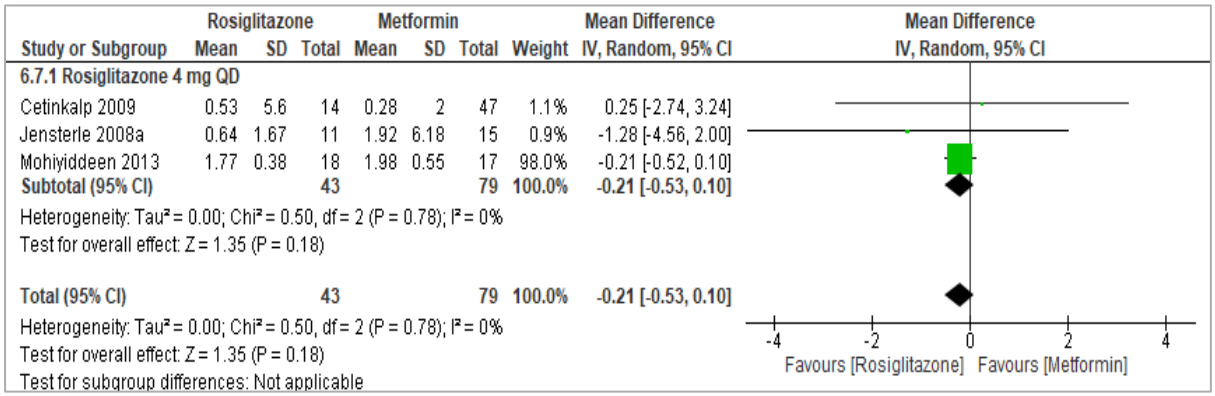
9.3.5 Effect of glitazones on CRP and lipid profiles

9.3.5.1 C-reactive protein (CRP)

9.3.5.1.1 Rosiglitazone versus Metformin

Three RCTs comparing rosiglitazone 4 mg QD with metformin showed no effect on the mean CRP (MD: -0.21 mg/L; 95% CI: -0.53, 0.10; I^2= 0 %, *p* = 0.18) (Figure 9-16) (low-grade evidence, table 16).

Figure 9-16: Forest plot of Rosiglitazone versus Metformin on CRP (mg/dL)

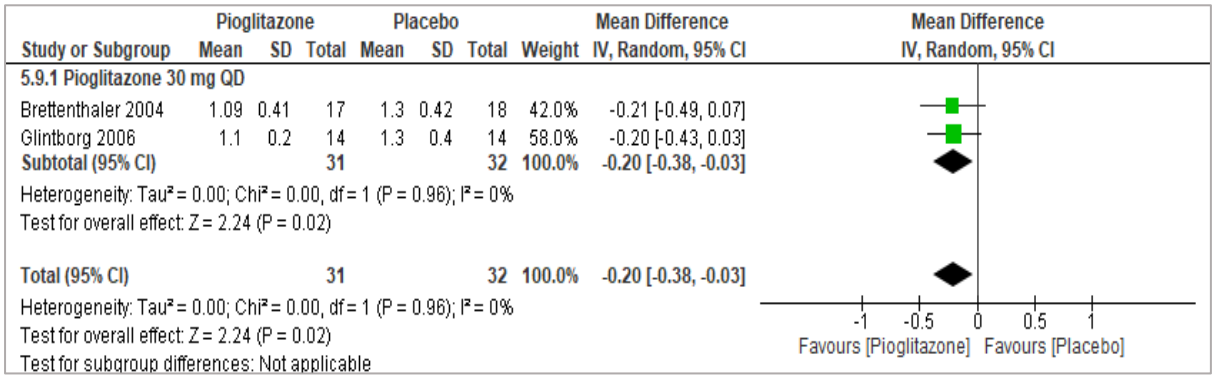


9.3.5.2 Triglycerides

9.3.5.2.1 Pioglitazone versus placebo

Two RCTs comparing pioglitazone 30 mg QD with placebo showed a significant reduction in the mean triglycerides by 0.20 mmol/L (95% CI: -0.38,-0.03; I^2= 0 %, *p* = 0.02) (Figure 9-17) (low-grade evidence, table 16).

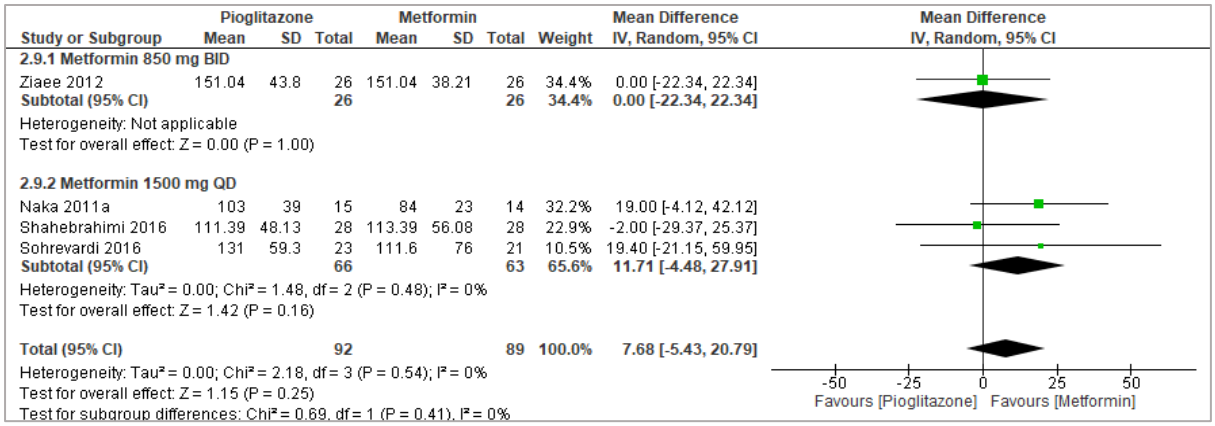
Figure 9-17: Forest plot of Pioglitazone versus placebo on TGs (mmol/L)



9.3.5.2.2 Pioglitazone versus Metformin

When pioglitazone was compared with various dosage of metformin no effect on the mean TGs was observed (MD: 7.68 mmol/L; 95%CI: -5.43, 20.79; I^2= 0 %, *p* = 0.25) (Figure 9-18) (very low-grade evidence, table 16).

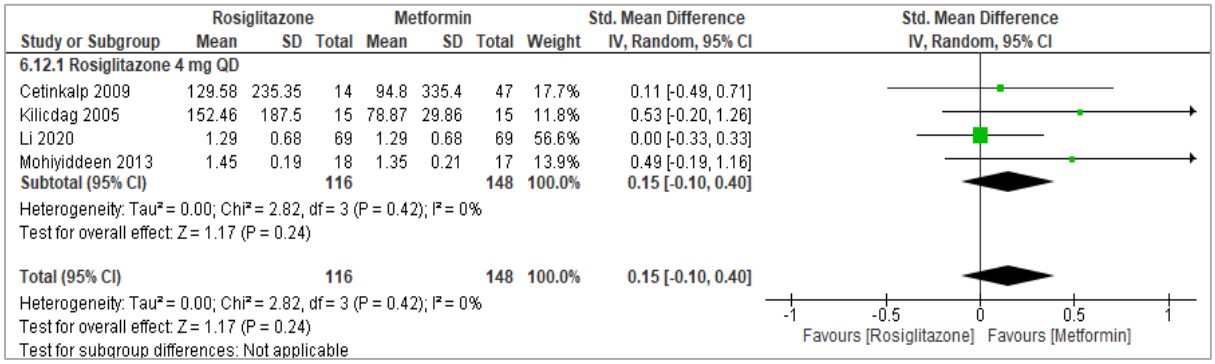
Figure 9-18: Forest plot of Pioglitazone versus Metformin on TGs (mmol/L)



9.3.5.2.3 Rosiglitazone versus Metformin

Four RCTs compared rosiglitazone 4 mg QD with metformin showed no effect on the mean TGs (SMD: 0.15; 95%CI: -0.10, 0.40; I^2= 0 %, *p* = 0.24) (Figure 9-19) (low-grade evidence, table 16).

Figure 9-19: Forest plot of Rosiglitazone versus Metformin on TGs

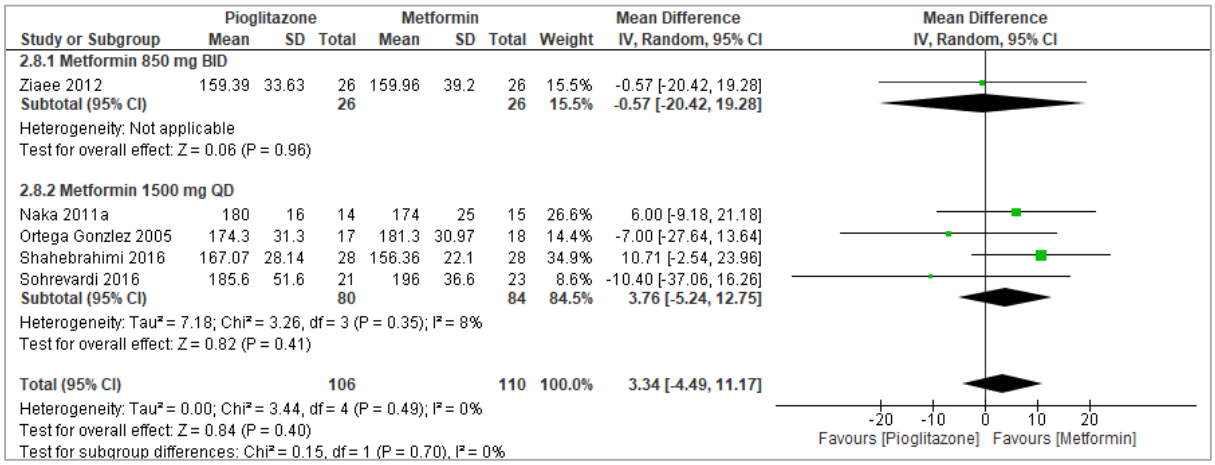


9.3.5.3 Total cholesterol (TC)

9.3.5.3.1 Pioglitazone versus Metformin

When metformin of various dosage was compared with pioglitazone no effect on the mean TC was observed (MD: 3.34 mmol/L; 95%CI: -4.49, 11.17; I^2= 0 %, *p* = 0.40) (Figure 9-20) (very low-grade evidence, table 16).

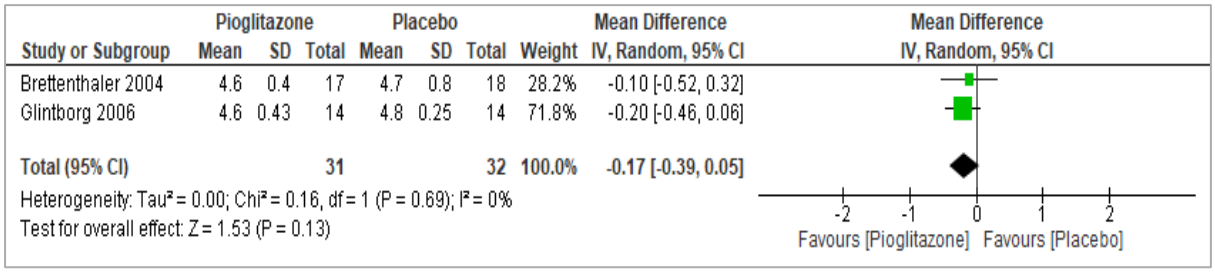
Figure 9-20: Forest plot of Pioglitazone versus Metformin on TC (mmol/L)



9.3.5.3.2 Pioglitazone versus placebo

Two RCTs compared pioglitazone 30 mg QD with placebo showed no effect on the mean TC (MD: -0.17 mmol/L; 95%CI: -0.39, 0.05; I^2= 0 %, *p* = 0.13) (Figure 9-21) (very low-grade evidence, table 16).

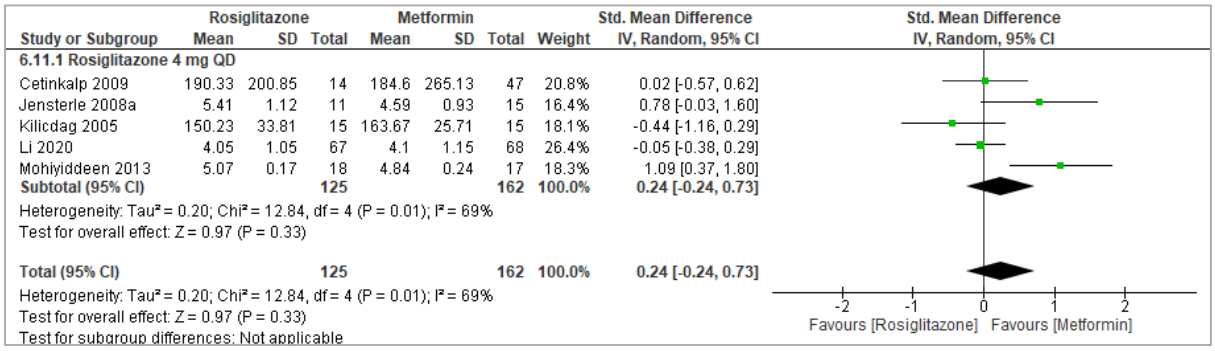
Figure 9-21: Forest plot of Pioglitazone versus placebo on TC (mmol/L)



9.3.5.3.3 Rosiglitazone versus Metformin

Five RCTs compared rosiglitazone 4 mg QD with metformin showed no significant effect on the mean TC (MD: 0.24 mmol/L; 95%CI: -0.24, 0.73; I^2= 69 %, *p* = 0.33) (Figure 9-22) (low-grade evidence, table 16).

Figure 9-22: Forest plot of Rosiglitazone versus Metformin on TC (mmol/L)

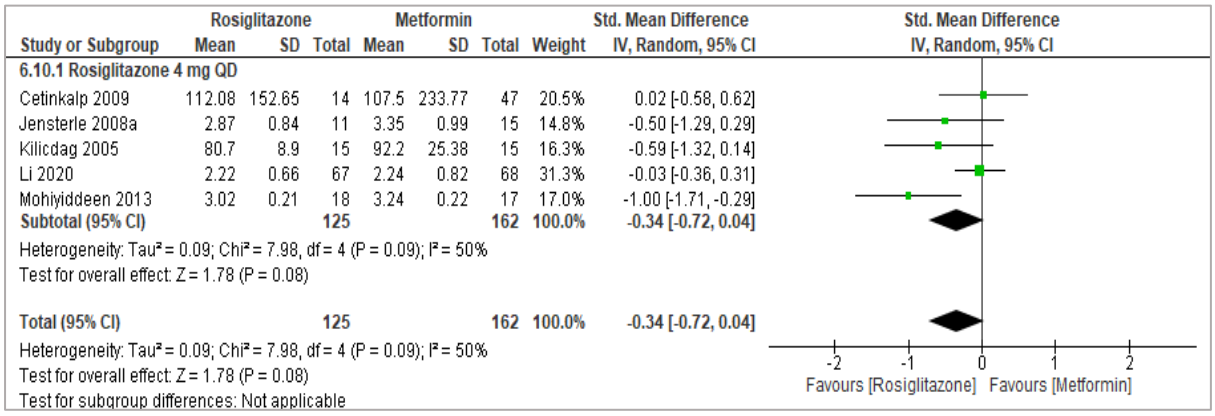


9.3.5.4 Low density lipoprotein cholesterol (LDL-C)

9.3.5.4.1 Rosiglitazone versus Metformin

Four RCTs comparing rosiglitazone 4 mg QD with metformin showed a significant reduction in the mean LDL-C (SMD: -0.34; 95% CI: -0.72, 0.04; I^2= 50 %, *p* = 0.08) (Figure 9-23) (low-grade evidence, table 16).

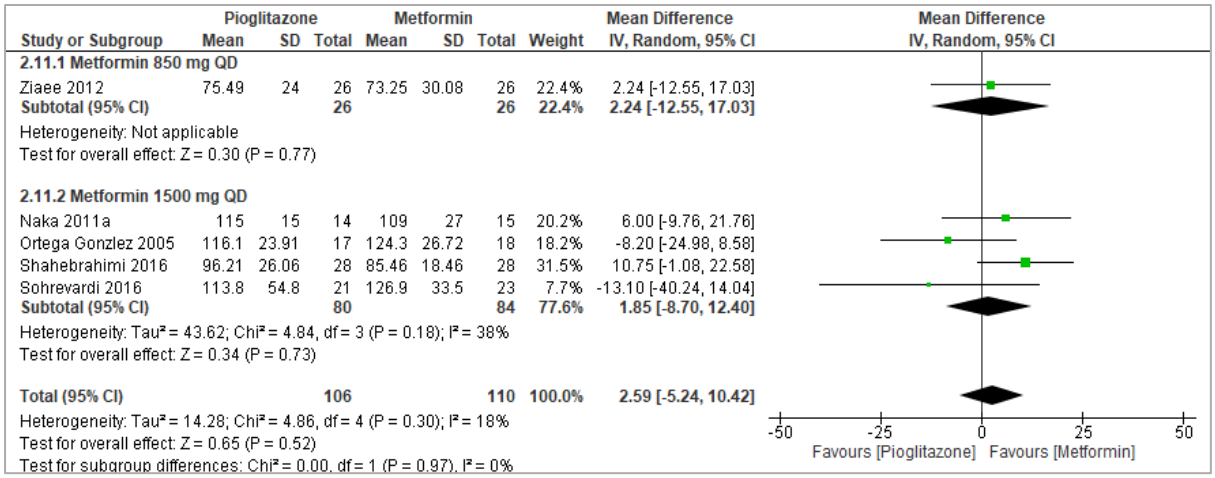
Figure 9-23: Forest plot of Rosiglitazone versus Metformin on LDL-C



9.3.5.4.2 Pioglitazone versus Metformin

Five RCTs compared metformin of various dosage and frequencies with pioglitazone showed no effect on the mean LDL-C (MD: 2.59 mmol/L; 95%CI: -5.24, 10.42; I^2= 18 %, *p* = 0.52) (Figure 9-24) (very low-grade evidence, table 16).

Figure 9-24: Forest plot of Pioglitazone versus Metformin on LDL-C (mmol/L)

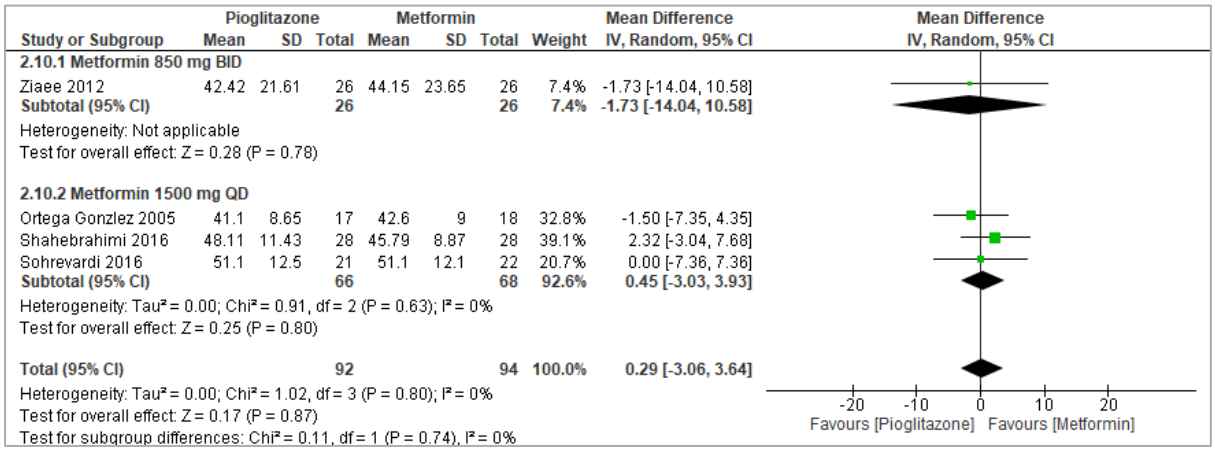


9.3.5.5 High density lipoprotein cholesterol (HDL-C)

9.3.5.5.1 Pioglitazone versus Metformin

Four RCTs compared metformin of various dosage and frequencies with pioglitazone showed no effect on the mean HDL-C (MD: 0.29 mmol/L; 95%CI: -3.06, 3.64; I^2= 0 %, *p* = 0.87) (Figure 9-25) (very low-grade evidence, table 16).

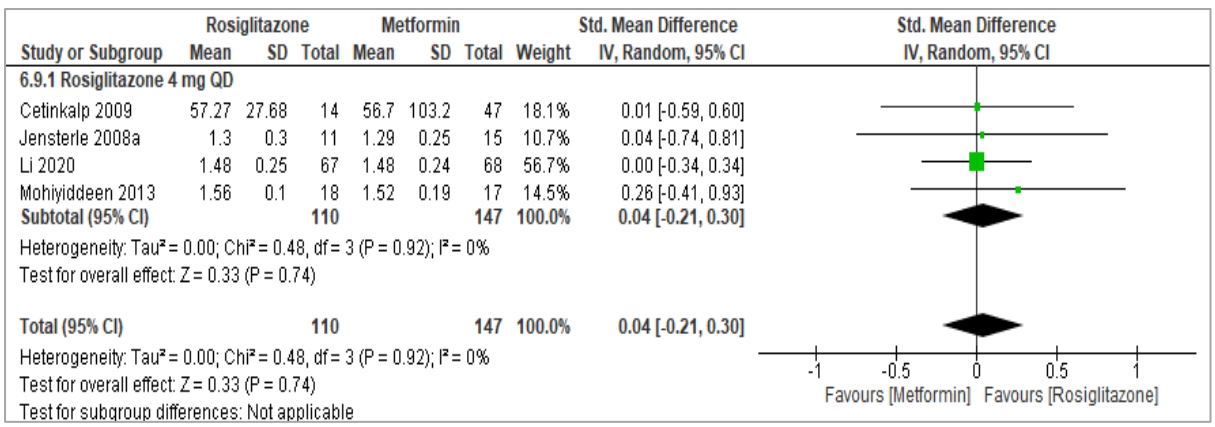
Figure 9-25: Forest plot of Pioglitazone versus Metformin on HDL-C (mmol/L)



9.3.5.5.2 Rosiglitazone versus Metformin

Four RCTs compared rosiglitazone 4 mg QD with metformin showed no effect on the mean HDL-C (SMD: 0.04; 95%CI: -0.21, 0.30; I^2= 0 %, *p* = 0.74) (Figure 9-26) (low-grade evidence, table 16).

Figure 9-26: Forest plot of Rosiglitazone versus Metformin on HDL-C



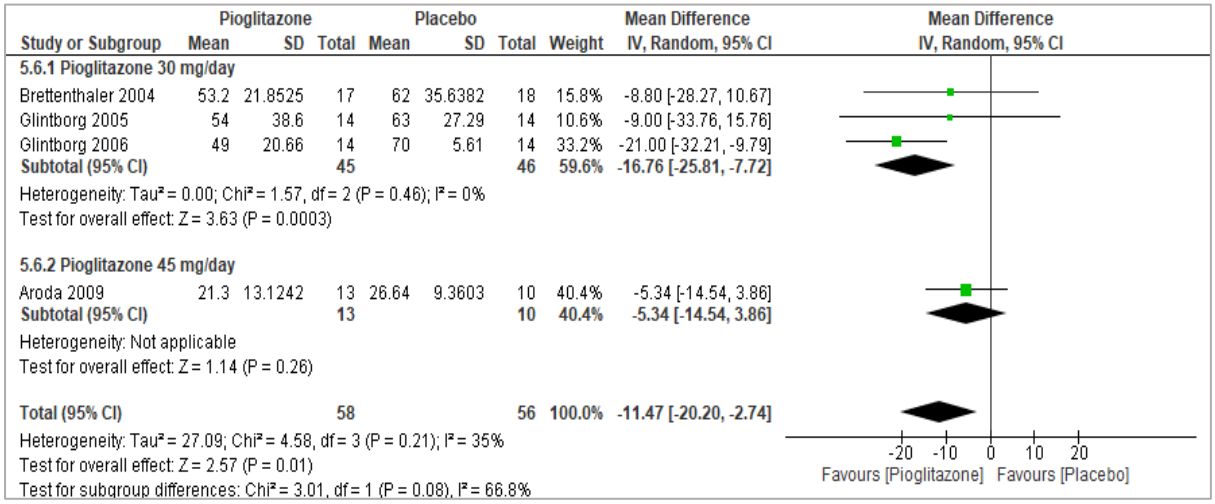
9.3.6 Effect of glitazones on the insulin resistance

9.3.6.1 Fasting insulin (FI)

9.3.6.1.1 Pioglitazone versus placebo

In three RCTs, pioglitazone 30 mg QD showed a significant reduction in the mean fasting insulin by 16.76 pmol/L (95 % CI: -25.81, -7.72) compared with placebo. One RCT compared pioglitazone 45 mg QD with placebo and showed no significant effect on the mean fasting insulin (MD: -5.34 pmol/L; 95% CI: -14.54, 3.86). Overall, pioglitazone compared with placebo significantly reduced the mean fasting insulin by 11.47 pmol/L (95% CI: -20.20, -2.74; I^2= 35 %, *p*= 0.01) (Figure 9-27) (very-low grade evidence, table 16).

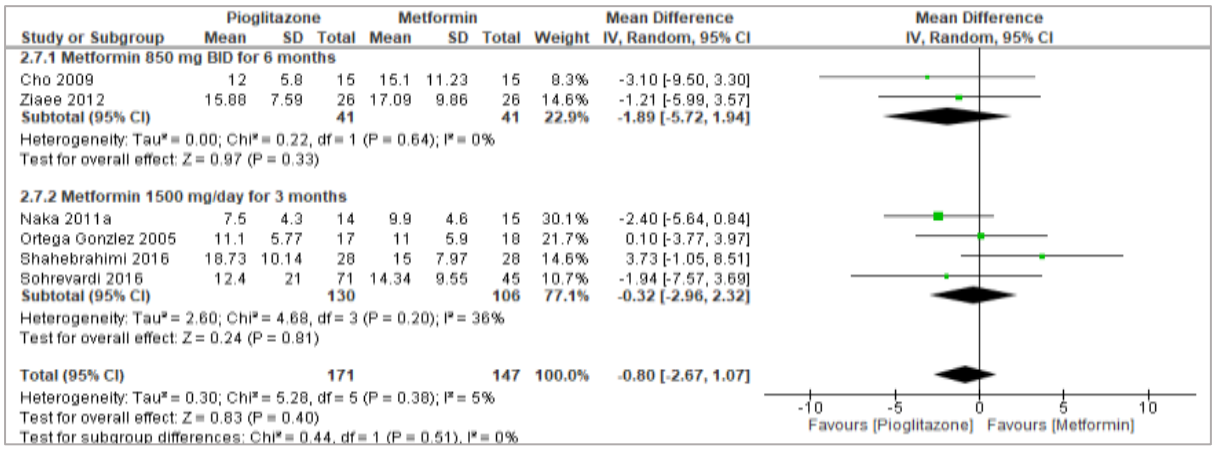
Figure 9-27: Forest plot of Pioglitazone versus placebo on FI (pmol/L)



9.3.6.1.2 Pioglitazone versus Metformin

Six RCTs compared various dosage and frequencies of metformin with pioglitazone showed no effect on the mean FI (MD: -0.80 pmol/L; 95%CI: -2.67, 1.07; I^2= 5 %, *p* = 0.40) (Figure 9-28) (very-low grade evidence, table 16).

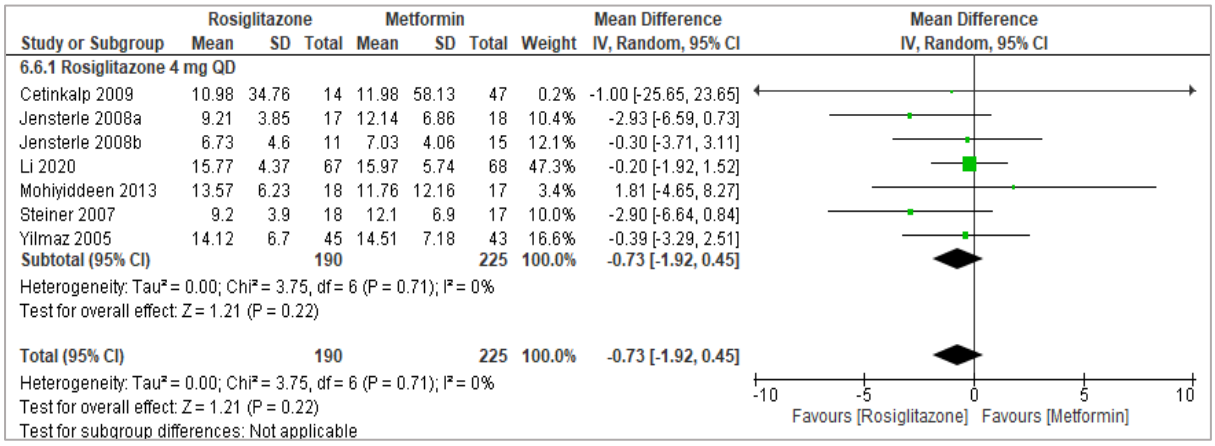
Figure 9-28: Forest plot of Pioglitazone versus Metformin on FI (pmol/L)



9.3.6.1.3 Rosiglitazone versus Metformin

In seven RCTs compared rosiglitazone 4 mg QD with metformin showed no effect on the mean FI (MD: -0.73 pmol/L; 95%CI: -1.92, 0.45; I^2= 0%, *p* = 0.22) (Figure 9-29) (low grade evidence, table 16).

Figure 9-29: Forest plot of Rosiglitazone versus Metformin on FI (pmol/L)

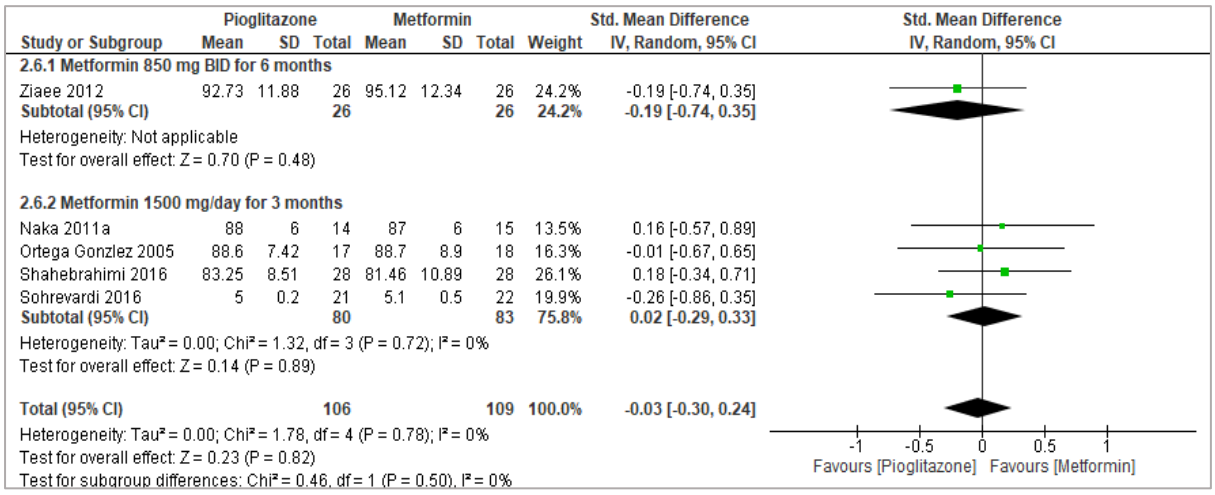


9.3.6.2 Fasting blood glucose (FBG)

9.3.6.2.1 Pioglitazone versus Metformin

Five RCTs compared metformin of various dosage and frequencies with pioglitazone showed no effect on the mean FBG (SMD: -0.03; 95%CI: -0.30, 0.24; I^2= 0%, *p* = 0.82) (Figure 9-30) (very low- grade evidence, table 16).

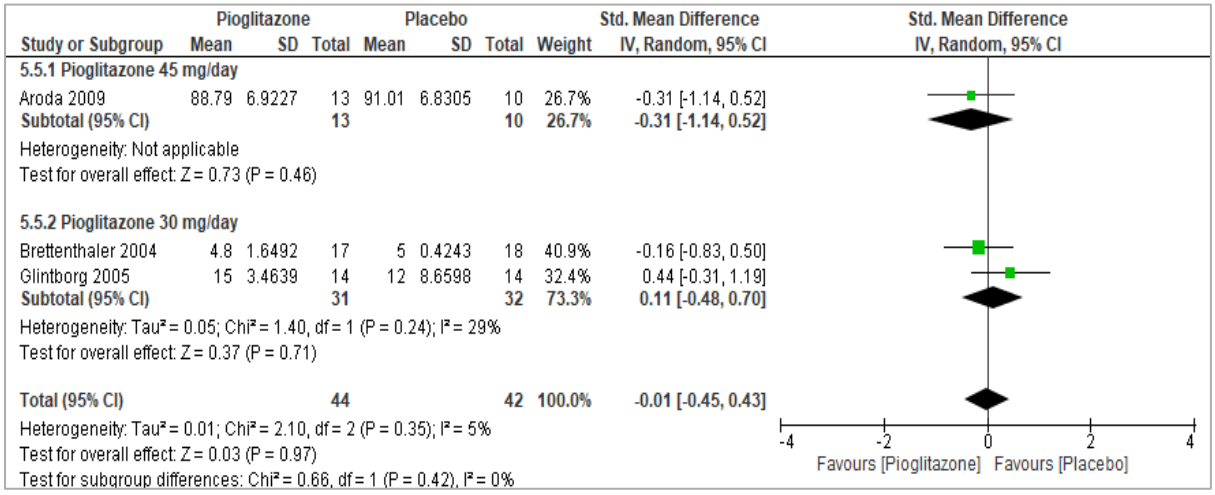
Figure 9-30: Forest plot of Pioglitazone versus Metformin on FBG



9.3.6.2.2 Pioglitazone versus placebo

Three RCTs compared various dosage of pioglitazone with placebo showed no effect on the mean FBG (SMD: -0.01; 95%CI: -0.45, 0.43; I^2= 5%, *p* = 0.97) (Figure 9-31) (low grade evidence, table 16).

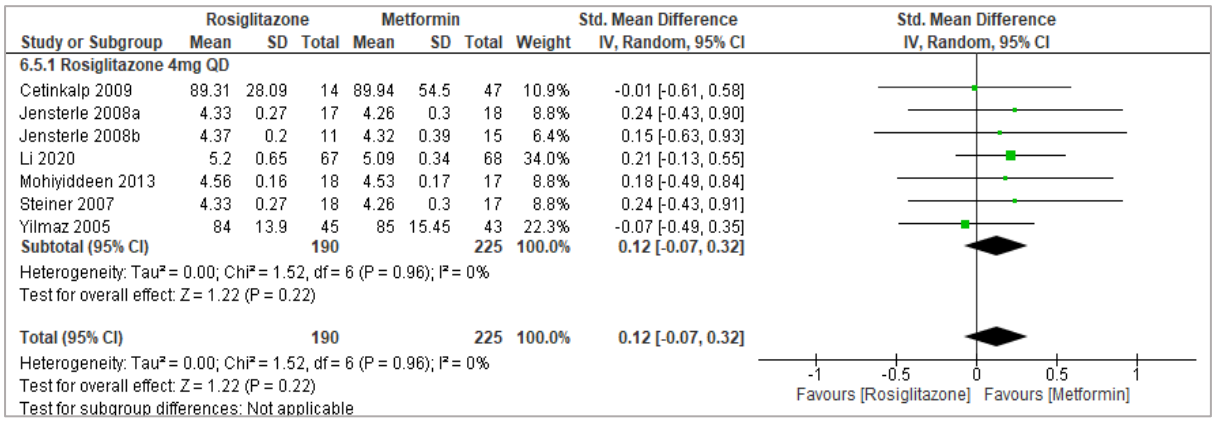
Figure 9-31: Forest plot of Pioglitazone versus placebo on FBG



9.3.6.2.3 Rosiglitazone versus Metformin

Seven RCTs compared rosiglitazone 4 mg QD with metformin showed no effect on the FBG (SMD: 0.12; 95%CI: -0.07, 0.32; I^2= 5%, *p* = 0.22) (Figure 9-32) (low grade evidence, table 16).

Figure 9-32: Forest plot of Rosiglitazone versus Metformin on FBG

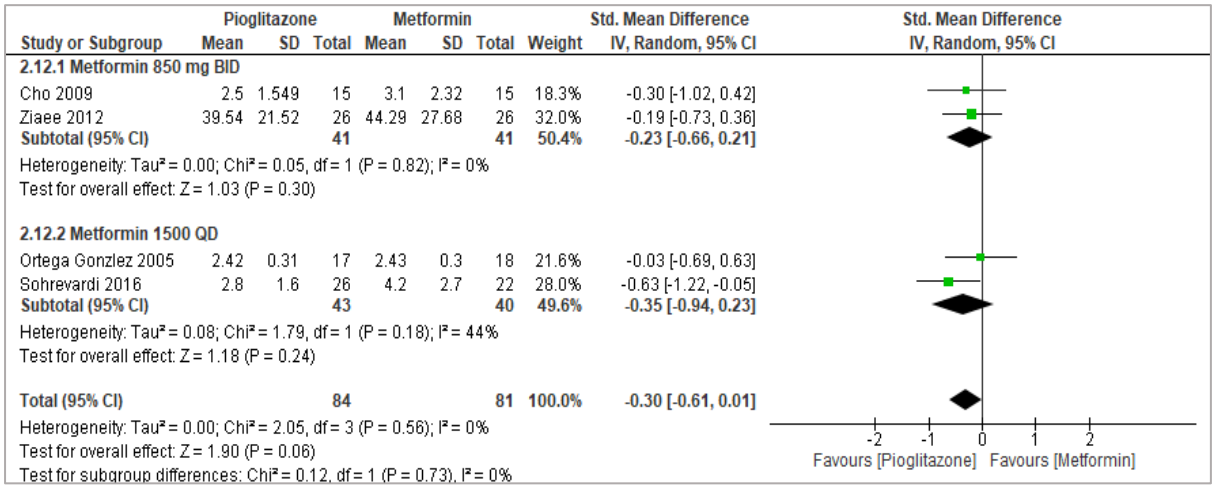


9.3.6.3 HOMA-IR

9.3.6.3.1 Pioglitazone versus Metformin

Two RCTs comparing pioglitazone 45 mg QD with metformin 850 mg BID showed no effect in the mean HOMA-IR (SMD: -0.23; 95% CI: -0.66, 0.21). In two RCTs compared pioglitazone 45 mg QD with metformin 1500 mg QD showed no effect on the mean HOMA-IR (SMD: -0.35; 95% CI: -0.94, 0.23). Overall, pioglitazone showed a significant reduction in HOMA-IR compared with metformin (SMD: -0.30; 95% CI: -0.61, 0.01; I^2= 35 %, p = 0.06) (Figure 9-33) (very-low grade evidence, table 16).

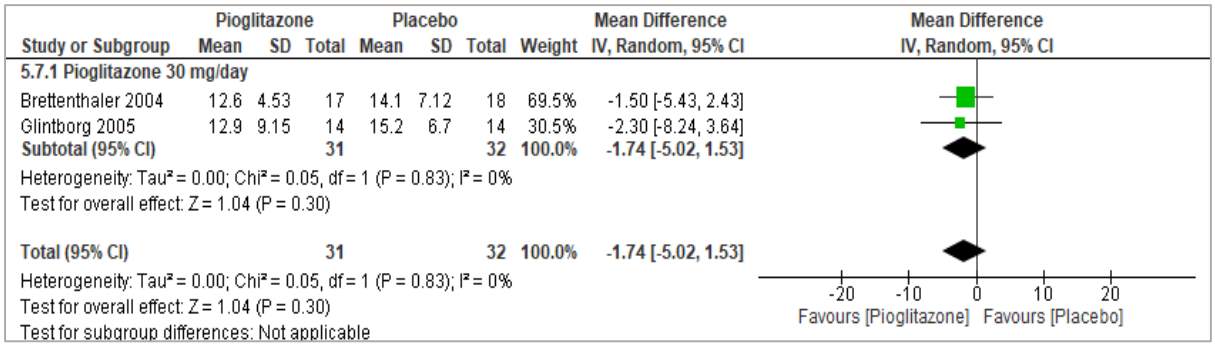
Figure 9-33: Forest plot of Pioglitazone versus Metformin on HOMA-IR



9.3.6.3.2 Pioglitazone versus placebo

Two RCTs compared pioglitazone 30 mg QD compared with placebo showed no effect on the mean HOMA-IR (MD:-1.74; 95%CI:-5.02, 1.53; I^2= 0 %, *p* = 0.30) (Figure 9-34) (very-low grade evidence, table 16).

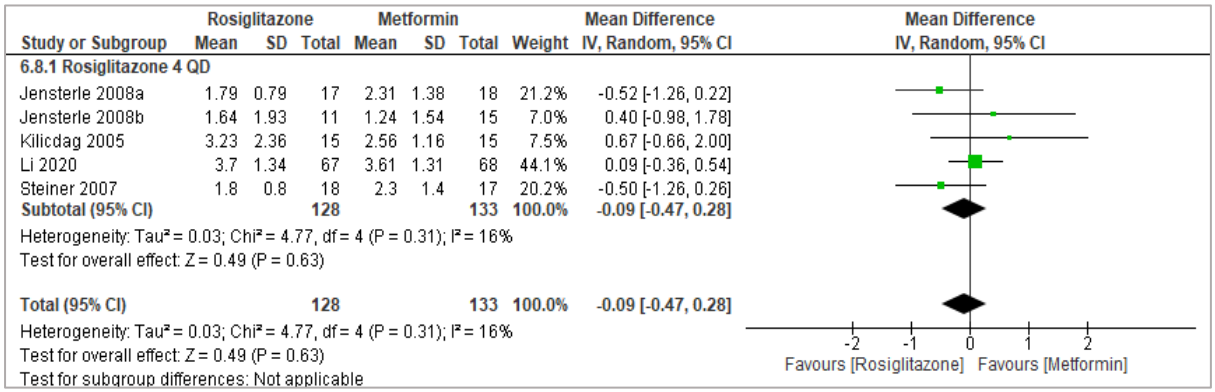
Figure 9-34: Forest plot of Pioglitazone versus placebo on HOMA-IR



9.3.6.3.3 Rosiglitazone versus Metformin

Five RCTs compared rosiglitazone 40 mg QD with metformin showed no effect on the mean HOMA-IR (MD: -0.09; 95%CI: -0.47, 0.28; I^2= 16 %, *p* = 0.63) (Figure 9-35) (moderate grade evidence, table 16).

Figure 9-35: Forest plot of Rosiglitazone versus Metformin on HOMA-IR



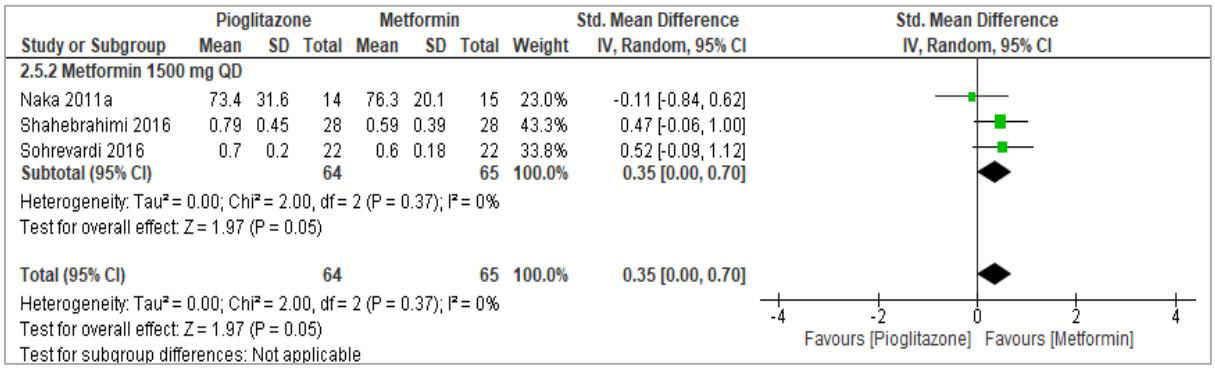
9.3.7 Effect of glitazones on androgen hormones

9.3.7.1 Total testosterone

9.3.7.1.1 Pioglitazone versus Metformin

Three RCTs comparing pioglitazone 45 mg QD with metformin 1500 mg QD. Metformin showed significant increase in the mean total testosterone level (SMD: 0.35; 95% CI: 0.00, 0.70; I^2= 0 %, *p* = 0.05)(Figure 9-36) (very low-grade evidence, table 16).

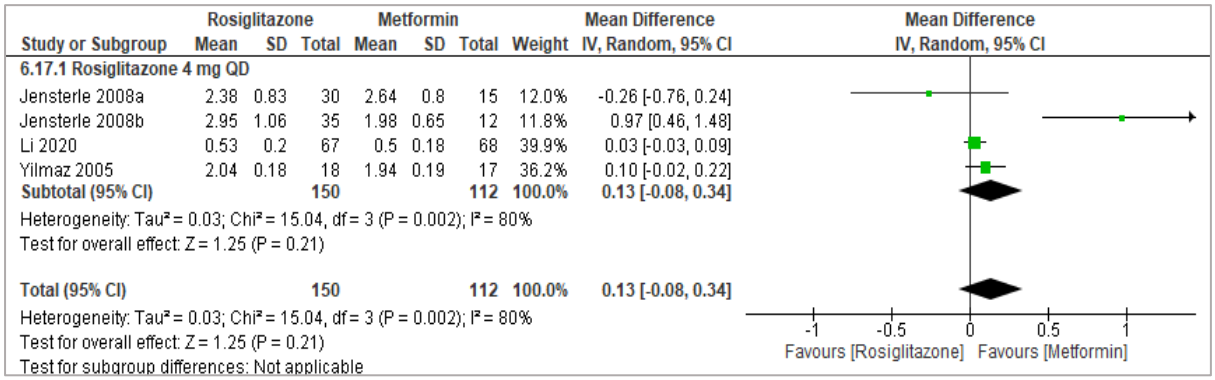
Figure 9-36: Forest plot of Pioglitazone versus Metformin on total testosterone



9.3.7.1.2 Rosiglitazone versus Metformin

Four RCTs compared rosiglitazone 4 mg QD compared with metformin showed no effect in the mean total testosterone (MD: 0.13 pmol/L; 95%CI: -0.08, 0.34; I^2= 80 %, *p* = 0.21)(Figure 9-37) (very low-grade evidence, table 16).

Figure 9-37: Forest plot of Rosiglitazone versus Metformin on total testosterone (pmol/L)

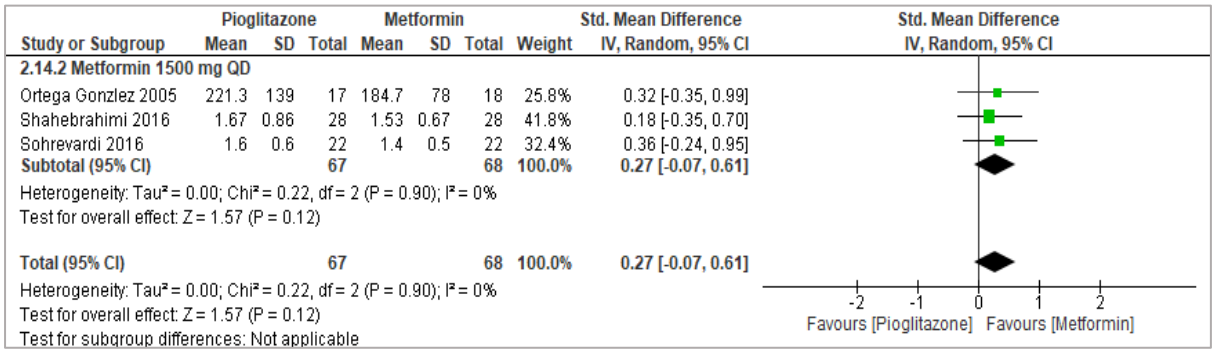


9.3.7.2 DHEAS

9.3.7.2.1 Pioglitazone versus Metformin

Three RCTs compared metformin 1500 mg QD with pioglitazone showed no effect on the mean DHEAS (SMD: 0.27; 95%CI: -0.07, 0.61; I^2= 0 %, *p* = 0.12)(Figure 9-38) (very low-grade evidence, table 16).

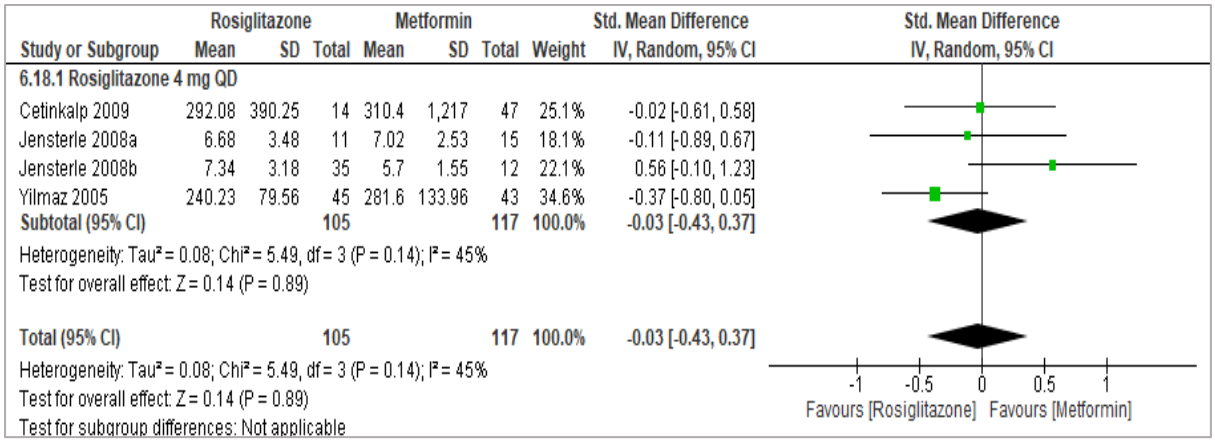
Figure 9-38: Forest plot of Pioglitazone versus Metformin on DHEAS



9.3.7.2.2 Rosiglitazone versus Metformin

Four RCTs compared rosiglitazone 4 mg QD with metformin showed no effect on the mean DHEAS (SMD: -0.03; 95%CI: -0.43, 0.37; I^2= 45 %, *p* = 0.89) (Figure 9-39) (very low-grade evidence, table 16).

Figure 9-39: Forest plot of Rosiglitazone versus Metformin on DHEAS

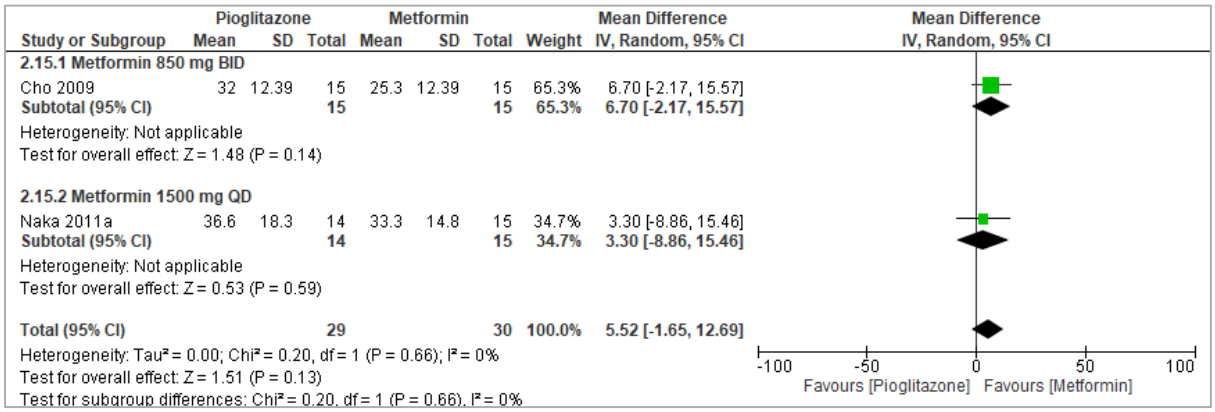


9.3.7.3 Sex hormone binding globulin (SHBG)

9.3.7.3.1 Pioglitazone versus Metformin

Two RCTs compared metformin of various dosage and frequencies with pioglitazone showed no effect on the means SHBG (MD: 5.52 mmol/L; 95%CI: -1.65, 12.69; I^2=0%, p = 0.13) (Figure 9-40) (very low-grade evidence, table 16).

Figure 9-40: Forest plot of Pioglitazone versus Metformin on SHBG (mmol/L)

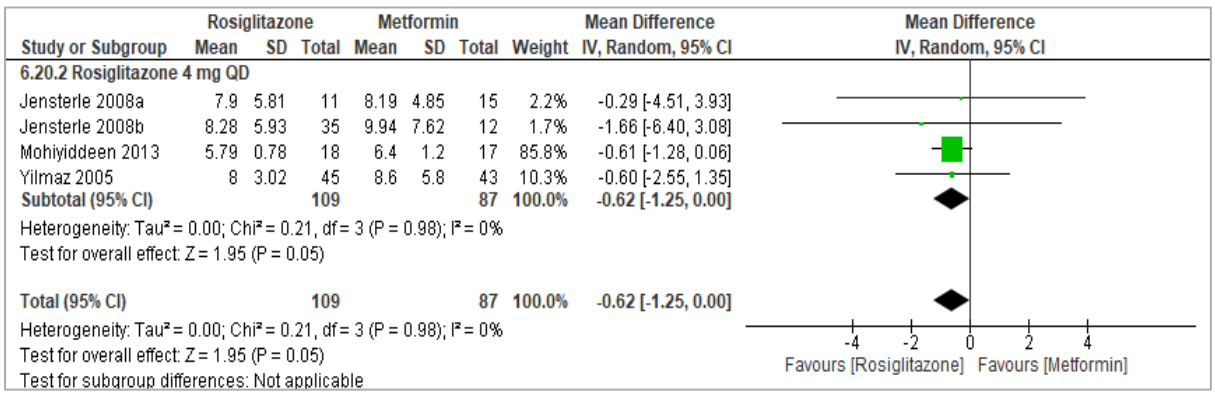


9.3.7.4 LH

9.3.7.4.1 Rosiglitazone versus Metformin

Four RCTs compared rosiglitazone 4 mg QD with metformin showed significant reduction in the mean LH (MD: -0.62 IU/L; 95%CI: -1.25, 0.00; I^2=0%, p = 0.05) (Figure 9-41) (very low-grade evidence, table 18).

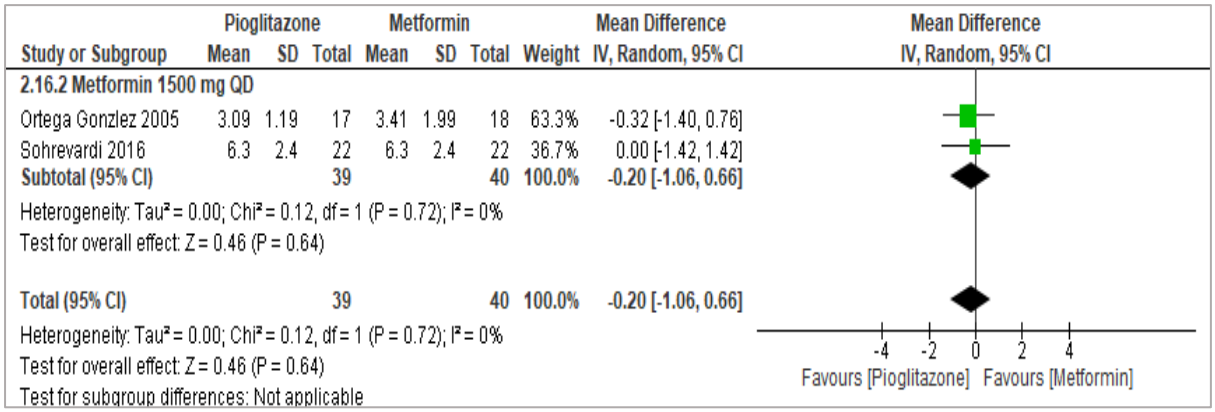
Figure 9-41: Forest plot of Rosiglitazone versus Metformin on LH (IU/L)



9.3.7.4.2 Pioglitazone versus Metformin

Two RCTs compared pioglitazone with metformin 1500 mg QD showed no effect on the mean LH (MD: -0.20 IU/L; 95%CI: -1.60, 0.66; I^2=0%, *p* = 0.64) (Figure 9-42) (very low-grade evidence, table 16).

Figure 9-42: Forest plot of Pioglitazone versus Metformin on LH (IU/L)

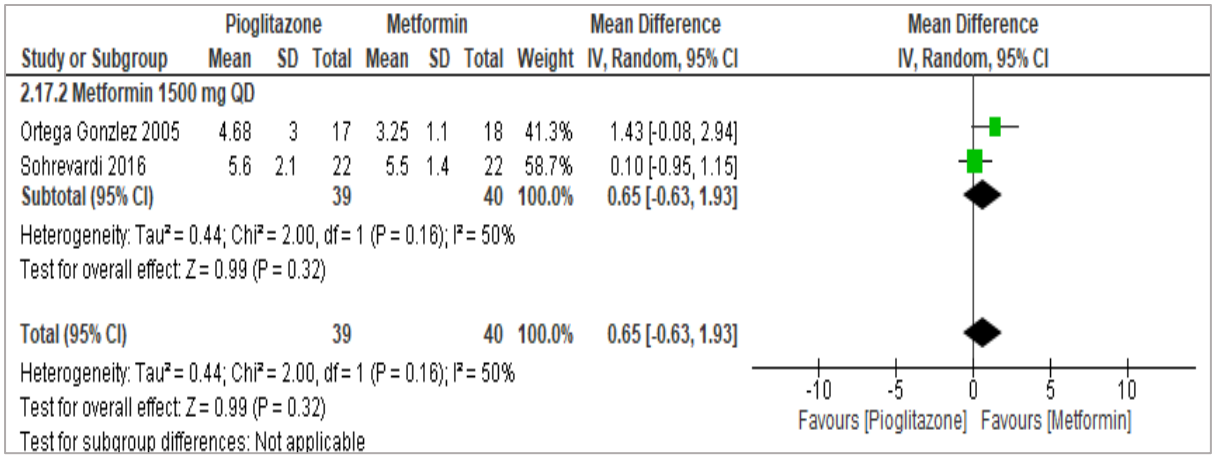


9.3.7.5 FSH

9.3.7.5.1 Pioglitazone versus Metformin

Two RCTs compared metformin 1500 mg QD with pioglitazone showed no effect in the mean FSH (MD: 0.65 IU/L; -0.63,1.93; I^2=50%, *p* = 0.32) (Figure 9-43) (very low-grade evidence, table 16).

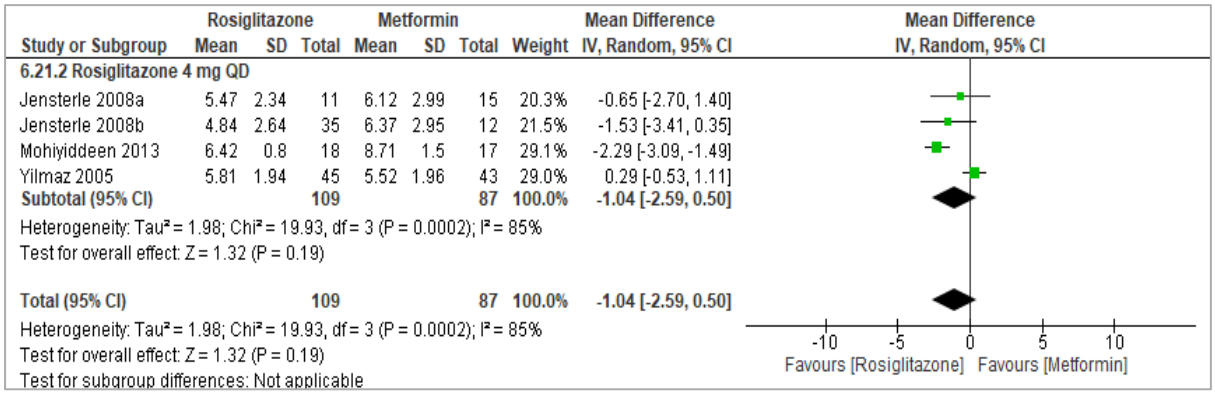
Figure 9-43: Forest plot of Pioglitazone versus Metformin on FSH (IU/L)



9.3.7.5.2 Rosiglitazone versus Metformin

Four RCTs compared rosiglitazone 4 mg DQ with metformin showed no effect in the mean FSH (MD: -1.04 IU/L; 95%CI: -2.59, 0.50; I^2=85%, *p* = 0.19) (Figure 9-44) (very low-grade evidence, table 18).

Figure 9-44: Forest plot of Rosiglitazone versus Metformin on FSH (IU/L)

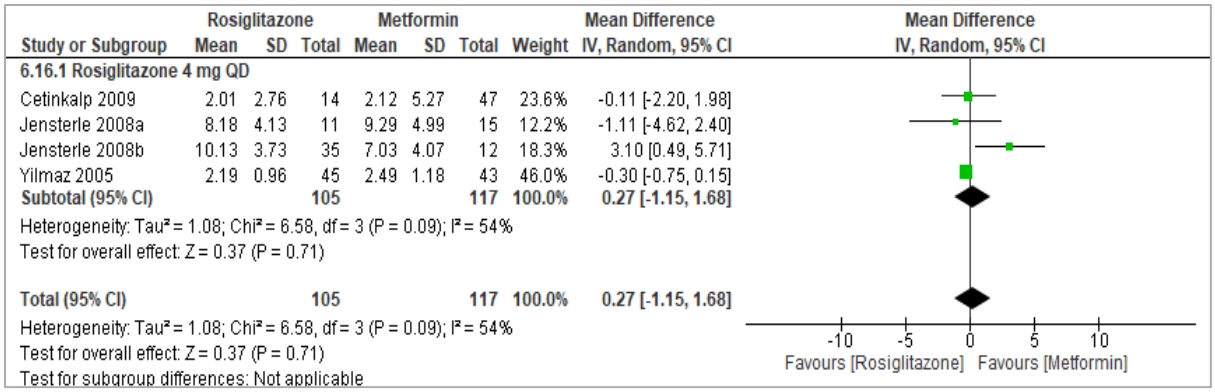


9.3.7.6 Free testosterone

9.3.7.6.1 Rosiglitazone versus Metformin

Four RCTs compared rosiglitazone 4 mg QD with metformin showed no effect in the mean free testosterone (MD: 0.27 pmol/L; 95%CI: -1.15,1.68; I^2=54%, *p* = 0.71) (Figure 9-45) (very low-grade evidence, table 18).

Figure 9-45: Forest plot of Rosiglitazone versus Metformin on free testosterone (pmol/L)



9.3.7.7 Androstenedione (A4)

9.3.7.7.1 Rosiglitazone versus Metformin

Three RCTs compared rosiglitazone 4 mg QD with metformin showed no effect in the mean A4 (MD: 1.03 nmol/L; 95%CI: -0.99, 3.06; I^2=75%, *p* = 0.32) (Figure 9-46) (very low-grade evidence, table 18).

Figure 9-46: Forest plot of Rosiglitazone versus Metformin on A4 (nmol/L)

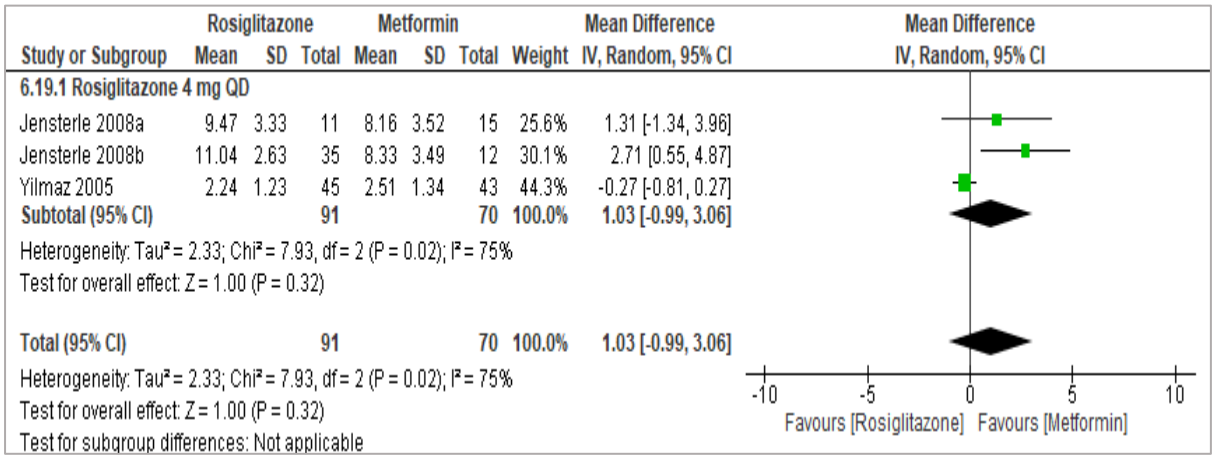


Table 16: Pioglitazone versus Metformin

Pioglitazone compared to Metformin in PCOS. **Patient or population:** PCOS **Setting:** **Intervention:** Pioglitazone **Comparison**: Metformin					
Outcome	**№ of participants (studies)**	**Certainty of the evidence (GRADE)**	**Relative effect (95% CI)**	**Anticipated absolute effects.**	
				Assumed risk.	
				Risk with Pioglitazone	**Risk difference with metformin**
Bodyweight	165 (4 RCTs)	⨁⨁◯◯ LOW a,b	-		**(Pioglitazone minus metformin)**
BMI	236 (6 RCTs)	⨁◯◯◯ VERY LOW a,c,d	-	The mean body weight ranged from 72.1-82.3	MD 1.62 higher (0.43 lower to 3.67 higher)
WC	85 (2 RCTs)	⨁◯◯◯ VERY LOW b,e	-	The mean BMI ranged from 27.4- 37.3	MD 0.85 higher (0.13 higher to 1.57 higher)
WHR	109 (3 RCTs)	⨁◯◯◯ VERY LOW b,f	-	The mean WC ranged from 88.1-90.36	MD 0.28 higher (4.66 higher to 4.10 lower)
FBG	215 (5 RCTs)	⨁◯◯◯ VERY LOW a,b,g	-	The mean WHR was 0.80-0.95	MD 0.02 lower (0 to 0.04 lower)
Fasting insulin	318 (6 RCTs)	⨁◯◯◯ VERY LOW a,b	-	The mean fasting Blood glucose was 5-92.73	MD 0.03 lower (0.30 lower to 0.24 higher)
Total Cholesterol	116 (5 RCTs)	⨁◯◯◯ VERY LOW a,b	-	The mean fasting insulin was 7.5- 18.73	MD 0.8 lower (1.07 higher to 2.67 lower)
Triglycerides	181 (4 RCTs)	⨁◯◯◯ VERY LOW a,b	-	The mean total Cholesterol was 156.36- 185.6	MD 3.34 higher (4.49 lower to 11.17 higher)
HDL	186 (4 RCTs)	⨁◯◯◯ VERY LOW a,b,h	-	The mean triglycerides was 103- 151.04	MD 7.68 higher (5.43 lower to 20.79 higher)
LDL	116(5 RCTs)	⨁◯◯◯ VERY LOW a,b	-	The mean HDL was 41.1-51.1	MD 0.29 higher (3.06 lower to 3.64 higher)
HOMA-IR	165 (4 RCTs)	⨁◯◯◯ VERY LOW a,b,g,l	-	The mean LDL was 73.25- 116.1	MD 2.59 higher (5.24 lower to 10.42 higher)
Total testosterone	129 (3RCTs)	⨁◯◯◯ VERY LOW a,c	-	The mean HOMA-IR was 2.42-39.54	MD 0.30 lower (0.61 lower to 0.01 higher)
DHEAS	145 (3RCTs)	⨁◯◯◯ VERY LOW a,c	-	The mean total testosterone range 0.6-0.73.4	SMD 0.35 higher (0.0 to 0.70 higher)
SHBG	59 (2RCTs)	⨁◯◯◯ VERY LOW a,c	-	The mean DHEAS range 1.4-221.3	SMD 0.27 higher(0.07 lower to 0.61 higher)
LH	79 (2RCTs)	⨁◯◯◯ VERY LOW a,c	-	The mean SHBG range 32-36.6	MD 5.52 higher (12.69 lower to 1.65 lower)
FSH	79 (2RCTs)	⨁◯◯◯ VERY LOW a,c	-	The mean LH range 3.08-6.3	MD 0.20 lower (1.06 lower to 0.66 higher)
				The mean FSH range 4.6-5.68	MD 0.65 higher (1.93 higher to 0.63 lower)

Table 17: Pioglitazone versus placebo

Pioglitazone compared to Placebo in PCOS. **Patient or population:** PCOS **Setting:** **Intervention:** Pioglitazone **Comparison:** Placebo					
Outcome	**№ of participants (studies)**	**Certainty of the evidence (GRADE)**	**Relative effect (95% CI)**	**Anticipated absolute effects.**	
				Assumed risk.	
				Risk with Placebo	**Risk difference with pioglitazone**
BMI	142 (5 RCTs)	⊕⊕◯◯ LOW d,e	-		**(Pioglitazone minus placebo)**
WC	77(3 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean BMI was 27.7-35.22	MD 2.56 Higher (1.77 higher to 3.34 higher)
WHR	78 (3 RCTs)	⊕⊕◯◯ LOW e,i	-	The mean WC was 85.4-105	MD 5.45 higher (2.18 higher to 8.71higher)
FBG	115 (4 RCTs)	⊕⊕◯◯ LOW c,e,g,l	-	The mean WHR was 0.84-0.89	MD 0.01 Lower (0.04 lower to 0.02 higher)
Fasting insulin	142 (5 RCTs)	⊕◯◯◯ VERY LOW c,e,g, i	-	The mean fasting Blood glucose was **5**-91.1	MD 0.12 lower (0.9 lower to 0.66 higher)
Total Cholesterol	63 (2 RCTs)	⊕◯◯◯ VERY LOW b,c,j	-	The mean fasting insulin was 62-70	MD 8.54 lower (15.22 lower to 1.86 lower)
Triglycerides	63 (2 RCTs)	⊕⊕◯◯ LOW I,k	-	The mean total Cholesterol was 4.7- 4.8.	MD 0.17 Lower (0.4 lower to 0.05 higher)
HOMA-IR	63 (2 RCTs)	⊕◯◯◯ VERY LOW c,e,j	-	The mean triglycerides was 1.3-1.3	MD 0.20 Lower (0.38 lower to 0.03 lower)
				The mean HOMA-IR was 14.1- 15.2	MD 1.74 Lower (5.02 lower to 1.53 higher)

Table 18: Rosiglitazone versus Metformin

Rosiglitazone compared to Metformin in PCOS. **Patient or population:** PCOS **Setting:** **Intervention:** Rosiglitazone **Comparison:** Metformin					
Outcome	**№ of participants (studies)**	**Certainty of the evidence (GRADE)**	**Relative effect (95% CI)**	**Anticipated absolute effects**	
				Assumed risk	
				Metformin	**Risk difference with rosiglitazone**
Bodyweight	212 (3 RCTs)	⨁◯◯◯ VERY LOW b,d,e	-		**(Rosiglitazone minus metformin)**
BMI	424 (8 RCTs)	⨁⨁⨁◯ MODERATE a	-	The mean body weight was 62.32-66.88	MD 1.95 higher (0.03 higher to 3.87 higher)
WC	408 (3 RCTs)	⨁⨁◯◯ LOW b,c	-	The mean BMI was 23.38- 29.12	MD 0.74 higher (0.07 higher to 1.41higher)
WHR	223 (2 RCTs)	⨁⨁◯◯ LOW b,f	-	The mean WC was 81.1-90.28	MD 0.09 higher (1.52 lower to 1.69 higher)
FBG	122 (3 RCTs)	⨁⨁◯◯ LOW b,d,e	-	The mean WHR was 0.85-0.89	MD 0.01 Higher (0.01 lower to 0.02 higher)
Fasting insulin	415 (7 RCTs)	⨁⨁◯◯ LOW a,f	-	The mean CRP was 0.28-1.98	MD 0.21 Lower (0.53 lower to 0.1 higher)
Total Cholesterol	415 (7 RCTs)	⨁⨁◯◯ LOW a,f	-	The mean FBG was 4.26-89.94	MD 0.12 Higher (0.07 lower to 0.32 higher)
Triglycerides	287 (5 RCTs)	⨁⨁◯◯ LOW a,c	-	The mean fasting insulin was 7.03-15.97	MD 0.73 lower (1.92 lower to 0.45 higher)
HDL	263 (4 RCTs)	⨁⨁◯◯ LOW d,e	-	The mean total Cholesterol was 4.1-184.6	SMD 0.24 higher (0.24 lower to 0.73 higher)
LDL	257 (4 RCTs)	⨁⨁◯◯ LOW a,g	-	The mean triglycerides was 1.29- 94.8	SMD 0.15 Higher (0.10 lower to 0.40 higher)
HOMA-IR	287 (5 RCTs)	⨁⨁◯◯ LOW a,e	-	The mean HDL was 1.29-1.52	SMD 0.04 higher(0.21 lower to 0.30 higher)
Total testosterone	261 (5 RCTs)	⨁⨁⨁◯ MODERATE b	-	The mean LDL was 2.24-107.5	MD 0.34 lower (0.72 lower to 0.04 lower)
DHEAS	222 (4 RCTs)	⨁◯◯◯ VERY LOW a,c	-	The mean HOMA-IR was 1.24-3.61	MD 0.09 lower (0.47 lower to 0.28 higher)
SHBG	262 (4 RCTs)	⨁◯◯◯ VERY LOW a,c	-	The mean free testosterone range 2.12-9.29	MD 0.27 higher (1.15 lower to 1.68 higher)
LH	222 (4 RCTs)	⨁◯◯◯ VERY LOW a,c	-	The mean total testosterone range 0.5-2.64	MD 0.13 higher (0.08 lower to 0.34 higher)
FSH	161 (3 RCTs)	⨁◯◯◯ VERY LOW a,c	-	The mean DHEAS range 5.7-310.4	MD 0.03 lower (0.43 lower to 0.37 higher)
				The mean A4 range 2.251- 4	MD 1.03 higher (0.99 lower to 3.06 higher)
				The mean LH range 6.4-9.94	MD 0.62 lower (0.00 to 1.25 lower)

Table 19: Rosiglitazone versus placebo

Rosiglitazone compared to Placebo in PCOS. **Patient or population:** PCOS **Setting:** **Intervention:** Rosiglitazone **Comparison:** Placebo					
Outcome	**№ of participants (studies)**	**Certainty of the evidence (GRADE)**	**Relative effect (95% CI)**	**Anticipated absolute effects.**	
				Assumed risk.	
				Risk with Placebo	**Risk difference with rosiglitazone**
BMI	153 (4 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-		**(Rosiglitazone minus placebo)**
Bodyweight	174 (3 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean BMI was 34.1-25.1	MD 0.30 lower (1.19 lower to 0.60 higher)
WHR	113 (3 RCTs)	⊕◯◯◯ VERY LOW a,b,c	-	The mean body weight was 62.32-66.88	MD 1.95 higher (0.03 higher to 3.87 higher)
				The mean WHR was 0.81-0.88	MD 0.08 lower (0.11 lower to 0.04)

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **MD:** Mean difference; **RCT:** randomised clinical trials; **BMI:** body mass index; **WHR**: waist-hip ratio; **WC:** waist circumference; **SMD:** standardised mean difference

GRADE Working Group grades of evidence.
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

Explanations
a. Two studies have an unclear risk of bias across five or more domains. One study has a high risk of performance bias. Thus, we downgraded one level.
b. A small number of participants with a wide confidence interval. So, we downgraded one level.
c. There is no overlapping of confidence interval between the studies, which could mean there are small studies with negative results been unreported. Thus, we downgraded one level.
d. Six studies showed an unclear risk of bias across more than five domains. One study has a high-performance bias. Thus we downgraded one level.
e. A small number of participants with a wide confidence interval. Thus, we downgraded one level.
f. One study has a high risk of performance bias. Thus, downgraded one level.
g. A considerable level of heterogeneity. Therefore, we downgraded one level.
h. Only two studies, and there is no overlapping of confidence intervals. So, we downgraded one level.
i. There is an unclear risk of bias across many domains in all the studies. One study has a high risk of performance bias. We downgraded one level.
j. unclear risk of bias across many domains of the studies. Therefore, we downgraded one level.
k. There is a wide range of confidence intervals across the studies with a significant effect of CL and MD. Thus, we downgraded one level.

9.4 Discussion

This review is the most comprehensive and up-to-date systematic review and meta-analysis on the effect of glitazones in women with PCOS with a comprehensive analysis reporting the effects of pioglitazone and rosiglitazone, either as add-on or monotherapy, on anthropometric outcomes, insulin resistance indices, lipid profiles, CRP and androgen hormones in women with PCOS. When pioglitazone and rosiglitazone were administered at various therapeutic doses and compared with placebo and metformin, there was a statistically significant increase in the mean body weight, BMI, and WC, and a significant reduction in triglycerides, LDL-C, fasting insulin, HOMA-IR and LH. Both pioglitazone and rosiglitazone had no effect on the mean fasting blood glucose, total cholesterol, HDL-C, CRP, SHBG, DHEAS, FSH, free testosterone and androstenedione. These findings are in accord with the findings of previous studies. A systematic review and meta-analysis of six randomised controlled trials showed pioglitazone was more effective in reducing fasting insulin and HOMA-IR (P = 0.02 and P = 0.014, respectively) and significantly increased the mean BMI compared to metformin (656). In another systematic review and meta-analysis of eleven RCTs comparing pioglitazone and metformin, pioglitazone significantly increased the mean BMI compared to metformin (P = 0.006) (393). This systematic review found that rosiglitazone significantly reduced the mean LH compared with metformin. These results will add to the existing evidence on the effect of TZDs. A similar effect was also reported in a network meta-analysis of 28 RCTs that compared the effect of rosiglitazone and pioglitazone on the hormonal parameters in PCOS, which showed a significant effect of rosiglitazone in reducing the mean LH compared to metformin (837). A recent network meta-analysis compared the efficacy of TZDs and metformin with respect to endocrine and metabolic profiles in women with PCOS. The results suggested a superior efficacy for the TZDs as an add-on therapy to metformin than monotherapy in ameliorating insulin resistance, lipid profile and testosterone levels

(838). Another network meta-analysis of 14 RCTs assessing the efficacy of TZDs in overweight women with PCOS reported that TZDs as add-on therapy to metformin had superior efficacy in improving hyperandrogenaemia compared to monotherapy (839). However, this systematic review and meta-analysis showed a superior effect of metformin on mean total testosterone than pioglitazone. There are several contraindications for TZDs, including heart failure due to its fluid retention effect (840). Also, due to its teratogenic potential, TZDs would not be the right choice for women with PCOS who are pregnant or actively seeking pregnancy (841). Thus, women should be switched to other insulin sensitisers such as metformin.

This study followed a comprehensive and systematic method to search for relevant databases and grey sources and only included RCTs. Several steps were taken to minimise the risk of bias, and we excluded observational studies and non-randomised clinical trials. This systematic review outlines the up-to-date evidence supporting the effectiveness of glitazones used in PCOS management; however, most RCTs were small, and the statistical power used to calculate sample size was not fully reported. Moreover, all the trials were of short duration; therefore, the long-term effects of TZDs in women with PCOS is unclear.

This systematic review also acknowledges the poor quality of the included trials, which is also reflected in the summary of evidence of the GRADE score. Due to the nature of the clinical trials, there was a significantly high level of heterogeneity and performance bias among the included studies. Based on our findings, it is clear that there is a lack of robust clinical trials assessing the efficacy of TZDs in the management of PCOS. Furthermore, trials examining the clinical effectiveness of pioglitazone and rosiglitazone were of low or very low quality. Therefore, the available data are insufficient to draw definite conclusions and propose recommendations for clinical practice. Furthermore, these trials were mainly of

small samples, which undermined the statistical power to calculate significant effects on the outcomes. Therefore, further clinical trials with robust design are needed to enable better-informed decisions and recommendations and draw guidelines for the effectiveness of pioglitazone and rosiglitazone used in women with PCOS.

9.5 Conclusion

Compared to metformin, pioglitazone and rosiglitazone are effective in reducing fasting insulin, triglycerides, LDL-C and LH. On the other hand, both pioglitazone and rosiglitazone significantly increased the mean body weight, BMI and WC. However, no significant effect was found on the mean FSH, CRP, DHEAS, total cholesterol and androstenedione. Thus, TZDs could be a reasonable alternative to metformin or as an add-on to metformin in metformin-resistance and metformin-intolerant women with PCOS.

10 Chapter 10: Living with polycystic ovary syndrome (LW-PCOS): A structured education programme for women with PCOS - a mixed-methods study

10.1 Introduction

Polycystic ovary syndrome (PCOS) is a prevalent endocrine and metabolic condition, affecting around 20% of women of reproductive age (506). PCOS is characterised by irregular periods, biochemical and clinical features of excess androgen and polycystic ovary morphology (60). Many women with PCOS experience difficulties maintaining healthy body weight and having a successful pregnancy (66, 134, 738). Furthermore, they are at increased risk of developing type 2 diabetes, metabolic syndrome, cardiovascular risk (320), anxiety and depression, which, in turn, compromise their quality of life (QoL) (134, 816). There are various diagnostic criteria for PCOS, including the Rotterdam-2003 criteria (597), the Androgen Excess Society criteria and the National Institutes of Health criteria 1990 (NIH)(38); the international evidence-based guidelines for the assessment and management of PCOS advocated the use of the Rotterdam-2003 diagnostic criteria (34, 38, 842). According to the Rotterdam criteria, to diagnose women with PCOS, at least two of the following three must be present: clinical or biochemical evidence of androgen excess, anovulation/oligo-ovulation, and polycystic ovarian morphology verified by ultrasound (34). However, despite these clear diagnostic criteria, PCOS often goes undiagnosed.

A recent study showed that it took more than two years for women diagnosed with PCOS before receiving a diagnosis (843). Furthermore, almost 1 in 2 women saw at least three healthcare professionals over two years before receiving a definite PCOS diagnosis (843). Women with PCOS also tend to get inadequate information about their condition and management, leading to confusion, feelings of guilt and lack of control (843-845). Research has shown that PCOS women have a high desire to know more about the

nature of the condition and its emotional and physical aspects (846). There is also a noticeable gap in knowledge among healthcare professionals, which highlights the need for raising awareness about the current diagnostic criteria and recommendations for managing PCOS (844). This was also addressed by the international evidence-based guidelines for the assessment and the management of PCOS-2018 (842),which recommended screening all women with PCOS for factors that have a detrimental impact on their QoL.

Management strategies for PCOS include lifestyle modifications such as diet and physical activity is the first-line treatment approach. This promotes weight loss in overweight and obese women with PCOS and may reduce their risk of developing diabetes and the metabolic syndrome (106). However, most of these available interventions require extensive input by a healthcare professional and have usually been conducted in secondary care facilities. On the other hand, most women with PCOS often receive care at the primary care level (GPs surgery) without referral to more specialist care. Therefore, there is a need for a pragmatic, structured education programme to empower women with PCOS by increasing their knowledge of PCOS, which will help them take control of their condition, and which can be implemented in the health system at both primary care and community level.

In recent years, there has been a significant recognition for the importance of integrating education as part of managing many chronic conditions. Thus, educating patients about self-management has become the focus of attention among healthcare professionals. This approach has been successful in patients with both type 1 and types 2 diabetes, and it was meant to acquire the necessary skills for day-to-day self-management of their condition (360, 847-849). There is, however, limited evidence on the use of structured education programmes tailored specifically to the characteristics and the needs of women

with PCOS. A recent study claimed that providing education in parallel to routine medical treatment can benefit women with PCOS. It can help them understand their condition, reduce their anxiety and improve their QoL (358).

10.2 Method

10.2.1 Ethical approval

The ethical approval process was explained in chapter 2, section 2.1.2.2.

10.2.2 Inclusion/exclusion criteria

The eligibility criteria were explained in chapter 2, section 2.1.2.4.

10.2.3 Participants and recruitment

This recruitment process was explained in chapter 2, section 2.1.2.3.

10.2.4 Design of the study and outcomes measured

The design of the study and the measured outcomes were explained in chapter 2, section 2.1.2.5.

10.2.5 Statistical analysis

The statistical analysis was explained in chapter 2, section 2.1.2.6.

10.3 Results

10.3.1 Results from the survey on patients' perspectives on the development of an educational programme for PCOS

A total of 320 participants were surveyed (210 online and 110 via post), Table 20. All respondents (n= 320)(100%)) had been previously diagnosed with PCOS and were Caucasians. The age distribution was as follows: 18-19 years (n=1/320 (0.3 %)), 19-30 years (n=131/320 (40.9%)), 31-40 years (n=127/320(39.6%)) and above 40 years (n=52/320 (16.3%)).The majority of the participants were employed 246/320 (76.9%), 29/320 (9%) unemployed, 20/320 (6.3%) were in full-time education, 4/320 (1.5%) were in formal training, and 11/320 (3.4%) did not specify their occupations. The mean age for diagnosis of PCOS was 21.8±5.4 years. The characteristics of the participants are presented in Table 20. The common themes identified from the survey are presented in Figure 10-1.

Table 20: Baseline characteristics of participants (n = 320) who completed the survey

Variables	Characteristics	Frequency (n)	Percentage (%)
Ethnicity	Caucasians	320	100%
Age group	18-19 years	1	0.3%
	19-30 years	131	40.9%
	31-40 years	127	39.6%
	>40 years	52	16.3%
Occupation	Employed	246	76.9%
	Unemployed	20	6.3%
	Full-time education	4	1.5%
	Formal training	11	3.4%

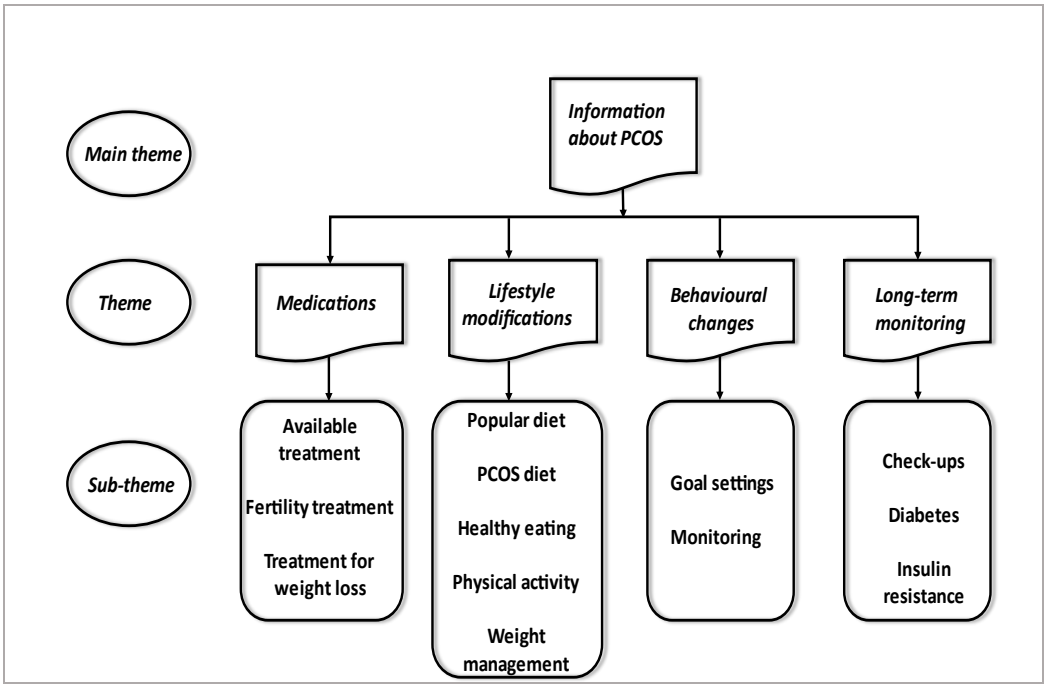


Figure 10-1: Theme and sub-theme identified from the survey

10.3.1.1 Confidence in managing PCOS

Women were asked what they thought might improve their confidence in managing their condition (multiple choice question). Most of the participants (n=224/320 (70%)) responded that information about the causes and the long-term consequences of PCOS would increase their confidence in managing their condition. For example, (n=193/320 (60.3%)) said long-term monitoring, including checks for diabetes, infertility and cancer, (n=163/320 (50.9%)) said medication and treatment options. Whilst 132/320 (41.3%) said psychological support and 37.8% (n=121/320) said behavioural strategies for weight loss (Figure 10-2).

Figure 10-2: Word clouds of the most reported theme about the confidence in managing PCOS

When women were asked to give further details, the following common themes were identified from the participant's quotes "in the participants' words":

10.3.1.1.1 Theme: help and support

10.3.1.1.1.1 Support from healthcare professionals with specialist knowledge in PCOS

"Support with weight loss that's not just information given out".

"Dedicated departments and specialists in the condition".[diagnosed at the age of 14].

"Having medical professionals be aware of PCOS, its prevalence, causes, symptoms, treatment options, and how it affects an individual's life negatively". [diagnosed at age of 14].

"Doctors knowing about the conditions and treatment options and not being prescribed hormonal birth control that makes insulin resistance worse (lots of GPs don't know this)".[diagnosed at age of 27].

"More support from dieticians, not just saying, well you have done your research we cannot help you. They need to be more informed than me. I need more information on balanced low carb diets for vegetarians".

10.3.1.1.1.2 Regular monitoring

"Nutritionists and regular testing at the doctors to see if the condition is improving or getting worse". [diagnosed at age of 22].

"Just to expand monitoring but also for GPs to discuss other areas it may impact that you may/may not have associated with it, to try and actually assess how you feel (increased prevalence of mental health issues) understanding the right diet/exercise to prevent potential harm by overdoing it".[diagnosed at age of 20].

10.3.1.1.1.3 Timely diagnosis

"When I was first diagnosed I wasn't told what I had. I was only told what I had years later. Being told what I had and education and support at that point would have changed the outcomes for me" [Symptoms started at age 17 years and diagnosed at age 21].

10.3.1.1.1.4 Peer support/ support group

"peer support or support group"[diagnosed at the age of 22].

10.3.1.1.1.5 Practical advice

"Totally, practical advice how to live with effects, i.e. dealing with shaving every day, kind of razors people use, depilatory creams, electrolysis".[diagnosed at the age of 30].

" Help to cope with daily challenges from this condition". [diagnosed age 17].

10.3.1.1.2 Theme: information and knowledge about PCOS

10.3.1.1.2.1 Subtheme: Aetiology and pathophysiology of PCOS

"Why do I have this condition?, how did it develop? Why isn't there a tablet to level out the hormones which would fix it".

"Investigation into the root causes of PCOS so you can identify the underlying issues that trigger your PCOS – e.g. Insulin resistance, inflammation or stress".

10.3.1.1.2.2 Subtheme: Long-term consequences of PCOS

"PCOS and the menopause, PCOS and long term health, PCOS and eating disorders, body image issues etc. There needs to be a more holistic, HAES based approach to guiding patients through diagnosis and long term management".

10.3.1.1.2.2.1 Fertility problems

“Information about fertility with PCOS and how to improve it beyond ‘lose weight’”.

"information on how using contraception such as the Implanon implant affects you and any potential for the future and getting pregnant".[diagnosed at the age of 35].

“ Information on PCOS beyond childbearing age. Everything seems geared towards fertility and having a baby, but symptoms continue after that, and no support is available. Similarly with peri/menopause and PCOS”.[diagnosed at the age of 17].

10.3.1.1.2.2.2 The PCOS impact on mental health

“Impact on mental health (with regards to anxiety etc). How changes in diet can impact symptoms. Is the condition subject to change over time? How can I manage it?”

10.3.1.2 The usefulness of an education programme

When women were asked about how useful it would be if an educational programme for PCOS were available, the majority (n=267/320 (83.5%)) said it would be very useful or useful, 13.4% (n=43/320) said somewhat would be helpful. In comparison, only 8/320 (2.5%) said it might not be useful, and 2/320 (0.6%) did not respond. Figure 10-3.

Figure 10-3: World clouds on the usefulness of the educational programme

When they were asked to give a reason for their answer, the following common themes were identified from the participant's quotes "in the participant's words":

10.3.1.2.1 Theme: not enough knowledge about PCOS

10.3.1.2.1.1 Subtheme: Lack of knowledge among healthcare professionals

"I've been very surprised how little information and advice has come via my GP and my gynaecologist. The changes that I have implemented to regulate my cycles and finally start ovulating were made based on the info I had read online. If I had only gone off of the info provided from my doctors, I would still be having bad symptoms and 5-month cycles. There is definitely a need for this information".

"There is so little knowledge about it, and not all GP will refer to gynae to get more understanding, but even the little bit of information they give you is not enough. You get whole education days, even weeks when your diabetic but a five-minute conversation and a leaflet when you have PCOS".

"Because at the GP, you virtually get no information".

10.3.1.2.1.2 Subtheme: Self-study to gain knowledge about PCOS

" I have had to do a considerable amount of personal study to understand the condition. Many GP's still do not seem to understand it or be able to provide support with losing weight or combatting the various issues connected to it"[diagnosed at age 25 years].

"When first diagnosed, I was given the pill and told to come back when I wanted kids. Everything I know has been through extensive research, and there is a wide range of information out there varying in quality and trustworthiness. I have gone through a lot of things that I wouldn't have needed to if there had been a structured education and care plan post-diagnosis, and I think something like this should exist for all patients".

" There isn't much knowledge around how to understand or manage PCOS, I think an educational course with support would be very helpful" [diagnosed at age 18].

10.3.1.3 Knowledge about PCOS

About one in 3 women (n=121/320 (37.8%)) did not have enough knowledge about PCOS, 140/320 (43.8%) and 59/320 (18.4%) claimed to know enough or very much enough about their condition, respectively. On the other hand, the vast majority (n=254/320(79.4%)) wanted to increase their

knowledge about PCOS and said they needed to know more, 46/320 (14,3%) said somewhat they are willing to increase their knowledge while only (n=20 (6.3%)) said they do not need to know.

10.3.1.4 PCOS symptoms

180/320 (56.3%) needed to know about the PCOS symptoms while a combined number of participants (n=140/320 (43.7%)) said either they somewhat needed to know, or they were not eager to know about PCOS-related symptoms.

10.3.1.5 The current clinical management strategies for PCOS

185/320 (57.8%) claimed they do not know about the current clinical management strategy for PCOS. In comparison, 50/320 (15.6%) said they somewhat know about the management strategy, and 85/320 (26.6%) said they have enough knowledge about the current management strategy for PCOS. The long-term consequences of PCOS was the most desirable topic (n=192/320 (60%)), followed by information on PCOS symptoms (n=109/320 (34%)). Nearly half of the participants (n=156/320 (49%)) managed their condition by a combination of lifestyle modifications and medications.

When women were asked whether they have anything else they would like to know, the following themes are identified, quoted from participants "in the participant's words":

10.3.1.5.1 Theme: managing PCOS

10.3.1.5.1.1 Subtheme: pharmacological interventions

“Is there are any other medication other than metformin”.

“ There isn't much knowledge around how to understand or manage PCOS, I think an educational course with support would be very helpful" [diagnosed at age 18].

“Information on conceiving with PCOS”.

10.3.1.6 Information about lifestyle modifications, including diet and exercise

Nearly two-thirds of the respondents (n=198/320 (61.9%)) were very much wanted to know more about healthy food choices and balanced diets, while 70/320 (21.9%) were somewhat needed to know, 49/320 (15.3%) did not wanted to know, and 3/320 (0.9%) did not stated.

Sixty per cent (n=192/320) of the participants were very much keen to know about the current recommendation of physical activity, and over a half (n=174/320 (54.4%)) wanted to know about ways to increase their physical activity levels. Over two-thirds of the participants (n=220/320 (68.8%)) wished to know about how to lose and maintain weight loss through changes in diet and exercise, and only 52 (16.3%) were not very keen to know about this strategy. Nearly half (n=159/320 (50%)) of the participants were very much keen to know about popular diets, including very-low-calorie diets, meal replacement products, low carb diets, ketogenic diets and intermittent fasting, while (n=48/320 (15.1%)) were keen to know and one-third (n=111/320 (34.9%)) were not very keen to know about popular diets. Figure 10-4.

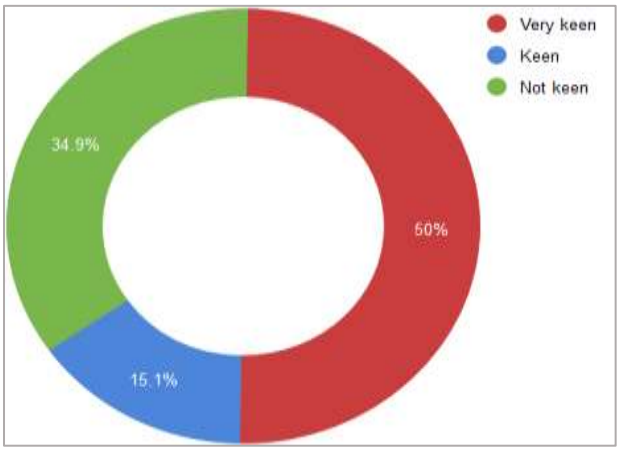


Figure 10-4: Popular diets

When participants were allowed to add anything else they would like to know about lifestyle modifications, the following common themes were identified from the participant's quotes "in the participant's words":

10.3.1.6.1 Theme: lifestyle modifications including diets and physical activity

10.3.1.6.1.1 A detailed programme to help and sustain lifestyle modifications

"I feel like I know about all this; you can read about it all. What I'd like to know of which one I should do to help with my PCOS and why it will help and a detailed programme helping me change my lifestyle".

"Lifestyle modifications need to be long term, not fad diets".

10.3.1.6.1.2 Emphasis on exercise

"I already use a lot of lifestyle modifications; I work with a dietician for food management. Knowledge of exercising safely and effectively would be good, some exercise is known to be ineffective for PCOS".

10.3.1.6.1.3 Myths around diet and exercise

"I saw a tier 3 weight management programme at Imperial, and although it was useful, I need to have information on diet and exercise with people who have PCOS as it is very different to the usual calorie control diet I have been trying for ages to do but not succeeding with. General advice about diet and exercise is not good enough. I hate being told that I am not doing enough when in fact, I am overdoing the exercise. All because of my PCOS".

"What's reasonable for a 'perfect normal diet'. Currently, no one told me about restricting dairy which I volunteered to do and improved my distressing bowel movements 10000 times. Being on a carb-free, 'clean' diet doesn't suit my lifestyle - does that mean I can never manage my PCOS? I'm a keen exerciser, however, I haven't found a routine that impacted my physical symptoms (weight gain, hair, periods etc) - which is best?".

"What the best way to eat and exercise is because there are too many contradictions online around the right way to eat and exercise for maximum results as you need to exercise to lose weight but shouldn't do too much because it causes inflammation and stress which then affects your insulin levels etc".

"There is a lot of information online that all suggest different diets that will supposedly help but there's no clear answer and it's difficult to work out which ones are healthy as most seem to be very restrictive and very likely lead to disordered eating".

"Education around healthy eating and increasing activity is crucial especially exercising for PCOS in light of some of the research that suggests too strenuous an activity can make symptoms worse. I am very wary

of any focus on diets per se as there is very little evidence for long term effectiveness of any diet and also focusing on diets encourages disordered eating and can be very triggering for some people".

10.3.1.6.1.4 Sub-theme: diets

10.3.1.6.1.4.1 Perception on popular diets

"I think popular diets should be introduced as tools that may work in the short term. There needs to be educated on how damaging diet culture is and again a more HAES led approach promoting healthy habits and changes rather than a constant focus on weight loss as the only goal when managing the condition".

"I would rather be told about a specific PCOS diet - one designed specifically for helping ease this condition, not just popular ones that are trending at that time (that do not work!)".

"What diets may be more appropriate than others for your type of PCOS (e.g. Fasting? Keto?) Do they make a difference? or is it just fewer calories? Could fasting be harmful".

10.3.1.6.1.4.2 The impact of diet on the PCOS-related consequences

"How food links with mood and how to manage blood sugar levels etc. Diet and fertility".

"What the best way to eat and exercise is because there are too many contradictions online around the right way to eat and exercise for maximum results as you need to exercise to lose weight but shouldn't do too much because it causes inflammation and stress which then affects your insulin levels etc".

10.3.1.6.1.4.3 Women's perceptions on dietary intervention

"Education around healthy eating and increasing activity is crucial, especially exercising for PCOS in light of some of the research that suggests too strenuous an activity can make symptoms worse. I am very worried of any focus on diets per se as there is very little evidence for long term effectiveness of any diet, and also focusing on diets encourages disordered eating and can be very triggering for some people".

"I think it's going to be triggered for a lot of women with PCOS if you push diet culture on to them. The majority of people with PCOS will have you dieted and tried everything to lose weight. You should focus on what a balanced diet made up of whole foods looks like and educate on good foods for PCOS rather than calories and restrictions. There is evidence to suggest that 96% of diets fail, and it shouldn't be recommended as a treatment for any illness".

"I think diets are very problematic for PCOS, and research shows that very-low-calorie diets, meal replacement products, low carb diets, ketogenic diets, intermittent fasting are all very problematic for PCOS - I am very concerned you are suggesting this to be included in evidence for people. This is setting people up to fail".

"There is a lot of information online that all suggest different diets that will supposedly help, but there's no clear answer, and it's difficult to work out which ones are healthy as most seem to be very restrictive and very likely lead to disordered eating".

10.3.1.6.1.5 Sub-theme: physical activity

"I currently do quite a bit of exercise, and at the moment, the weight loss I had been having had halted, and I've actually gained over the last few months, So balance as I don't know whether I've eaten worse than I think or whether the exercise has caused raised cortisol".

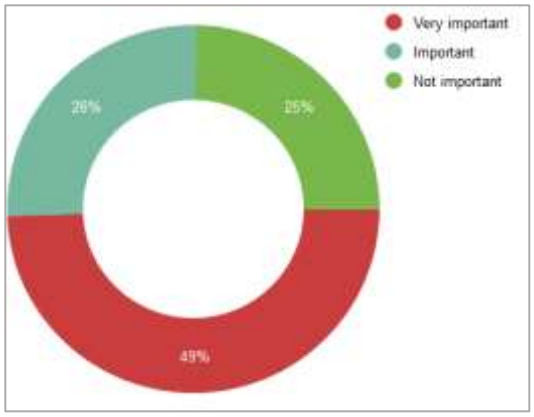
10.3.1.6.1.6 Sub-theme: weight management

"Weight gain as well! Had lean PCOS and always struggled with gaining weight, not the main issue for women with PCOS but for those who are thin only being told how to lose weight can be negative and cause confusing body esteem".

"With having PCOS, it is harder to lose weight. If I know how my body loses weight and how it gains weight with my condition, I would therefore know what proper exercise would benefit the most".

10.3.1.7 Information about behavioural change techniques

Over half of the participants, 189/320 (59%) were keen to know about these techniques, and 135/320 (42.2%) of the participants were very keen to know about goal setting relevant to physical activity and diet. Nearly a half (n=152/320 (47.5%) were thought learning how to monitor weight regularly would be important, and 128/320 (40%) of the participants said information on keeping records of what you eat, or drink would be important. Figure 10-5. Overall, nearly a half (n=158/320 (49%) of the participants agreed that keeping records of daily activities would be very important, 83/320 (26%) said it would be essential, and 78/320 (25%) did not think it would be important. Over a third (n=119/320 (37%)) of the participants thought taking part in exercise or educational group would be very beneficial, 80/320 (25%) said it would be beneficial, and 121/320 (38%) said it would not be beneficial in helping them to lose weight. Figure 10-6.



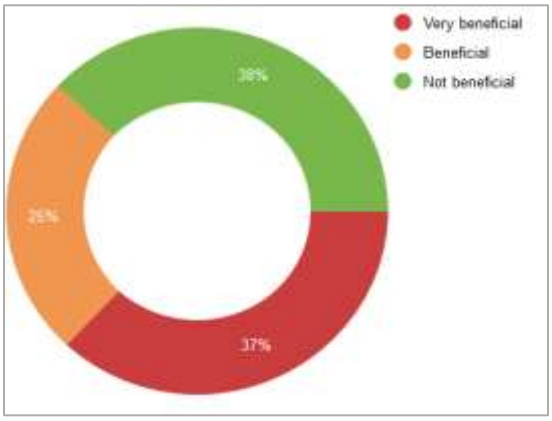


Figure 10-5: Keeping records **Figure 10-6:** Group exercise

The following are the common themes identified and quoted from the participants about the behavioural changes techniques:

10.3.1.7.1 Theme: behavioural changes

10.3.1.7.1.1 Sub-theme: monitoring and tracking diets

"Diets and food tracking lead to unhealthy and disordered eating. Very often, nutritional advice has been patronising and, at worst incorrect. I have had far more success on my own managing my condition than through medical professionals whose first-line treatment is the pill-which is not a treatment of the underlying condition but a way to mask symptoms. Management of stress, self-esteem counselling, enjoyable movement and sustainable eating patterns, targeted supplements have worked far better than anything else. Living with PCOS has left me with a deep distrust of GPS, gynaecologists, dieticians and fitness 'experts' who tend to take the approach that women with PCOS just need educating when in fact, it's the other way around".

10.3.1.7.1.2 Sub-theme: goal setting

10.3.1.7.1.2.1.1 Challenges of setting goals

“How to sustain changes to lifestyle, especially diet changes when socialising or eating out”.

"My overall health has always declined with "lifestyle modifications". The obsession with weight management is unhealthy and cruel. I think for this reason, women don't know about what else PCOS does to us. Because the weight increase is pushed upon us and expected to be our greatest issue It is not".

"Not enough focus is given to not losing weight, particularly for those of us who are already slim!".

"I would like information on how to not become obsessed with dieting and weight and instead find ways on how to incorporate it into my daily lifestyle".

10.3.1.8 Information about PCOS symptoms

Participants were asked about any symptoms they were experiencing and how they were concerned that these symptoms affected their lives. Weight gain was by far the most commonly reported symptom by 225/320 (70.3%) of the participants, followed by excess body hair (68%) and binge eating (63.8%), respectively. Similarly, over half (n=189/320 (59%)) of these participants reported weight gain as their primary concern, while just over a quarter (n= 90/320 (28%)) reported acne as the least worrying symptom (Table 20).

Table 21: The most reported symptoms among women with PCOS

Symptom	*Overall = n (%)*	*Major concern = n (%)*	*Minor concern = n (%)*
Irregular period (oligomenorrhea)	169 (52.8%)	128 (40%)	12 (3.8%)
Absence of period (amenorrhoea)	88 (27.5%)	71(22.2%)	18 (5.6%)
Excess body or facial hair (hirsutism)	218 (68%)	129 (40.3%)	19 (5.9%)
Acne (spots)	125 (39%)	67 (20.9%)	90 (28%)
Difficulty with fertility/ falling pregnant	119 (37.2%)	137 (42.8%)	25 (7.8%)
Weight gain/difficulty losing weight	225 (70.3%)	189 (59%)	43 (13.4%)
Mood-swings	165 (51.5%)	115 (35.9%)	77 (24%)
Male-pattern baldness	54 (16.9%)	44 (13.75%)	40 (12.5%)
Binge eating	204 (63.6%)	147 (45.9%)	71 (22.2%)

10.3.1.9 Information on medications

Metformin was by far the most common treatment prescribed for these women, with over a half (55%) already on metformin for a mean duration of 4.7±3.0 years. However, a considerable percentage were not on any kind of pharmacological treatment (Figure 10-7). Nearly a third of participants (31.5%) did not have enough information about available treatments for PCOS, while only 14/320 (4.4%) claimed to know

enough about it. However, most of the participants (79.7%) expressed their interest to know more about the pharmacological treatments available for PCOS.

Figure 10-7: Word clouds for the information on treatment

Participants were allowed to express themselves on what else they need to know with regards to the treatment of PCOS; the following themes were identified "in the participant's words":

10.3.1.9.1 Theme: PCOS treatment

10.3.1.9.1.1 Sub-theme: lack of information about PCOS treatment

"I don't feel like there is treatment, I paid to see a specialist privately, and the treatment is Metformin; however, that doesn't treat all the issues I have which relate to my PCOS".

"I researched my own weight loss treatment (low GI diet), but it would have been nice for alternative treatments to be provided from the Dr other than "go on the pill" or "lose weight" (and then not providing guidance for PCOS specific diets to help me lose weight)".

"Guidance and information on potential infertility before you are ready to conceive".

"I want to know everything. I should have to research it all myself. I want the information provided to me and healthcare professionals so together we can come up with treatment plans and be monitored and adjusted accordingly".

10.3.1.9.1.1.1 Information on metformin

"Why do they use metformin? The dangers of taking it and alternative treatments".

10.3.1.9.1.1.2 Information on fertility treatment

"Because I was on Depo Provera as a contraceptive, I had no periods and haven't since starting that 20 years ago. I was only diagnosed by chance when I had visited the GP, thinking I was perimenopausal, and my blood showed that I had PCOS. I appreciate that I was diagnosed during the pandemic, but I have had no information and support with I since they realised I was a too high risk to take the combined pill, which they did prescribe for me until I queried it due to my age and high BMI after reading about it on the internet".

10.3.1.9.1.2 Sub-theme: the lack of offering effective PCOS treatment

"None currently, but before having children, I was on the contraceptive pill from age 16 until age 37. It's odd to be experiencing a normal cycle so late into adulthood".

"I am not on any medications; however, I do have the marina coil to stop the heavy periods I was having that would last many months then go away for many months".

"Previously on the pill but have had to stop taking this die to try to get pregnant".

"Not on any medication but taking a few supplements".

"I am no longer on any prescribed medication. I follow the natural route and take Inositol, Magnesium, Saw Palmetto and Vit D3".

"Not currently on medication. Have taken Metformin in the past".

"Was metformin for years I stopped after I had my son as decided I wanted to give the body a break, and the doctors didn't seem to know much about taking it forever".

10.3.1.10 Information on response to treatment

Participants were also asked how they felt after being offered treatment and whether this affected their symptoms. Overall, a very small proportion of participants felt that their symptoms improved after treatment. Conversely, a great proportion of participants were dissatisfied with the treatment offered to

them. Only 13/320 (4%) participants managed to lose weight, while 135/320 participants (42.18%) admitted they had not been able to lose weight effectively (Table 22).

Table 22: Response to management among women with PCOS

Symptoms	***Improved = n (%)***	***Not improved = n (%)***
Irregular period (oligomenorrhea)	49 (15.3%)	91 (28.4%)
Absence of period (amenorrhoea)	50 (15.6%)	93 (29%)
Excess body or facial hair (hirsutism)	20 (6.25%)	146 (45.6%)
Acne (spots)	20 (6.25%)	105 (32.8%)
Difficulty with fertility/ falling pregnant	28 (8.75%)	129 (40.3%)
Weight gain/difficulty losing weight	13 (4%)	135 (42.18%)
Mood-swings	20 (6.25%)	115 (35.9%)
Male-pattern baldness	15 (4.68%)	105 (32.8%)
Binge eating	12 (3.75%)	124 (38.75%)

When women were allowed to express themselves, the following themes were identified:

10.3.1.10.1 Theme: response to treatment

10.3.1.10.1.1 The adverse event associated with PCOS treatment

"Anything! Metformin didn't make any impact other than cause a very aggressive stomach reaction for months".

"Currently not taking anything as it upset my stomach when they upped my dosage, but I was taking metformin for a year, currently taking supplements to try and help like inositol".

"Not just giving out the contraceptive pill as your only method to "manage" your period".

10.3.1.11 Long-term monitoring and health checks available for PCOS

When women were asked whether they would like to know more about the long-term monitoring and health checks available for PCOS, including checks for diabetes, heart problems, infertility, and cancer, 295/320 (92.2%) expressed their willingness to know more about this topic, only 6/320 (1.9%) said they are not keen to know about the long-term monitoring. The following are the most identified theme from the participant's response regarding the long-term monitoring:

10.3.1.11.1 Theme: lack of long-term monitoring

"Since being diagnosed nearly seven years ago, I have not been offered any check-ups or monitoring. All the Drs told me was to come back when I wanted to try for children or if I thought my pelvic pain was getting worse. I had to figure out everything else either by myself or through the help of Verity, the PCOS charity".

"I feel like the doctors don't care, they do one blood test a year, but I never am asked how I'm managing, for example, my last period was over 5 months ago, I struggle with weight, but there is no information from them as what I should do, I just feel like I'm left to struggle with it alone, especially as I was told when I was first diagnosed to look up what it was on the internet and to come back when I wanted to have children, it's upsetting and infuriating".

"I have not been offered any long term monitoring for my PCOS. I find that when I do make appointments to discuss any particular issues (irregular periods, insulin resistance), I feel as if I am wasting the GPS time as they are expected issues related to PCOS and don't find I am given any help in managing them".

"It's in my file at the doctor's, but there's never been a mention of monitoring basically (I feel) because my BMI is normal".

10.3.1.12 Experience living with PCOS

When participants were asked to express their feeling regarding their experience living with PCOS, the majority reported that they felt worried, embarrassed, helpless and depressed (Figure 10-8).

Figure 10-8: Word clouds on the experience living with PCOS

The following are the most common identified themes "in the participant's words" about the women's experience of living with PCOS:

10.3.1.12.1 Theme: experience living with PCOS

10.3.1.12.1.1 Adapt living with PCOS

"I'm currently just living with it: full symptoms, no treatment, no lifestyle changes. One of your previous mentions knowledge from an expert (GP) GPs are NOT experts. Hardly any have Any useful information to give and just tell you to lose weight. So I just live with it because I can't be bothered to stress about fighting for alternative treatments".

10.3.1.12.1.2 Struggling to live with PCOS

"It is hard living with it. It has affected my social life, my family life, my mental health, my work. The professionals I've seen haven't got enough knowledge on it, which has meant I have spent so much time and money researching and reading up on it for myself to understand my condition better, which has severely affected my mental health".

10.3.1.12.1.3 Sub-theme: PCOS impact on daily life

"It has a daily life impact, especially when on medication. It can affect where you go, who with, preparations for clothes, how you feel, disheartening and when a cyst ruptures - words do not explain the pain and sweats and contemplation for hospital every time".

"It's not just a case of having irregular/absent periods, and not being able to conceive - there is far more to it than that. It's a serious, long term condition that still doesn't get enough recognition".

10.3.1.12.1.4 The psychological impact of PCOS

10.3.1.12.1.4.1 Depression

"I have spent my life being conscious of facial hair and being overweight - sometimes feeling depressed about it. Never found a GP who is interested or knowledgeable".

10.3.1.12.1.4.2 Frustration

"It's frustrating; nobody seems to know anything. Every problem I go to GPS about (weight, ovary pain, hair loss) seems to boil down to these words " it's probably just because you have PCOS, we can run a test if you want, but it's more than likely something you'll have to learn to live with as it's a symptom of your condition." having Pcos is tiring, you get dismissed all the time, especially if you aren't trying for children. It's like nobody cares about your treatment or symptoms etc. unless you're trying to reproduce. Just a list

of steps on what to try or what to do or whom to talk to would have been a godsend not only for younger me but me now".

"For a long time, I got very frustrated with my periods. It was regular or sometimes too regular. The amount of pain was unbearable to the point where I couldn't walk. Or I would be screaming curled up in a ball on the floor. I would also like to know how my PCOS is affected by contraception and the contraceptive pill and conceptive implant. Since having the implant, it has greatly reduced the pain and heaviness of my period. It has made it, so I have 2 a month. This is bothersome to a point, but I am dealing with it and managing it as the benefits outweigh the negative. I still want to know what the effect of the hormone has on my own PCOS hormone level. Why do I have 2 periods a month, and if that reduces my chances of conceiving a child in the future. Does my PCOS mean I have to start thinking about children soon? Or chances for freezing eggs. Etc".

10.3.1.12.1.4.3 Lack of confidence

"It hugely impacts on your confidence and moods. I felt quite depressed and time and helpless".

"I feel like I don't belong in this world, I don't feel like a woman. It's been a very lonely life living with PCOS".

10.3.1.12.1.4.4 Low self-esteem

"It's been a nightmare. Diagnosed at 15, then undiagnosed in my 20s. Now in my 30s, having absent and horrendously heavy periods and struggling with infertility. The only thing that a go has ever said was to lose weight and try just eating 400 calories per day. I've lived with excess body hair, obesity, disordered eating, low self-esteem and depression for many years with no support. Please don't let this happen to others".

Overall, women with PCOS were very keen to know more about the long-term monitoring for the health problems associated with PCOS and the available treatment. On the other hand, more information concerning PCOS symptoms, diagnosis, and effectively losing weight was the least favourite among women with PCOS. Figure 10-9.

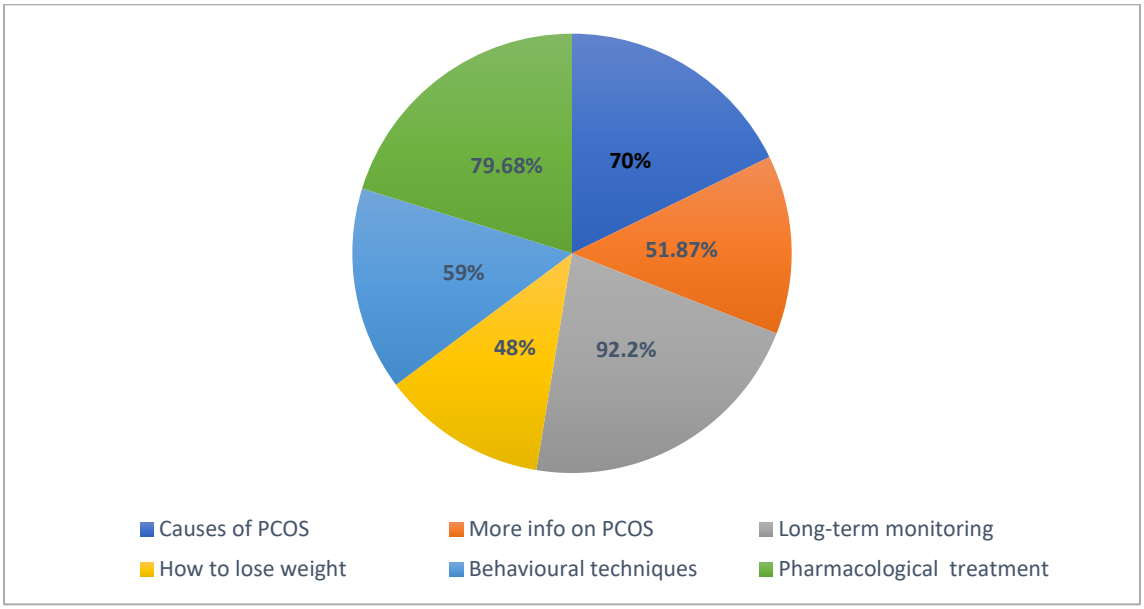


Figure 10-9: patient's perspectives on developing an educational programme for PCOS

10.3.2 Results of the pilot study of the educational intervention

Thirty participants from our PCOS clinic were recruited for the pilot study, of which 11/30 (36.6%) attended the face-to-face educational session, and 19/30 (63.4%) completed the educational session online (self-directed study) after signing a written informed consent form. The online session was delivered to mitigate Covid-19 regulations. Before starting the session, women were given a pre-pilot evaluation form designed to capture their expectations, knowledge and perceptions before participating in the programme. They were given opportunities to ask questions, discuss and share their experience during the session. By the end of the session, a post-pilot questionnaire that also captured their satisfaction, knowledge, skills and illness perceptions were given to participants. Their analysis is as follow:

10.3.2.1 Expectations and satisfaction

Before the beginning of the session, 15/30 (50%) of the participants were expected the session to be beneficial, 11/30 (36.6%) participants expected the session to be engaging, and 19/30 (63.4%) participants expected the session to be informative. By the end of the session, 11/30 (36.6%) of the participants claimed it was effortless to access the programme, 12/30 (40%) very satisfied, 9/30 (30%) satisfied, and only 1/30(3%) was not satisfied with the delivery of the session. Only 5/30 (16.7%) said the duration was perfect, 10/30 (33.3%) said the duration of the session was just right, and 14/30 (46.7%) rated the time of the session as very long. When participants were asked about the quality of the presented materials, 17/30 (56.6%) were very satisfied with the quality of the educational materials, and 13/30 (46.6%) said the message was very clear. Overall, 15/30 (50%) of the participants were very satisfied, 8/30 (26.6%) were satisfied, and only 5/30 (16.7%) were not satisfied with the educational session.

10.3.2.2 Knowledge about PCOS

Before the start of the session, 20/30 (66.7%) of the participants did not have enough knowledge about PCOS and how it is diagnosed. There was no statistically significant difference in participant's knowledge about PCOS and its related symptoms, long-term consequences and the current PCOS treatment before and after the educational session. Table 23.

Table 23: PCOS knowledge evaluation

Variables	***Pre-pilot N (%)***	***Post-pilot N(%)***	***(p- value)***
What PCOS is, and how is it diagnosed?	20/30 (66.7%)	22/30 (73.3%)	*p = 0.64*
PCOS symptoms	12/30 (40%)	15/30 (50%)	*p = 0.193*
Long-term consequences	5/30 (16.6%)	9/30 (30%)	*p= 0.496*
Current treatment options	4/30 (13.3%)	6/30 (20%)	*p = 1.0*

10.3.2.3 Skills development

Only 2/30 (6.6%) of participants have enough knowledge on the behavioural strategies of how to lose weight or to maintain weight loss. Similarly, only 3/30 (10%) of the women knew about healthy food choices, balanced diets and physical activities that help lose weight. However, after the educational session, 13/30 (43.3%) of the participants gained skills in setting goals, and 17/30 (56.6%) gained self-monitoring and self-efficacy skills.

10.3.2.4 Illness perception

When women were asked about how often they think about their illness, 14/30 (46.6%) reported that they were frequently thinking about their condition, 7/30(23.3%) were occasionally thinking, while 9/30(30%) claimed they do not think much about it. Most participants believed that stress and environmental factors were the leading cause of their condition (36.60% versus 33.30%, respectively). Figure 10-10. Nearly half (46.6%) of the participants believed their condition was permanent and would be with them for the rest of their lives. While 33.3% think their condition will lead to significant health consequences in their lives. On the other hand, only 6.6% of participants believed their condition was manageable or would improve with time, Figure 10-11.

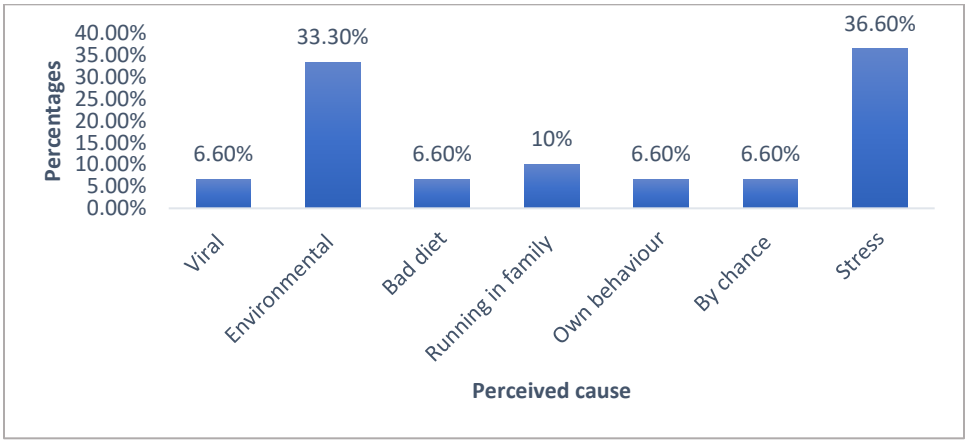


Figure 10-10: The perceived causes of PCOS

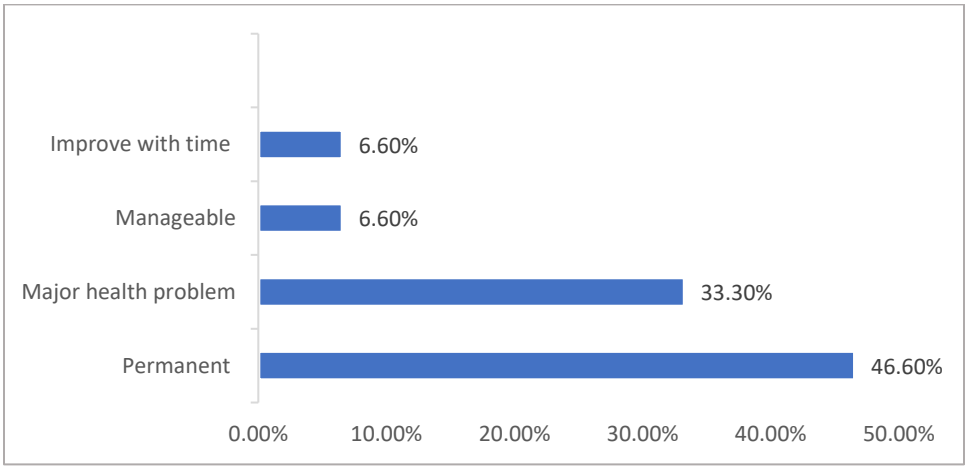


Figure 10-11: Illness perception among women with PCOS

10.4 Discussion

The study found an increase in willingness for women to know more about PCOS. Information regarding the PCOS symptoms, PCOS-related long-term health consequences and behavioural strategies for effectively losing weight or maintaining weight loss were highly valued. However, the study also identified a hesitancy among clinicians in diagnosing PCOS early and a delay in offering effective treatment.

To our knowledge, there are very few structured education studies for women with PCOS that address their complex needs. Therefore, direct comparisons between the existing literature are somewhat

limited. However, in the SUCCESS study, a single-centre structured education for women with PCOS found that a single exposure to a structured education programme improved women's understanding of their condition and quality of life. The SUCCESS study was mainly focused on how structured education will improve the QOL and improve physical activity in women with PCOS. However, it did not improve the level of their physical activity, biochemical markers and BMI (358).

Studies of lifestyle interventions in women with PCOS have mixed outcomes. A systematic review and meta-analysis of randomised controlled trials comparing lifestyle interventions including diet, physical activity and behavioural therapy, concluded that lifestyle intervention did improve body weight (MD -3.47 kg [95% CI] -4.94 to -2.00, $P < 0.00001$), waist circumference (MD -1.95 cm [95% CI] -3.34 to -0.57, $P = 0.006$) and androgen level (MD -0.27 nmol/L, [95% CI] -0.46 -0.09, $P = 0.004$) with no effect on quality of life and treatment satisfaction (850). A study of women allocated for six months supervised exercise programme showed a significant decrease in homocysteine level, a marker for atherosclerosis (851).

A recent survey of 493 women with PCOS evaluating the perceptions and the experience of lifestyle interventions reported that even though 83% of the respondents adjusted their physical activity and diet to improve their health, only 13% achieved these goals (852). Structured education for other conditions showed a significant improvement in the quality of life. A structured group education programme for newly diagnosed patients with type 2 diabetes showed a significant improvement in the beliefs about the illness but not in the biochemical parameters (853). A feasibility study of improving physical activity in obese osteoarthritic adults without PCOS showed a significant improvement in self-efficacy in performing lower-extremity exercise (854).

In the current study, most women reported feeling “lost, helpless, frustrated, ashamed and annoyed” living with PCOS. This, however, highlights the significant psychological impact that women with PCOS experience and indicate the lack of support for these women. We also reported the delay in the diagnosis of PCOS. In a cross-sectional study of 1385 women with PCOS, 33.6% of participants waited more than two years and saw more than three healthcare professionals before the PCOS was diagnosed. Only 32.5% were satisfied with their diagnosis experience, and 15.6% were happy with the information received (843). In our study, we report that 13 (43.3%) of the participants gained the skill of how to set goals and 17 (56.6%) gained self-monitoring skills. In a narrative review, incorporating behavioural and psychological strategies including goal setting and self-monitoring improved weight management outcomes in women with PCOS (855). These psychological factors are significant barriers to successful management in PCOS. Thus, it is imperative to address such barriers. Therefore, it is expected that introducing an education programme similar to ours during the diagnosis of PCOS could improve psychological well-being and improve satisfaction among women with PCOS. In the current study we did not find any statistically significant difference in participant’s knowledge about PCOS and its related symptoms, long-term consequences and the current PCOS treatment before and after the educational session. This could be due to how sensitive is talking about PCOS among young women particularly in a group gathering because they are not familiar with each others. However, this could be overcomed with implementing more than one group session to allow for acclimatisation.

10.4.1 Strength and limitations

The study emphasised the feasibility of implementing an education programme for women with PCOS, perhaps at diagnosis. However, the study also affirmed the delay in diagnosing PCOS and the hesitancy to offer effective treatment for women with PCOS. A limitation to the study is that it is a pilot study, and

a more definitive study with large sample size and longer duration is needed. We also found a significant difference in the response rate for women who took the face-to-face education programme and the once who did self-directed online study. This, however, reflects the impact of covid-19 on the acceptance of face-to-face meetings.

10.5 Conclusion and clinical implication

The study outcomes assert the importance of providing information in a structured patient-centred approach and should be offered as part of PCOS management. Implementing a single exposure to an educational programme could provide valuable skills to women with PCOS. Thus, it will help them to live with their condition.

10.5.1 Future direction

As a next step, we plan to apply to fund a more definitive study, and if found beneficial, we will roll out this programme for all women with PCOS. We also plan to set up a PCOS support group to support women in the region. The study also identified the lack of a validated tool to properly evaluate PCOS women's perspectives and experience living with the condition. Therefore, a future study adopting validated assessment tools is needed.

11 Chapter 11: General summary and future directions

11.1 General summary

PCOS is one of the most prevalent endocrine disorders, particularly in women of reproductive age (20). PCOS is characterised by excess androgen, the condition's hallmark and the main driver for its clinical and biochemical manifestations (58). Besides the reproductive dysfunction, PCOS is also associated with an array of metabolic disturbances, including insulin resistance, increased body weight, increased inflammatory markers, impaired glucose tolerance, impaired QoL, abnormal lipid profiles and increased risk of T2DM and CVD (406). Currently, the recommendation from the international evidence-based guideline for the assessment and management of PCOS is to increase the focus on education, lifestyle intervention, emotional well-being, and the QoL of women with PCOS. It also emphasised the importance of evidence-based medical therapy (707). Accordingly, lifestyle modification, including diet and physical activity, is the first-line management for PCOS. However, this is beyond the scope of this thesis.

On the other hand, pharmacological management is the second-line therapy usually used to treat fertility issues, excess androgen and reduce the long-term consequences of PCOS (96). Although these pharmacological interventions have been evaluated in several small-sized RCTs and their actual impacts on PCOS remain elusive. Therefore, this thesis aimed to evaluate the impact of the different pharmacological treatments in PCOS management.

In this research work, the use of atorvastatin, metformin, saxagliptin, rosiglitazone, and pioglitazone as monotherapy or add-on therapy with various dosage, duration and frequencies were associated with a significant reduction in the parameters of lipid profiles. There was also a reduction in the level of CRP. These findings highlight the importance of the effective use of the various agents targeting dyslipidaemia

and CRP in PCOS, an effect meant to reduce the substantial CVD risk associated with PCOS. Women with PCOS also suffer from insulin resistance; it has been estimated that 50-70% of women with PCOS have insulin resistance which increases the risk of T2DM and impaired glucose tolerance (506). Administering various pharmacological interventions including metformin, acarbose, pioglitazone and exenatide alone or as add-on therapy of various dosage, duration or frequencies were associated with a significant reduction in the mean parameters of the insulin resistance, including FBG, FI and HOMA-IR. However, the thesis did not establish any significant effect for these agents on the mean HOMA-B. This also emphasises the need to effectively address insulin resistance in women with PCOS by using these agents as a standalone treatment or in combination.

Increased body weight is a prominent feature of PCOS, and around 50% of women with PCOS are either overweight or obese (706). Obesity aggravates PCOS features such as excessive hair growth, infertility, pregnancy complications and insulin resistance, culminating in an increased metabolic risk associated with PCOS (78). Therefore, tackling body weight issues in PCOS is not just improving the fertility outcomes but also preventing the long-term health risk associated with PCOS and will improve the QoL of women with PCOS. Therapeutic options such as metformin, orlistat, acarbose and sitagliptin of various dosages and for different durations and frequencies had a significant impact on the anthropometric indices of women with PCOS. They were all associated with a significant reduction in the mean body weight, BMI, WC and WHR. Conversely, pioglitazone and rosiglitazone of various dosage, duration and frequencies both alone and combined with other therapies showed a significant increase in the anthropometric indices in women with PCOS. Therefore, the thesis interrogated the use of pioglitazone and rosiglitazone particularly in overweight and obese women with PCOS.

Hyperandrogenism is one of three diagnostic criteria for its diagnosis, and common features for PCOS include anovulation, menstrual irregularity, acne and hirsutism (66, 738). Therefore, management strategies for PCOS are primarily based on managing the androgen-related symptoms. Therapeutic options including metformin, dexamethasone, flutamide, finasteride and the combined oral contraceptives in various dosage duration and frequencies significantly reduced the androgen hormones in PCOS. The use of these pharmacological agents has subsequently been associated with an improvement in PCOS associated symptoms. However, the thesis did not find any significant effect of rosiglitazone, pioglitazone, cabergoline or statins on the level of androgen hormones in women with PCOS.

Fertility problems in PCOS account for up to 80% of anovulatory infertility (110, 506). Fertility treatments such as letrozole and CC alone or added to metformin have effectively increased the ovulation, pregnancy and live birth rates compared with other treatments. Therefore, in infertile women with PCOS seeking conception, these pharmacological agents are tangible options.

Moreover, in the second section of the studies, the thesis also finds a gap in the PCOS knowledge among the vast majority of women with PCOS. Thus, the thesis also asserts the need for integrating education into the management plan for PCOS. Implementing a single exposure to an educational programme added valuable skills to women with PCOS, which will help them live with their condition.

11.2 Future directions

This research work assessed and evaluated the effects of the different pharmacological interventions and the prospect of integrating a structured education in PCOS management. The research has demonstrated the effects of the various therapeutic agents in improving PCOS related symptoms, and the PCOS related long-term complications. However, most of the RCTs included in the meta-analysis were of a small sample size, and there was significant heterogeneity amongst the included studies. Thus, further large scale and properly designed RCTs for the various pharmacological interventions used to manage PCOS are needed. The thesis also highlights the need for a comparative analysis for the differing therapeutic agents used in PCOS management. To do this, network meta-analysis after properly assessing for heterogeneity would be recommended.

While research work found that implementing a structured education will provide valuable skills necessary to help live with PCOS, there is a lack of support for women living with PCOS. As a result, the thesis accentuates the need for setting up a local support group for women with PCOS. A future definitive trial for structured education is needed. Overall, there is significantly conflicting evidence about the genuine global, regional and local prevalence of women with PCOS. Consequently, establishing a local database platform for women with PCOS is highly recommended. The author acknowledges the obstacles this idea might face; however, beginning locally is a starting point.

12 Chapter 12: References

1. Kumarendran B, O'Reilly MW, Manolopoulos KN, Toulis KA, Gokhale KM, Sitch AJ, et al. Polycystic ovary syndrome, androgen excess, and the risk of nonalcoholic fatty liver disease in women: A longitudinal study based on a United Kingdom primary care database. PLoS Med. 2018;15(3):e1002542.
2. Vallisnieri A. Istoria Della Generazione Dell'Uomo, E Degli Animali, Se Sia Da'Vermicelli Spermatici, O Dalle Uova... Di Antonio Vallisneri... Alla Sacra Cesarea Real Cattolica Maestà di Carlo VI. Imperador De'Romani, Re di Germania, delle Spagne, d'Ungheria, e di Boemia, Arciduca d'Austria, & Con Un Trattato Nel Fine Della Sterilita. & Con La Critica de'Superflui: Hertz; 1721.
3. Chéreau DA. Mémoires pour servir à l'étude des maladies des ovaires. Premier mémoire contenant: 1° les considérations anatomiques et physiologiques; 2° l'agénésie et les vices de conformation des ovaires; 3° l'inflammation aiguë des ovaires, ovarite aiguë, par Achille Chéreau: Fortin, Masson; 1844.
4. Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. American Journal of Obstetrics and Gynecology. 1935;29(2):181-91.
5. Greenblatt RB. Cortisone in treatment of the hirsute woman. Obstetrical & Gynecological Survey. 1955;10(2):237-9.
6. Jones GE, Howard J, Langford H. The use of cortisone in follicular phase disturbances. Fertility and Sterility. 1953;4(1):49-62.
7. Mc AJ, Ingersoll FM, Worcester J. The urinary excretion of interstitial-cell and follicle-stimulating hormone activity by women with diseases of the reproductive system. J Clin Endocrinol Metab. 1958;18(11):1202-15.
8. Yalow RS, Berson SA. Immunoassay of endogenous plasma insulin in man. J Clin Invest. 1960;39:1157-75.
9. Ferriman D, Gallwey J. Clinical assessment of body hair growth in women. The Journal of Clinical Endocrinology & Metabolism. 1961;21(11):1440-7.
10. Yen SS, Vela P, Rankin J. Inappropriate secretion of follicle-stimulating hormone and luteinizing hormone in polycystic ovarian disease. J Clin Endocrinol Metab. 1970;30(4):435-42.
11. Burghen GA, Givens JR, Kitabchi AE. Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease. J Clin Endocrinol Metab. 1980;50(1):113-6.
12. Reaven GM. Role of insulin resistance in human disease (syndrome X): an expanded definition. Annu Rev Med. 1993;44:121-31.
13. Sharpless JL. Polycystic ovary syndrome and the metabolic syndrome. Clinical Diabetes. 2003;21(4):154-61.
14. Daniilidis A, Dinas K. Long term health consequences of polycystic ovarian syndrome: A review Analysis. Hippokratia. 2009;13(2):90-2.
15. Acmaz G, Albayrak E, Acmaz B, Baser M, Soyak M, Zararsiz G, et al. Level of anxiety, depression, self-esteem, social anxiety, and quality of life among the women with polycystic ovary syndrome. Scientific World Journal. 2013;2013:851815.
16. Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod. 2016;31(12):2841-55.
17. Palep-Singh M, Picton HM, Vrotsou K, Maruthini D, Balen AH. South Asian women with polycystic ovary syndrome exhibit greater sensitivity to gonadotropin stimulation with reduced fertilization and ongoing pregnancy rates than their Caucasian counterparts. Eur J Obstet Gynecol Reprod Biol. 2007;134(2):202-7.
18. Ding T, Baio G, Hardiman PJ, Petersen I, Sammon C. Diagnosis and management of polycystic ovary syndrome in the UK (2004-2014): a retrospective cohort study. BMJ Open. 2016;6(7):e012461.
19. Michelmore KF, Balen AH, Dunger DB, Vessey MP. Polycystic ovaries and associated clinical and biochemical features in young women. Clin Endocrinol (Oxf). 1999;51(6):779-86.
20. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metab. 2004;89(6):2745-9.

21. Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R. Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. J Clin Endocrinol Metab. 1998;83(9):3078-82.
22. Asuncion M, Calvo RM, San Millan JL, Sancho J, Avila S, Escobar-Morreale HF. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. J Clin Endocrinol Metab. 2000;85(7):2434-8.
23. Diamanti-Kandarakis E, Kouli CR, Bergiele AT, Filandra FA, Tsianateli TC, Spina GG, et al. A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. J Clin Endocrinol Metab. 1999;84(11):4006-11.
24. March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod. 2010;25(2):544-51.
25. Boyle JA, Cunningham J, O'Dea K, Dunbar T, Norman RJ. Prevalence of polycystic ovary syndrome in a sample of Indigenous women in Darwin, Australia. Med J Aust. 2012;196(1):62-6.
26. Kumarapeli V, Seneviratne Rde A, Wijeyaratne CN, Yapa RM, Dodampahala SH. A simple screening approach for assessing community prevalence and phenotype of polycystic ovary syndrome in a semi-urban population in Sri Lanka. Am J Epidemiol. 2008;168(3):321-8.
27. Chen X, Yang D, Mo Y, Li L, Chen Y, Huang Y. Prevalence of polycystic ovary syndrome in unselected women from southern China. Eur J Obstet Gynecol Reprod Biol. 2008;139(1):59-64.
28. Vutyavanich T, Khaniyao V, Wongtra-Ngan S, Sreshthaputra O, Sreshthaputra R, Piromlertamorn W. Clinical, endocrine and ultrasonographic features of polycystic ovary syndrome in Thai women. J Obstet Gynaecol Res. 2007;33(5):677-80.
29. Azziz R. Diagnostic criteria for polycystic ovary syndrome: a reappraisal. Fertil Steril. 2005;83(5):1343-6.
30. Rotterdam EA-SPCWG. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004;81(1):19-25.
31. Rotterdam EA-SPcwg. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19(1):41-7.
32. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril. 2009;91(2):456-88.
33. Lujan ME, Chizen DR, Pierson RA. Diagnostic criteria for polycystic ovary syndrome: pitfalls and controversies. J Obstet Gynaecol Can. 2008;30(8):671-9.
34. Azziz R. Controversy in clinical endocrinology: diagnosis of polycystic ovarian syndrome: the Rotterdam criteria are premature. J Clin Endocrinol Metab. 2006;91(3):781-5.
35. Campbell S. A short history of sonography in obstetrics and gynaecology. Facts Views Vis Obgyn. 2013;5(3):213-29.
36. Nikokavoura EA, Johnston KL, Broom J, Wrieden WL, Rolland C. Weight loss for women with and without polycystic ovary syndrome following a very low-calorie diet in a community-based setting with trained facilitators for 12 weeks. Diabetes Metab Syndr Obes. 2015;8:495-503.
37. Chang WY, Knochenhauer ES, Bartolucci AA, Azziz R. Phenotypic spectrum of polycystic ovary syndrome: clinical and biochemical characterization of the three major clinical subgroups. Fertil Steril. 2005;83(6):1717-23.
38. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. J Clin Endocrinol Metab. 2006;91(11):4237-45.
39. Hahn S, Tan S, Elsenbruch S, Quadbeck B, Herrmann BL, Mann K, et al. Clinical and biochemical characterization of women with polycystic ovary syndrome in North Rhine-Westphalia. Horm Metab Res. 2005;37(7):438-44.

40. Lowenstein EJ. Diagnosis and management of the dermatologic manifestations of the polycystic ovary syndrome. Dermatol Ther. 2006;19(4):210-23.
41. Azziz R, Sanchez LA, Knochenhauer ES, Moran C, Lazenby J, Stephens KC, et al. Androgen excess in women: experience with over 1000 consecutive patients. J Clin Endocrinol Metab. 2004;89(2):453-62.
42. Wijeyaratne CN, Balen AH, Barth JH, Belchetz PE. Clinical manifestations and insulin resistance (IR) in polycystic ovary syndrome (PCOS) among South Asians and Caucasians: is there a difference? Clin Endocrinol (Oxf). 2002;57(3):343-50.
43. Khan MJ, Ullah A, Basit S. Genetic basis of polycystic ovary syndrome (PCOS): current perspectives. Appl Clin Genet. 2019;12:249-60.
44. Jahanfar S, Eden JA, Warren P, Seppala M, Nguyen TV. A twin study of polycystic ovary syndrome. Fertil Steril. 1995;63(3):478-86.
45. Vink JM, Sadrzadeh S, Lambalk CB, Boomsma DI. Heritability of polycystic ovary syndrome in a Dutch twin-family study. J Clin Endocrinol Metab. 2006;91(6):2100-4.
46. Franks S, Gilling-Smith C, Gharani N, McCarthy M. Pathogenesis of polycystic ovary syndrome: evidence for a genetically determined disorder of ovarian androgen production. Hum Fertil (Camb). 2000;3(2):77-9.
47. Gharani N, Waterworth DM, Batty S, White D, Gilling-Smith C, Conway GS, et al. Association of the steroid synthesis gene CYP11a with polycystic ovary syndrome and hyperandrogenism. Hum Mol Genet. 1997;6(3):397-402.
48. Lamminen T, Huhtaniemi I. A common genetic variant of luteinizing hormone; relation to normal and aberrant pituitary-gonadal function. Eur J Pharmacol. 2001;414(1):1-7.
49. Liu N, Ma Y, Wang S, Zhang X, Zhang Q, Zhang X, et al. Association of the genetic variants of luteinizing hormone, luteinizing hormone receptor and polycystic ovary syndrome. Reprod Biol Endocrinol. 2012;10:36.
50. Hogeveen KN, Cousin P, Pugeat M, Dewailly D, Soudan B, Hammond GL. Human sex hormone-binding globulin variants associated with hyperandrogenism and ovarian dysfunction. J Clin Invest. 2002;109(7):973-81.
51. Siegel S, Futterweit W, Davies TF, Concepcion ES, Greenberg DA, Villanueva R, et al. AC/T single nucleotide polymorphism at the tyrosine kinase domain of the insulin receptor gene is associated with polycystic ovary syndrome. Fertility and Sterility. 2002;78(6):1240-3.
52. Diamanti-Kandarakis E, Palioniko G, Alexandraki K, Bergiele A, Koutsouba T, Bartzis M. The prevalence of 4G5G polymorphism of plasminogen activator inhibitor-1 (PAI-1) gene in polycystic ovarian syndrome and its association with plasma PAI-1 levels. European Journal of Endocrinology. 2004;150(6):793-8.
53. Rutkowska AZ, Diamanti-Kandarakis E. Polycystic ovary syndrome and environmental toxins. Fertil Steril. 2016;106(4):948-58.
54. Soave I, Occhiali T, Assorgi C, Marci R, Caserta D. Environmental toxin exposure in polycystic ovary syndrome women and possible ovarian neoplastic repercussion. Curr Med Res Opin. 2020;36(4):693-703.
55. Witchel SF, Oberfield SE, Pena AS. Polycystic ovary syndrome: pathophysiology, presentation, and treatment with emphasis on adolescent girls. J Endocr Soc. 2019;3(8):1545-73.
56. Wu Y, Zhong G, Chen S, Zheng C, Liao D, Xie M. Polycystic ovary syndrome is associated with anogenital distance, a marker of prenatal androgen exposure. Hum Reprod. 2017;32(4):937-43.
57. Pan Z, Zhu F, Zhou K. A systematic review of anogenital distance and gynecological disorders: endometriosis and polycystic ovary syndrome. Front Endocrinol (Lausanne). 2021;12:696879.
58. Baskind NE, Balen AH. Hypothalamic-pituitary, ovarian and adrenal contributions to polycystic ovary syndrome. Best Pract Res Clin Obstet Gynaecol. 2016;37:80-97.
59. Liao B, Qiao J, Pang Y. Central regulation of PCOS: abnormal neuronal-reproductive-metabolic circuits in PCOS pathophysiology. Front Endocrinol (Lausanne). 2021;12:667422.
60. Rosenfield RL, Ehrmann DA. The pathogenesis of polycystic ovary syndrome (PCOS): The hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocr Rev. 2016;37(5):467-520.
61. Li T, Zhang T, Cui T, Yang Y, Liu R, Chen Y, et al. Involvement of endogenous testosterone in hepatic steatosis in women with polycystic ovarian syndrome. J Steroid Biochem Mol Biol. 2020;204:105752.

62. Grymowicz M, Rudnicka E, Podfigurna A, Napierala P, Smolarczyk R, Smolarczyk K, et al. Hormonal effects on hair follicles. Int J Mol Sci. 2020;21(15).
63. Abbott DH, Dumesic DA, Levine JE. Hyperandrogenic origins of polycystic ovary syndrome - implications for pathophysiology and therapy. Expert Rev Endocrinol Metab. 2019;14(2):131-43.
64. Saadia Z. Follicle Stimulating Hormone (LH: FSH) ratio in Polycystic Ovary Syndrome (PCOS) - obese vs. non- obese women. Med Arch. 2020;74(4):289-93.
65. Deswal R, Nanda S, Dang AS. Association of luteinizing hormone and LH receptor gene polymorphism with susceptibility of polycystic ovary syndrome. Syst Biol Reprod Med. 2019;65(5):400-8.
66. Ye W, Xie T, Song Y, Zhou L. The role of androgen and its related signals in PCOS. J Cell Mol Med. 2021;25(4):1825-37.
67. Hammond GL, Wu TS, Simard M. Evolving utility of sex hormone-binding globulin measurements in clinical medicine. Curr Opin Endocrinol Diabetes Obes. 2012;19(3):183-9.
68. Cupisti S, Kajaia N, Dittrich R, Duezenli H, M WB, Mueller A. Body mass index and ovarian function are associated with endocrine and metabolic abnormalities in women with hyperandrogenic syndrome. Eur J Endocrinol. 2008;158(5):711-9.
69. Baglietto L, English DR, Hopper JL, MacInnis RJ, Morris HA, Tilley WD, et al. Circulating steroid hormone concentrations in postmenopausal women in relation to body size and composition. Breast Cancer Res Treat. 2009;115(1):171-9.
70. Lin XF, Wu RR, Du J, Liao YC, Du Y, Ye Y, et al. Exploring the significance of sex hormone-binding globulin examination in the treament of women with polycystic ovarian syndrome (PCOS). Clin Exp Obstet Gynecol. 2015;42(3):315-20.
71. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature. 2001;414(6865):799-806.
72. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev. 2012;33(6):981-1030.
73. Lee J, Kim MS. The role of GSK3 in glucose homeostasis and the development of insulin resistance. Diabetes Res Clin Pract. 2007;77 Suppl 1:S49-57.
74. Choi K, Kim YB. Molecular mechanism of insulin resistance in obesity and type 2 diabetes. Korean J Intern Med. 2010;25(2):119-29.
75. Sam S. Obesity and polycystic ovary syndrome. Obes Manag. 2007;3(2):69-73.
76. Rizk AY, Bedaiwy MA, Al-Inany HG. N-acetyl-cysteine is a novel adjuvant to clomiphene citrate in clomiphene citrate-resistant patients with polycystic ovary syndrome. Fertil Steril. 2005;83(2):367-70.
77. Gill S, Gemmell A, Colleran R, Bt Zanuri N, O'Brien H, Poobalan A. Does metformin combined with clomiphene citrate improve fertility related outcomes in clomiphene resistant women with PCOS: A systematic review. Middle East Fertility Society Journal. 2014;19(2):81-8.
78. Motta AB. The role of obesity in the development of polycystic ovary syndrome. Curr Pharm Des. 2012;18(17):2482-91.
79. Diamanti-Kandarakis E, Piperi C. Genetics of polycystic ovary syndrome: searching for the way out of the labyrinth. Hum Reprod Update. 2005;11(6):631-43.
80. Legro RS, Bentley-Lewis R, Driscoll D, Wang SC, Dunaif A. Insulin resistance in the sisters of women with polycystic ovary syndrome: association with hyperandrogenemia rather than menstrual irregularity. J Clin Endocrinol Metab. 2002;87(5):2128-33.
81. Carmina E, Legro RS, Stamets K, Lowell J, Lobo RA. Difference in body weight between American and Italian women with polycystic ovary syndrome: influence of the diet. Hum Reprod. 2003;18(11):2289-93.
82. Virtue S, Vidal-Puig A. Adipose tissue expandability, lipotoxicity and the metabolic syndrome--an allostatic perspective. Biochim Biophys Acta. 2010;1801(3):338-49.
83. Duleba AJ, Dokras A. Is PCOS an inflammatory process? Fertil Steril. 2012;97(1):7-12.

84. Escobar-Morreale HF, Villuendas G, Botella-Carretero JI, Sancho J, San Millan JL. Obesity, and not insulin resistance, is the major determinant of serum inflammatory cardiovascular risk markers in pre-menopausal women. Diabetologia. 2003;46(5):625-33.
85. Lefebvre P, Bringer J, Renard E, Boulet F, Clouet S, Jaffiol C. Influences of weight, body fat patterning and nutrition on the management of PCOS. Hum Reprod. 1997;12 Suppl 1:72-81.
86. Dokras A. Cardiovascular disease risk in women with PCOS. Steroids. 2013;78(8):773-6.
87. Guzick DS. Cardiovascular risk in PCOS. The Journal of Clinical Endocrinology & Metabolism. 2004;89(8):3694-5.
88. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004;364(9438):937-52.
89. Wild RA, Carmina E, Diamanti-Kandarakis E, Dokras A, Escobar-Morreale HF, Futterweit W, et al. Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. J Clin Endocrinol Metab. 2010;95(5):2038-49.
90. Mannerås-Holm L, Baghaei F, Holm G, Janson PO, Ohlsson C, Lönn M, et al. Coagulation and fibrinolytic disturbances in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2011;96(4):1068-76.
91. Christian RC, Dumesic DA, Behrenbeck T, Oberg AL, Sheedy PF, Fitzpatrick LA. Prevalence and predictors of coronary artery calcification in women with polycystic ovary syndrome. The Journal of Clinical Endocrinology & Metabolism. 2003;88(6):2562-8.
92. Sutton-Tyrrell K, Wildman R, Matthews K, Chae C, Lasley B, Brockwell S, et al. Sex-hormone-binding globulin and the free androgen index are related to cardiovascular risk factors in multiethnic premenopausal and perimenopausal women enrolled in the Study of Women Across the Nation (SWAN) Circulation. 2005; 111: 1242–1249. doi: 10.1161/01. CIR.
93. Talbott EO, Zborowski JV, Rager JR, Boudreaux MY, Edmundowicz DA, Guzick DS. Evidence for an association between metabolic cardiovascular syndrome and coronary and aortic calcification among women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2004;89(11):5454-61.
94. Christian RC, Dumesic DA, Behrenbeck T, Oberg AL, Sheedy PF, 2nd, Fitzpatrick LA. Prevalence and predictors of coronary artery calcification in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2003;88(6):2562-8.
95. Calderon-Margalit R, Siscovick D, Merkin SS, Wang E, Daviglus ML, Schreiner PJ, et al. Prospective association of polycystic ovary syndrome with coronary artery calcification and carotid-intima-media thickness: the coronary artery risk development in young adults women's study. Arterioscler Thromb Vasc Biol. 2014;34(12):2688-94.
96. Vryonidou A, Papatheodorou A, Tavridou A, Terzi T, Loi V, Vatalas IA, et al. Association of hyperandrogenemic and metabolic phenotype with carotid intima-media thickness in young women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2005;90(5):2740-6.
97. Oliver-Williams C, Vassard D, Pinborg A, Schmidt L. Risk of cardiovascular disease for women with polycystic ovary syndrome: results from a national Danish registry cohort study. Eur J Prev Cardiol. 2020.
98. Oliver-Williams C, Vassard D, Pinborg A, Schmidt L. Polycystic ovary syndrome as a novel risk factor for atrial fibrillation: results from a national Danish registry cohort study. Eur J Prev Cardiol. 2020:2047487320922927.
99. Shroff R, Kerchner A, Maifeld M, Van Beek EJR, Jagasia D, Dokras A. Young obese women with polycystic ovary syndrome have evidence of early coronary atherosclerosis. The Journal of Clinical Endocrinology & Metabolism. 2007;92(12):4609-14.
100. Zhou Y, Wang X, Jiang Y, Ma H, Chen L, Lai C, et al. Association between polycystic ovary syndrome and the risk of stroke and all-cause mortality: insights from a meta-analysis. Gynecol Endocrinol. 2017;33(12):904-10.

101. Wild S, Pierpoint T, McKeigue P, Jacobs H. Cardiovascular disease in women with polycystic ovary syndrome at long-term follow-up: a retrospective cohort study. Clin Endocrinol (Oxf). 2000;52(5):595-600.
102. Berni TR, Morgan CL, Rees DA. Women with polycystic ovary syndrome have an increased risk of major cardiovascular events: a population study. J Clin Endocrinol Metab. 2021;106(9):e3369-e80.
103. Merz CN, Shaw LJ, Azziz R, Stanczyk FZ, Sopko G, Braunstein GD, et al. Cardiovascular disease and 10-Year mortality in postmenopausal women with clinical features of polycystic ovary syndrome. J Womens Health (Larchmt). 2016;25(9):875-81.
104. Zhang J, Xu JH, Qu QQ, Zhong GQ. Risk of cardiovascular and cerebrovascular events in polycystic ovarian syndrome women: A meta-analysis of cohort studies. Front Cardiovasc Med. 2020;7:552421.
105. Wild RA, Carmina E, Diamanti-Kandarakis E, Dokras A, Escobar-Morreale HF, Futterweit W, et al. Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: A Consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. The Journal of Clinical Endocrinology & Metabolism. 2010;95(5):2038-49.
106. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, et al. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2013;98(12):4565-92.
107. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Erratum. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod. 2019;34(2):388.
108. Hart R. PCOS and infertility. Panminerva Med. 2008;50(4):305-14.
109. Melo AS, Ferriani RA, Navarro PA. Treatment of infertility in women with polycystic ovary syndrome: approach to clinical practice. Clinics (Sao Paulo). 2015;70(11):765-9.
110. Thessaloniki EA-SPCWG. Consensus on infertility treatment related to polycystic ovary syndrome. Fertil Steril. 2008;89(3):505-22.
111. Kaluzna M, Nomejko A, Slowinska A, Wachowiak-Ochmanska K, Pikosz K, Ziemnicka K, et al. Lower sexual satisfaction in women with polycystic ovary syndrome and metabolic syndrome. Endocr Connect. 2021;10(9):1035-44.
112. De Frene V, Verhofstadt L, Loeys T, Stuyver I, Buysse A, De Sutter P. Sexual and relational satisfaction in couples where the woman has polycystic ovary syndrome: a dyadic analysis. Hum Reprod. 2015;30(3):625-31.
113. Bhagavath B, Carson SA. Ovulation induction in women with polycystic ovary syndrome: an update. Am J Obstet Gynecol. 2012;206(3):195-8.
114. Imani B, Eijkemans MJ, te Velde ER, Habbema JD, Fauser BC. A nomogram to predict the probability of live birth after clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. Fertil Steril. 2002;77(1):91-7.
115. Brown J, Farquhar C, Beck J, Boothroyd C, Hughes E. Clomiphene and anti-oestrogens for ovulation induction in PCOS. Cochrane Database Syst Rev. 2009(4):Cd002249.
116. Kousta E, White D, Franks S. Modern use of clomiphene citrate in induction of ovulation. Human Reproduction Update. 1997;3(4):359-65.
117. Messinis IE. Ovulation induction: a mini review. Hum Reprod. 2005;20(10):2688-97.
118. Eijkemans MJC, Polinder S, Mulders AGMGJ, Laven JSE, Habbema JDF, Fauser BCJM. Individualized cost-effective conventional ovulation induction treatment in normogonadotrophic anovulatory infertility (WHO group 2). Human Reproduction. 2005;20(10):2830-7.
119. Palomba S, Falbo A, Zullo F. Management strategies for ovulation induction in women with polycystic ovary syndrome and known clomifene citrate resistance. Curr Opin Obstet Gynecol. 2009;21(6):465-73.
120. Marshall JC, Dunaif A. Should all women with PCOS be treated for insulin resistance? Fertil Steril. 2012;97(1):18-22.
121. DeUgarte CM, Bartolucci AA, Azziz R. Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. Fertil Steril. 2005;83(5):1454-60.

122. Thessaloniki EA-SPCWG. Consensus on infertility treatment related to polycystic ovary syndrome. Hum Reprod. 2008;23(3):462-77.
123. Smith V, Osianlis T, Vollenhoven B. Prevention of ovarian hyperstimulation syndrome: A Review. Obstet Gynecol Int. 2015;2015:514159.
124. White DM, Polson DW, Kiddy D, Sagle P, Watson H, Gilling-Smith C, et al. Induction of ovulation with low-dose gonadotropins in polycystic ovary syndrome: an analysis of 109 pregnancies in 225 women. J Clin Endocrinol Metab. 1996;81(11):3821-4.
125. Farquhar C, Lilford RJ, Marjoribanks J, Vandekerckhove P. Laparoscopic 'drilling' by diathermy or laser for ovulation induction in anovulatory polycystic ovary syndrome. Cochrane Database Syst Rev. 2007(3):CD001122.
126. Farquhar C, Lilford RJ, Marjoribanks J, Vandekerckhove P. Laparoscopic "drilling" by diathermy or laser for ovulation induction in anovulatory polycystic ovary syndrome. Cochrane Database Syst Rev. 2005(3):CD001122.
127. Farquhar C, Vandekerckhove P, Arnot M, Lilford R. Laparoscopic "drilling" by diathermy or laser for ovulation induction in anovulatory polycystic ovary syndrome. Cochrane Database Syst Rev. 2000(2):CD001122.
128. Bordewijk EM, Ng KYB, Rakic L, Mol BWJ, Brown J, Crawford TJ, et al. Laparoscopic ovarian drilling for ovulation induction in women with anovulatory polycystic ovary syndrome. Cochrane Database Syst Rev. 2020;2:CD001122.
129. Al-Inany HG, Youssef MA, Ayeleke RO, Brown J, Lam WS, Broekmans FJ. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. Cochrane Database Syst Rev. 2016;4:CD001750.
130. Al-Inany HG, Youssef MA, Aboulghar M, Broekmans F, Sterrenburg M, Smit J, et al. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. Cochrane Database Syst Rev. 2011(5):CD001750.
131. Palomba S, de Wilde MA, Falbo A, Koster MP, La Sala GB, Fauser BC. Pregnancy complications in women with polycystic ovary syndrome. Hum Reprod Update. 2015;21(5):575-92.
132. Boomsma CM, Fauser BC, Macklon NS. Pregnancy complications in women with polycystic ovary syndrome. Semin Reprod Med. 2008;26(1):72-84.
133. Liu S, Mo M, Xiao S, Li L, Hu X, Hong L, et al. Pregnancy outcomes of women with polycystic ovary syndrome for the first in vitro fertilization treatment: A retrospective cohort study with 7678 patients. Front Endocrinol (Lausanne). 2020;11:575337.
134. Kamalanathan S, Sahoo JP, Sathyapalan T. Pregnancy in polycystic ovary syndrome. Indian J Endocrinol Metab. 2013;17(1):37-43.
135. Chakraborty P, Goswami SK, Rajani S, Sharma S, Kabir SN, Chakravarty B, et al. Recurrent pregnancy loss in polycystic ovary syndrome: role of hyperhomocysteinemia and insulin resistance. PLoS One. 2013;8(5):e64446.
136. Morin-Papunen L, Rantala AS, Unkila-Kallio L, Tiitinen A, Hippelainen M, Perheentupa A, et al. Metformin improves pregnancy and live-birth rates in women with polycystic ovary syndrome (PCOS): a multicenter, double-blind, placebo-controlled randomized trial. J Clin Endocrinol Metab. 2012;97(5):1492-500.
137. Sterling L, Liu J, Okun N, Sakhuja A, Sierra S, Greenblatt E. Pregnancy outcomes in women with polycystic ovary syndrome undergoing in vitro fertilization. Fertil Steril. 2016;105(3):791-7 e2.
138. Issat T, Nowicka MA, Jakimiuk AJ. [Polycystic ovary syndrome (PCOS) and gestational diabetes mellitus (GDM) risk]. Ginekol Pol. 2015;86(5):392-5.
139. Ashrafi M, Sheikhan F, Arabipoor A, Hosseini R, Nourbakhsh F, Zolfaghari Z. Gestational diabetes mellitus risk factors in women with polycystic ovary syndrome (PCOS). Eur J Obstet Gynecol Reprod Biol. 2014;181:195-9.
140. Bals-Pratsch M, Grosser B, Seifert B, Ortmann O, Seifarth C. Early onset and high prevalence of gestational diabetes in PCOS and insulin resistant women before and after assisted reproduction. Exp Clin Endocrinol Diabetes. 2011;119(6):338-42.
141. Feng L, Lin XF, Wan ZH, Hu D, Du YK. Efficacy of metformin on pregnancy complications in women with polycystic ovary syndrome: a meta-analysis. Gynecol Endocrinol. 2015;31(11):833-9.

142. Lovvik TS, Carlsen SM, Salvesen O, Steffensen B, Bixo M, Gomez-Real F, et al. Use of metformin to treat pregnant women with polycystic ovary syndrome (PregMet2): a randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol. 2019;7(4):256-66.
143. de Vries MJ, Dekker GA, Schoemaker J. Higher risk of preeclampsia in the polycystic ovary syndrome. A case control study. Eur J Obstet Gynecol Reprod Biol. 1998;76(1):91-5.
144. Schneider D, Gonzalez JR, Yamamoto M, Yang J, Lo JC. The association of polycystic ovary syndrome and gestational hypertensive disorders in a diverse community-based cohort. J Pregnancy. 2019;2019:9847057.
145. Mills G, Badeghiesh A, Suarthana E, Baghlaf H, Dahan MH. Polycystic ovary syndrome as an independent risk factor for gestational diabetes and hypertensive disorders of pregnancy: a population-based study on 9.1 million pregnancies. Hum Reprod. 2020;35(7):1666-74.
146. Robinson SL, Yeung EH. Polycystic ovary syndrome and preterm birth-what's going on? Fertil Steril. 2021;115(2):326-7.
147. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75-84.
148. Yamamoto M, Feigenbaum SL, Crites Y, Escobar GJ, Yang J, Ferrara A, et al. Risk of preterm delivery in non-diabetic women with polycystic ovarian syndrome. J Perinatol. 2012;32(10):770-6.
149. Valgeirsdottir H, Sundstrom Poromaa I, Kunovac Kallak T, Vanky E, Akhter T, Roos N, et al. Polycystic ovary syndrome and extremely preterm birth: A nationwide register-based study. PLoS One. 2021;16(2):e0246743.
150. Kaing A, Jaswa EA, Diamond MP, Legro RS, Cedars MI, Huddleston HG. Highly elevated level of antimullerian hormone associated with preterm delivery in polycystic ovary syndrome patients who underwent ovulation induction. Fertil Steril. 2021;115(2):438-46.
151. Hu KL, Liu FT, Xu H, Li R, Qiao J. High antimullerian hormone levels are associated with preterm delivery in patients with polycystic ovary syndrome. Fertil Steril. 2020;113(2):444-52 e1.
152. Hsu JY, James KE, Bormann CL, Donahoe PK, Pepin D, Sabatini ME. Mullerian-inhibiting substance/anti-mullerian hormone as a predictor of preterm birth in polycystic ovary syndrome. J Clin Endocrinol Metab. 2018;103(11):4187-96.
153. Freeman AM, Pennings N. Insulin Resistance. StatPearls. Treasure Island (FL)2021.
154. Calcaterra V, De Silvestri A, Schneider L, Acunzo M, Vittoni V, Meraviglia G, et al. Acanthosis nigricans in children and adolescents with type 1 diabetes or obesity: The potential interplay role between insulin resistance and excess weight. Children (Basel). 2021;8(8).
155. Jeanes YM, Reeves S. Metabolic consequences of obesity and insulin resistance in polycystic ovary syndrome: diagnostic and methodological challenges. Nutr Res Rev. 2017;30(1):97-105.
156. Longo M, Zatterale F, Naderi J, Parrillo L, Formisano P, Raciti GA, et al. Adipose Tissue dysfunction as determinant of obesity-associated metabolic complications. Int J Mol Sci. 2019;20(9).
157. DeFronzo RA, Gunnarsson R, Bjorkman O, Olsson M, Wahren J. Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. J Clin Invest. 1985;76(1):149-55.
158. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes. 1981;30(12):1000-7.
159. Navale AM, Paranjape AN. Glucose transporters: physiological and pathological roles. Biophys Rev. 2016;8(1):5-9.
160. Mueckler M, Thorens B. The SLC2 (GLUT) family of membrane transporters. Mol Aspects Med. 2013;34(2-3):121-38.
161. Smith U, Kahn BB. Adipose tissue regulates insulin sensitivity: role of adipogenesis, de novo lipogenesis and novel lipids. J Intern Med. 2016;280(5):465-75.

162. Dimitriadis G, Mitrou P, Lambadiari V, Maratou E, Raptis SA. Insulin effects in muscle and adipose tissue. Diabetes Research and Clinical Practice. 2011;93:S52-S9.
163. Sam S, Dunaif A. Polycystic ovary syndrome: syndrome XX? Trends Endocrinol Metab. 2003;14(8):365-70.
164. Diamanti-Kandarakis E, Papavassiliou AG. Molecular mechanisms of insulin resistance in polycystic ovary syndrome. Trends Mol Med. 2006;12(7):324-32.
165. Gürgan T, Yarah H. Hyperandrogenism and insulin resistance. New Trends in Reproductive Medicine: Springer; 1996. p. 169-75.
166. Dasgupta A, Khan A, Banerjee U, Ghosh M, Pal M, Chowdhury KM, et al. Predictors of insulin resistance and metabolic complications in polycystic ovarian syndrome in an eastern Indian population. Indian J Clin Biochem. 2013;28(2):169-76.
167. Dumesic DA, Phan JD, Leung KL, Grogan TR, Ding X, Li X, et al. Adipose insulin resistance in normal-weight women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2019;104(6):2171-83.
168. Ezeh U, Chen IY, Chen YH, Azziz R. Adipocyte insulin resistance in PCOS: Relationship with GLUT-4 expression and whole-body glucose disposal and beta-cell Function. J Clin Endocrinol Metab. 2020;105(7).
169. Behboudi-Gandevani S, Ramezani Tehrani F, Rostami Dovom M, Farahmand M, Bahri Khomami M, Noroozzadeh M, et al. Insulin resistance in obesity and polycystic ovary syndrome: systematic review and meta-analysis of observational studies. Gynecol Endocrinol. 2016;32(5):343-53.
170. Morrison SA, Goss AM, Azziz R, Raju DA, Gower BA. Peri-muscular adipose tissue may play a unique role in determining insulin sensitivity/resistance in women with polycystic ovary syndrome. Hum Reprod. 2017;32(1):185-92.
171. Ciaraldi TP, el-Roeiy A, Madar Z, Reichart D, Olefsky JM, Yen SS. Cellular mechanisms of insulin resistance in polycystic ovarian syndrome. J Clin Endocrinol Metab. 1992;75(2):577-83.
172. Dunaif A, Segal KR, Shelley DR, Green G, Dobrjansky A, Licholai T. Evidence for distinctive and intrinsic defects in insulin action in polycystic ovary syndrome. Diabetes. 1992;41(10):1257-66.
173. Rosenbaum D, Haber RS, Dunaif A. Insulin resistance in polycystic ovary syndrome: decreased expression of GLUT-4 glucose transporters in adipocytes. American Journal of Physiology-Endocrinology And Metabolism. 1993;264(2):E197-E202.
174. Lystedt E, Westergren H, Brynhildsen J, Lindh-Astrand L, Gustavsson J, Nystrom FH, et al. Subcutaneous adipocytes from obese hyperinsulinemic women with polycystic ovary syndrome exhibit normal insulin sensitivity but reduced maximal insulin responsiveness. Eur J Endocrinol. 2005;153(6):831-5.
175. Qiu HY, Chu YL, Li M, Sun YY, Li HF. [Tyrosine phosphorylation and protein expression of insulin receptor substrate-2 in the adipose tissue from patients with polycystic ovary syndrome]. Zhonghua Fu Chan Ke Za Zhi. 2005;40(2):116-9.
176. Wang Z, Gu X, Yang D, Kuang J. Expression of insulin receptor substrate 1 and phosphorylation of tyrosine in adipose tissue of polycystic ovary syndrome. Zhonghua fu chan ke za zhi. 2005;40(2):120-3.
177. Chu YL, Sun YY, Qiu HY, Li HF. [Tyrosine phosphorylation and protein expression of insulin receptor substrate-1 in the patients with polycystic ovary syndrome]. Zhonghua Fu Chan Ke Za Zhi. 2004;39(3):176-9.
178. Rosenbaum D, Haber RS, Dunaif A. Insulin resistance in polycystic ovary syndrome: decreased expression of GLUT-4 glucose transporters in adipocytes. Am J Physiol. 1993;264(2 Pt 1):E197-202.
179. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev. 1997;18(6):774-800.
180. Book CB, Dunaif A. Selective insulin resistance in the polycystic ovary syndrome. J Clin Endocrinol Metab. 1999;84(9):3110-6.
181. Venkatesan AM, Dunaif A, Corbould A. Insulin resistance in polycystic ovary syndrome: progress and paradoxes. Recent Prog Horm Res. 2001;56:295-308.
182. Dunaif A, Xia J, Book CB, Schenker E, Tang Z. Excessive insulin receptor serine phosphorylation in cultured fibroblasts and in skeletal muscle. A potential mechanism for insulin resistance in the polycystic ovary syndrome. J Clin Invest. 1995;96(2):801-10.

183. Dunaif A, Wu X, Lee A, Diamanti-Kandarakis E. Defects in insulin receptor signaling in vivo in the polycystic ovary syndrome (PCOS). Am J Physiol Endocrinol Metab. 2001;281(2):E392-9.
184. Corbould A, Kim YB, Youngren JF, Pender C, Kahn BB, Lee A, et al. Insulin resistance in the skeletal muscle of women with PCOS involves intrinsic and acquired defects in insulin signaling. Am J Physiol Endocrinol Metab. 2005;288(5):E1047-54.
185. Reaven E, Tsai L, Azhar S. Intracellular events in the "selective" transport of lipoprotein-derived cholesteryl esters. J Biol Chem. 1996;271(27):16208-17.
186. Chaffin CL, Dissen GA, Stouffer RL. Hormonal regulation of steroidogenic enzyme expression in granulosa cells during the peri-ovulatory interval in monkeys. Mol Hum Reprod. 2000;6(1):11-8.
187. Seto-Young D, Zajac J, Liu HC, Rosenwaks Z, Poretsky L. The role of mitogen-activated protein kinase in insulin and insulin-like growth factor I (IGF-I) signaling cascades for progesterone and IGF-binding protein-1 production in human granulosa cells. J Clin Endocrinol Metab. 2003;88(7):3385-91.
188. Munir I, Yen HW, Geller DH, Torbati D, Bierden RM, Weitsman SR, et al. Insulin augmentation of 17alpha-hydroxylase activity is mediated by phosphatidyl inositol 3-kinase but not extracellular signal-regulated kinase-1/2 in human ovarian theca cells. Endocrinology. 2004;145(1):175-83.
189. Nelson-Degrave VL, Wickenheisser JK, Hendricks KL, Asano T, Fujishiro M, Legro RS, et al. Alterations in mitogen-activated protein kinase kinase and extracellular regulated kinase signaling in theca cells contribute to excessive androgen production in polycystic ovary syndrome. Mol Endocrinol. 2005;19(2):379-90.
190. Garzo VG, Dorrington JH. Aromatase activity in human granulosa cells during follicular development and the modulation by follicle-stimulating hormone and insulin. Am J Obstet Gynecol. 1984;148(5):657-62.
191. Goodarzi MO, Quinones MJ, Azziz R, Rotter JI, Hsueh WA, Yang H. Polycystic ovary syndrome in Mexican-Americans: prevalence and association with the severity of insulin resistance. Fertil Steril. 2005;84(3):766-9.
192. Vrbikova J, Bendlova B, Hill M, Vankova M, Vondra K, Starka L. Insulin sensitivity and beta-cell function in women with polycystic ovary syndrome. Diabetes Care. 2002;25(7):1217-22.
193. Song DK, Hong YS, Sung YA, Lee H. Insulin resistance according to beta-cell function in women with polycystic ovary syndrome and normal glucose tolerance. PLoS One. 2017;12(5):e0178120.
194. Flier JS, Minaker KL, Landsberg L, Young JB, Pallotta J, Rowe JW. Impaired in vivo insulin clearance in patients with severe target-cell resistance to insulin. Diabetes. 1982;31(2):132-5.
195. Gutch M, Kumar S, Razi SM, Gupta KK, Gupta A. Assessment of insulin sensitivity/resistance. Indian J Endocrinol Metab. 2015;19(1):160-4.
196. Robert JJ. [Methods for the measurement of insulin resistance. Hyperinsulinemic euglycemic clamp]. Presse Med. 1995;24(15):730-4.
197. Coates PA, Luzio SD, Brunel P, Owens DR. Comparison of estimates of insulin sensitivity from minimal model analysis of the insulin-modified frequently sampled intravenous glucose tolerance test and the isoglycemic hyperinsulinemic clamp in subjects with NIDDM. Diabetes. 1995;44(6):631-5.
198. Rossner SM, Neovius M, Mattsson A, Marcus C, Norgren S. HOMA-IR and QUICKI: decide on a general standard instead of making further comparisons. Acta Paediatr. 2010;99(11):1735-40.
199. Keskin M, Kurtoglu S, Kendirci M, Atabek ME, Yazici C. Homeostasis model assessment is more reliable than the fasting glucose/insulin ratio and quantitative insulin sensitivity check index for assessing insulin resistance among obese children and adolescents. Pediatrics. 2005;115(4):e500-3.
200. Diamanti-Kandarakis E, Christakou CD, Kandaraki E, Alexandraki KI. Early onset adiposity: a pathway to polycystic ovary syndrome in adolescents? Hormones (Athens). 2007;6(3):210-7.
201. Cedars MI, Steingold KA, de Ziegler D, Lapolt PS, Chang RJ, Judd HL. Long-term administration of gonadotropin-releasing hormone agonist and dexamethasone: assessment of the adrenal role in ovarian dysfunction. Fertil Steril. 1992;57(3):495-500.
202. Sorensen AE, Udesen PB, Wissing ML, Englund ALM, Dalgaard LT. MicroRNAs related to androgen metabolism and polycystic ovary syndrome. Chem Biol Interact. 2016;259(Pt A):8-16.

203. Inoue T, Okamoto K, Moriyama T, Takahashi T, Shimizu K, Miyama A. Effect of Yersinia enterocolitica ST on cyclic guanosine 3',5'-monophosphate levels in mouse intestines and cultured cells. Microbiol Immunol. 1983;27(2):159-66.
204. Louwers YV, de Jong FH, van Herwaarden NA, Stolk L, Fauser BC, Uitterlinden AG, et al. Variants in SULT2A1 affect the DHEA sulphate to DHEA ratio in patients with polycystic ovary syndrome but not the hyperandrogenic phenotype. J Clin Endocrinol Metab. 2013;98(9):3848-55.
205. Nolan JJ, Ludvik B, Beerdsen P, Joyce M, Olefsky J. Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. N Engl J Med. 1994;331(18):1188-93.
206. Rutter MK, Meigs JB, Sullivan LM, D'Agostino RB, Sr., Wilson PW. Insulin resistance, the metabolic syndrome, and incident cardiovascular events in the Framingham Offspring Study. Diabetes. 2005;54(11):3252-7.
207. Teede HJ, Hutchison SK, Zoungas S. The management of insulin resistance in polycystic ovary syndrome. Trends Endocrinol Metab. 2007;18(7):273-9.
208. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27(5):1047-53.
209. Legro RS, Kunselman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. J Clin Endocrinol Metab. 1999;84(1):165-9.
210. Celik C, Abali R, Bastu E, Tasdemir N, Tasdemir UG, Gul A. Assessment of impaired glucose tolerance prevalence with hemoglobin A(1)c and oral glucose tolerance test in 252 Turkish women with polycystic ovary syndrome: a prospective, controlled study. Hum Reprod. 2013;28(4):1062-8.
211. Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care. 1999;22(1):141-6.
212. Moran LJ, Misso ML, Wild RA, Norman RJ. Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update. 2010;16(4):347-63.
213. Chen X, Yang D, Li L, Feng S, Wang L. Abnormal glucose tolerance in Chinese women with polycystic ovary syndrome. Hum Reprod. 2006;21(8):2027-32.
214. Talbott EO, Zborowski JV, Rager JR, Kip KE, Xu X, Orchard TJ. Polycystic ovarian syndrome (PCOS): a significant contributor to the overall burden of type 2 diabetes in women. J Womens Health (Larchmt). 2007;16(2):191-7.
215. Ding T, Hardiman PJ, Petersen I, Baio G. Incidence and prevalence of diabetes and cost of illness analysis of polycystic ovary syndrome: a Bayesian modelling study. Hum Reprod. 2018;33(7):1299-306.
216. Norman RJ, Masters L, Milner CR, Wang JX, Davies MJ. Relative risk of conversion from normoglycaemia to impaired glucose tolerance or non-insulin dependent diabetes mellitus in polycystic ovarian syndrome. Hum Reprod. 2001;16(9):1995-8.
217. Celik C, Tasdemir N, Abali R, Bastu E, Yilmaz M. Progression to impaired glucose tolerance or type 2 diabetes mellitus in polycystic ovary syndrome: a controlled follow-up study. Fertil Steril. 2014;101(4):1123-8 e1.
218. Legro RS, Gnatuk CL, Kunselman AR, Dunaif A. Changes in glucose tolerance over time in women with polycystic ovary syndrome: a controlled study. J Clin Endocrinol Metab. 2005;90(6):3236-42.
219. Huang PL. A comprehensive definition for metabolic syndrome. Dis Model Mech. 2009;2(5-6):231-7.
220. Carmina E. Metabolic syndrome in polycystic ovary syndrome. Minerva Ginecol. 2006;58(2):109-14.
221. Khorshidi A, Azami M, Tardeh S, Tardeh Z. The prevalence of metabolic syndrome in patients with polycystic ovary syndrome: A systematic review and meta-analysis. Diabetes Metab Syndr. 2019;13(4):2747-53.
222. Ehrmann DA, Liljenquist DR, Kasza K, Azziz R, Legro RS, Ghazzi MN, et al. Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2006;91(1):48-53.
223. Azziz R. How prevalent is metabolic syndrome in women with polycystic ovary syndrome? Nat Clin Pract Endocrinol Metab. 2006;2(3):132-3.

224. Apridonidze T, Essah PA, Iuorno MJ, Nestler JE. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2005;90(4):1929-35.
225. Coviello AD, Legro RS, Dunaif A. Adolescent girls with polycystic ovary syndrome have an increased risk of the metabolic syndrome associated with increasing androgen levels independent of obesity and insulin resistance. J Clin Endocrinol Metab. 2006;91(2):492-7.
226. Glueck CJ, Morrison JA, Friedman LA, Goldenberg N, Stroop DM, Wang P. Obesity, free testosterone, and cardiovascular risk factors in adolescents with polycystic ovary syndrome and regularly cycling adolescents. Metabolism. 2006;55(4):508-14.
227. Kazemi M, Pierson RA, Lujan ME, Chilibeck PD, McBreairty LE, Gordon JJ, et al. Comprehensive evaluation of type 2 diabetes and cardiovascular disease risk profiles in reproductive-age women with polycystic ovary syndrome: a large canadian cohort. J Obstet Gynaecol Can. 2019;41(10):1453-60.
228. Bluher M. Obesity: global epidemiology and pathogenesis. Nat Rev Endocrinol. 2019;15(5):288-98.
229. Engin A. The definition and prevalence of obesity and metabolic syndrome. Adv Exp Med Biol. 2017;960:1-17.
230. Pietrobelli A. OBESITY | Definition, etiology and assessment. In: Caballero B, editor. Encyclopedia of Human Nutrition (Second Edition). Oxford: Elsevier; 2005. p. 389-92.
231. Rezaianzadeh A, Namayandeh SM, Sadr SM. National cholesterol education program adult treatment panel III versus international diabetic federation definition of metabolic syndrome, which one is associated with diabetes mellitus and coronary artery disease? Int J Prev Med. 2012;3(8):552-8.
232. Gambacciani M, Ciaponi M, Cappagli B, Piaggesi L, De Simone L, Orlandi R, et al. Body weight, body fat distribution, and hormonal replacement therapy in early postmenopausal women. J Clin Endocrinol Metab. 1997;82(2):414-7.
233. Sanchez-Garcia S, Garcia-Pena C, Duque-Lopez MX, Juarez-Cedillo T, Cortes-Nunez AR, Reyes-Beaman S. Anthropometric measures and nutritional status in a healthy elderly population. BMC Public Health. 2007;7:2.
234. Glueck CJ, Goldenberg N. Characteristics of obesity in polycystic ovary syndrome: Etiology, treatment, and genetics. Metabolism. 2019;92:108-20.
235. Legro RS. Obesity and PCOS: implications for diagnosis and treatment. Semin Reprod Med. 2012;30(6):496-506.
236. Holte J, Bergh T, Gennarelli G, Wide L. The independent effects of polycystic ovary syndrome and obesity on serum concentrations of gonadotrophins and sex steroids in premenopausal women. Clin Endocrinol (Oxf). 1994;41(4):473-81.
237. Bouchard C, Despres JP, Mauriege P. Genetic and nongenetic determinants of regional fat distribution. Endocr Rev. 1993;14(1):72-93.
238. Zheng SH, Li XL. Visceral adiposity index as a predictor of clinical severity and therapeutic outcome of PCOS. Gynecol Endocrinol. 2016;32(3):177-83.
239. Blouin K, Boivin A, Tchernof A. Androgens and body fat distribution. J Steroid Biochem Mol Biol. 2008;108(3-5):272-80.
240. Kwon H, Kim D, Kim JS. Body fat distribution and the risk of incident metabolic syndrome: a Longitudinal cohort study. Sci Rep. 2017;7(1):10955.
241. Barbosa-Desongles A, Hernandez C, Simo R, Selva DM. Testosterone induces cell proliferation and cell cycle gene overexpression in human visceral preadipocytes. Am J Physiol Cell Physiol. 2013;305(3):C355-9.
242. Cawthorn WP, Scheller EL, MacDougald OA. Adipose tissue stem cells meet preadipocyte commitment: going back to the future. J Lipid Res. 2012;53(2):227-46.
243. Chazenbalk G, Singh P, Irge D, Shah A, Abbott DH, Dumesic DA. Androgens inhibit adipogenesis during human adipose stem cell commitment to preadipocyte formation. Steroids. 2013;78(9):920-6.
244. Dicker A, Ryden M, Naslund E, Muehlen IE, Wiren M, Lafontan M, et al. Effect of testosterone on lipolysis in human pre-adipocytes from different fat depots. Diabetologia. 2004;47(3):420-8.

245. Luque-Ramirez M, Alvarez-Blasco F, Mendieta-Azcona C, Botella-Carretero JI, Escobar-Morreale HF. Obesity is the major determinant of the abnormalities in blood pressure found in young women with the polycystic ovary syndrome. J Clin Endocrinol Metab. 2007;92(6):2141-8.
246. Luque-Ramirez M, Marti D, Fernandez-Duran E, Alpanes M, Alvarez-Blasco F, Escobar-Morreale HF. Office blood pressure, ambulatory blood pressure monitoring, and echocardiographic abnormalities in women with polycystic ovary syndrome: role of obesity and androgen excess. Hypertension. 2014;63(3):624-9.
247. Li N, Ma R, Wang S, Zhao Y, Wang P, Yang Z, et al. The potential role of testosterone in hypertension and target organ damage in hypertensive postmenopausal women. Clin Interv Aging. 2019;14:743-52.
248. Huang JH, Chiu WC, Hsu MI, Chen YJ. Effects of androgen on vascular and inflammatory biomarkers in a female hypertensive population. Gynecol Endocrinol. 2013;29(4):340-4.
249. Wu CC, Cheng J, Zhang FF, Gotlinger KH, Kelkar M, Zhang Y, et al. Androgen-dependent hypertension is mediated by 20-hydroxy-5,8,11,14-eicosatetraenoic acid-induced vascular dysfunction: role of inhibitor of kappaB Kinase. Hypertension. 2011;57(4):788-94.
250. Connelly PJ, Casey H, Montezano AC, Touyz RM, Delles C. Sex steroids receptors, hypertension, and vascular ageing. J Hum Hypertens. 2021.
251. Moretti C, Lanzolla G, Moretti M, Gnessi L, Carmina E. Androgens and hypertension in men and women: a unifying view. Curr Hypertens Rep. 2017;19(5):44.
252. Mellembakken JR, Mahmoudan A, Morkrid L, Sundstrom-Poromaa I, Morin-Papunen L, Tapanainen JS, et al. Higher blood pressure in normal weight women with PCOS compared to controls. Endocr Connect. 2021;10(2):154-63.
253. Kadi H, Avci E, Usta A, Demirtas AO. Morning blood pressure surge and its relation to insulin resistance in patients of reproductive age with polycystic ovary syndrome. Reprod Biol Endocrinol. 2018;16(1):75.
254. Zachurzok-Buczynska A, Szydlowski L, Gawlik A, Wilk K, Malecka-Tendera E. Blood pressure regulation and resting heart rate abnormalities in adolescent girls with polycystic ovary syndrome. Fertil Steril. 2011;96(6):1519-25.
255. Legato MJ. Dyslipidemia, gender, and the role of high-density lipoprotein cholesterol: implications for therapy. Am J Cardiol. 2000;86(12A):15L-8L.
256. National Cholesterol Education Program Expert Panel on Detection E, Treatment of High Blood Cholesterol in A. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002;106(25):3143-421.
257. Hong Y, Yang D, Liu W, Zhao X, Chen X, Li L. Dyslipidemia in relation to body mass index and insulin resistance in Chinese women with polycystic ovary syndrome. J Biol Regul Homeost Agents. 2011;25(3):365-74.
258. Mandrelle K, Kamath MS, Bondu DJ, Chandy A, Aleyamma T, George K. Prevalence of metabolic syndrome in women with polycystic ovary syndrome attending an infertility clinic in a tertiary care hospital in south India. J Hum Reprod Sci. 2012;5(1):26-31.
259. Essah PA, Nestler JE, Carmina E. Differences in dyslipidemia between American and Italian women with polycystic ovary syndrome. J Endocrinol Invest. 2008;31(1):35-41.
260. Rocha MP, Marcondes JA, Barcellos CR, Hayashida SA, Curi DD, da Fonseca AM, et al. Dyslipidemia in women with polycystic ovary syndrome: incidence, pattern and predictors. Gynecol Endocrinol. 2011;27(10):814-9.
261. Florakis D, Diamanti-Kandarakis E, Katsikis I, Nassis GP, Karkanaki A, Georgopoulos N, et al. Effect of hypocaloric diet plus sibutramine treatment on hormonal and metabolic features in overweight and obese women with polycystic ovary syndrome: a randomized, 24-week study. Int J Obes (Lond). 2008;32(4):692-9.
262. Moran LJ, Noakes M, Clifton PM, Tomlinson L, Galletly C, Norman RJ. Dietary composition in restoring reproductive and metabolic physiology in overweight women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2003;88(2):812-9.

263. Yilmaz M, Biri A, Bukan N, Karakoc A, Sancak B, Toruner F, et al. Levels of lipoprotein and homocysteine in non-obese and obese patients with polycystic ovary syndrome. Gynecol Endocrinol. 2005;20(5):258-63.
264. Tsouma I, Kouskouni E, Demeridou S, Boutsikou M, Hassiakos D, Chasiakou A, et al. Lipid lipoprotein profile alterations in Greek infertile women with polycystic ovaries: influence of adipocytokines levels. In Vivo. 2014;28(5):935-9.
265. Warnick GR, Nauck M, Rifai N. Evolution of methods for measurement of HDL-cholesterol: from ultracentrifugation to homogeneous assays. Clin Chem. 2001;47(9):1579-96.
266. Martin SS, Khokhar AA, May HT, Kulkarni KR, Blaha MJ, Joshi PH, et al. HDL cholesterol subclasses, myocardial infarction, and mortality in secondary prevention: the lipoprotein investigators collaborative. Eur Heart J. 2015;36(1):22-30.
267. Joshi PH, Toth PP, Lirette ST, Griswold ME, Massaro JM, Martin SS, et al. Association of high-density lipoprotein subclasses and incident coronary heart disease: The Jackson heart and framingham offspring cohort studies. Eur J Prev Cardiol. 2016;23(1):41-9.
268. Heinecke JW. The HDL proteome: a marker--and perhaps mediator--of coronary artery disease. J Lipid Res. 2009;50 Suppl:S167-71.
269. Talbott E, Guzick D, Clerici A, Berga S, Detre K, Weimer K, et al. Coronary heart disease risk factors in women with polycystic ovary syndrome. Arterioscler Thromb Vasc Biol. 1995;15(7):821-6.
270. Conway GS, Agrawal R, Betteridge DJ, Jacobs HS. Risk factors for coronary artery disease in lean and obese women with the polycystic ovary syndrome. Clin Endocrinol (Oxf). 1992;37(2):119-25.
271. Hevonoja T, Pentikäinen MO, Hyvönen MT, Kovanen PT, Ala-Korpela M. Structure of low density lipoprotein (LDL) particles: Basis for understanding molecular changes in modified LDL. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. 2000;1488(3):189-210.
272. Berneis K, Rizzo M, Lazzarini V, Fruzzetti F, Carmina E. Atherogenic lipoprotein phenotype and low-density lipoproteins size and subclasses in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2007;92(1):186-9.
273. Ivanova EA, Myasoedova VA, Melnichenko AA, Grechko AV, Orekhov AN. Small dense low-density lipoprotein as biomarker for atherosclerotic diseases. Oxid Med Cell Longev. 2017;2017:1273042.
274. Dejager S, Pichard C, Giral P, Bruckert E, Federspield MC, Beucler I, et al. Smaller LDL particle size in women with polycystic ovary syndrome compared to controls. Clin Endocrinol (Oxf). 2001;54(4):455-62.
275. Berneis K, Rizzo M, Lazzaroni V, Fruzzetti F, Carmina E. Atherogenic lipoprotein phenotype and low-density lipoproteins size and subclasses in omen with polycystic ovary syndrome. The Journal of Clinical Endocrinology & Metabolism. 2007;92(1):186-9.
276. Holte J, Bergh T, Berne C, Lithell H. Serum lipoprotein lipid profile in women with the polycystic ovary syndrome: relation to anthropometric, endocrine and metabolic variables. Clinical Endocrinology. 1994;41(4):463-71.
277. Gardner CD, Fortmann SP, Krauss RM. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. JAMA. 1996;276(11):875-81.
278. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA. 1988;260(13):1917-21.
279. Huang J, Gu JX, Bao HZ, Li SS, Yao XQ, Yang M, et al. Elevated serum small dense low-density lipoprotein cholesterol may increase the risk and severity of coronary heart disease and predict cardiovascular events in patients with type 2 diabetes mellitus. Dis Markers. 2021;2021:5597028.
280. Tsai MY, Steffen BT, Guan W, McClelland RL, Warnick R, McConnell J, et al. New automated assay of small dense low-density lipoprotein cholesterol identifies risk of coronary heart disease: the Multi-ethnic Study of Atherosclerosis. Arterioscler Thromb Vasc Biol. 2014;34(1):196-201.
281. Liou L, Kaptoge S. Association of small, dense LDL-cholesterol concentration and lipoprotein particle characteristics with coronary heart disease: A systematic review and meta-analysis. PLoS One. 2020;15(11):e0241993.

282. Pirwany IR, Fleming R, Greer IA, Packard CJ, Sattar N. Lipids and lipoprotein subfractions in women with PCOS: relationship to metabolic and endocrine parameters. Clin Endocrinol (Oxf). 2001;54(4):447-53.
283. Kim JJ, Chae SJ, Choi YM, Hwang KR, Song SH, Yoon SH, et al. Atherogenic changes in low-density lipoprotein particle profiles were not observed in non-obese women with polycystic ovary syndrome. Hum Reprod. 2013;28(5):1354-60.
284. Rajkhowa M, Neary RH, Kumpatla P, Game FL, Jones PW, Obhrai MS, et al. Altered composition of high density lipoproteins in women with the polycystic ovary syndrome. J Clin Endocrinol Metab. 1997;82(10):3389-94.
285. Macut D, Panidis D, Glisic B, Spanos N, Petakov M, Bjekic J, et al. Lipid and lipoprotein profile in women with polycystic ovary syndrome. Can J Physiol Pharmacol. 2008;86(4):199-204.
286. Einbinder Y, Biron-Shental T, Agassi-Zaitler M, Tzadikevitch-Geffen K, Vaya J, Khatib S, et al. High-density lipoproteins (HDL) composition and function in preeclampsia. Arch Gynecol Obstet. 2018;298(2):405-13.
287. Hasselwander O, McEneny J, McMaster D, Fogarty DG, Nicholls DP, Maxwell AP, et al. HDL composition and HDL antioxidant capacity in patients on regular haemodialysis. Atherosclerosis. 1999;143(1):125-33.
288. Zhang J, Fan P, Liu H, Bai H, Wang Y, Zhang F. Apolipoprotein A-I and B levels, dyslipidemia and metabolic syndrome in south-west Chinese women with PCOS. Hum Reprod. 2012;27(8):2484-93.
289. Dullaart RP, Sluiter WJ, Dikkeschei LD, Hoogenberg K, Van Tol A. Effect of adiposity on plasma lipid transfer protein activities: a possible link between insulin resistance and high density lipoprotein metabolism. Eur J Clin Invest. 1994;24(3):188-94.
290. Tikkanen MJ, Nikkila EA. Regulation of hepatic lipase and serum lipoproteins by sex steroids. Am Heart J. 1987;113(2 Pt 2):562-7.
291. Ohmura H, Mokuno H, Sawano M, Hatsumi C, Mitsugi Y, Watanabe Y, et al. Lipid compositional differences of small, dense low-density lipoprotein particle influence its oxidative susceptibility: possible implication of increased risk of coronary artery disease in subjects with phenotype B. Metabolism. 2002;51(9):1081-7.
292. Ek I, Arner P, Ryden M, Holm C, Thorne A, Hoffstedt J, et al. A unique defect in the regulation of visceral fat cell lipolysis in the polycystic ovary syndrome as an early link to insulin resistance. Diabetes. 2002;51(2):484-92.
293. Subramanian S, Chait A. Hypertriglyceridemia secondary to obesity and diabetes. Biochim Biophys Acta. 2012;1821(5):819-25.
294. Klop B, Elte JW, Cabezas MC. Dyslipidemia in obesity: mechanisms and potential targets. Nutrients. 2013;5(4):1218-40.
295. Dumesic DA, Tulberg A, McNamara M, Grogan TR, Abbott DH, Naik R, et al. Serum testosterone to androstenedione ratio predicts metabolic health in normal-weight polycystic ovary syndrome women. J Endocr Soc. 2021;5(11):bvab158.
296. O'Reilly MW, Kempegowda P, Walsh M, Taylor AE, Manolopoulos KN, Allwood JW, et al. AKR1C3-mediated adipose androgen generation drives lipotoxicity in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2017;102(9):3327-39.
297. O'Reilly M, Gathercole L, Capper F, Arlt W, Tomlinson J. Effect of insulin on AKR1C3 expression in female adipose tissue: in-vivo and in-vitro study of adipose androgen generation in polycystic ovary syndrome. Lancet. 2015;385 Suppl 1:S16.
298. McGrath KC, McRobb LS, Heather AK. Androgen therapy and atherosclerotic cardiovascular disease. Vasc Health Risk Manag. 2008;4(1):11-21.
299. Kozarsky KF, Donahee MH, Glick JM, Krieger M, Rader DJ. Gene transfer and hepatic overexpression of the HDL receptor SR-BI reduces atherosclerosis in the cholesterol-fed LDL receptor-deficient mouse. Arterioscler Thromb Vasc Biol. 2000;20(3):721-7.
300. Leiva A, Verdejo H, Benitez ML, Martinez A, Busso D, Rigotti A. Mechanisms regulating hepatic SR-BI expression and their impact on HDL metabolism. Atherosclerosis. 2011;217(2):299-307.

301. Herbst KL, Amory JK, Brunzell JD, Chansky HA, Bremner WJ. Testosterone administration to men increases hepatic lipase activity and decreases HDL and LDL size in 3 wk. Am J Physiol Endocrinol Metab. 2003;284(6):E1112-8.
302. Tan KC, Shiu SW, Kung AW. Alterations in hepatic lipase and lipoprotein subfractions with transdermal testosterone replacement therapy. Clin Endocrinol (Oxf). 1999;51(6):765-9.
303. Croston GE, Milan LB, Marschke KB, Reichman M, Briggs MR. Androgen receptor-mediated antagonism of estrogen-dependent low density lipoprotein receptor transcription in cultured hepatocytes. Endocrinology. 1997;138(9):3779-86.
304. Iverius PH, Brunzell JD. Relationship between lipoprotein lipase activity and plasma sex steroid level in obese women. J Clin Invest. 1988;82(3):1106-12.
305. Legro RS, Kunselman AR, Dunaif A. Prevalence and predictors of dyslipidemia in women with polycystic ovary syndrome. Am J Med. 2001;111(8):607-13.
306. Taghibiglou C, Carpentier A, Van Iderstine SC, Chen B, Rudy D, Aiton A, et al. Mechanisms of hepatic very low density lipoprotein overproduction in insulin resistance. Evidence for enhanced lipoprotein assembly, reduced intracellular ApoB degradation, and increased microsomal triglyceride transfer protein in a fructose-fed hamster model. J Biol Chem. 2000;275(12):8416-25.
307. Diamanti-Kandarakis E, Papavassiliou AG, Kandarakis SA, Chrousos GP. Pathophysiology and types of dyslipidemia in PCOS. Trends Endocrinol Metab. 2007;18(7):280-5.
308. Niu Z, Lin N, Gu R, Sun Y, Feng Y. Associations between insulin resistance, free fatty acids, and oocyte quality in polycystic ovary syndrome during in vitro fertilization. J Clin Endocrinol Metab. 2014;99(11):E2269-76.
309. Zhang J, Zhang Y, Liu H, Bai H, Wang Y, Jiang C, et al. Antioxidant properties of high-density lipoproteins are impaired in women with polycystic ovary syndrome. Fertil Steril. 2015;103(5):1346-54.
310. Fernandez-Sanchez A, Madrigal-Santillan E, Bautista M, Esquivel-Soto J, Morales-Gonzalez A, Esquivel-Chirino C, et al. Inflammation, oxidative stress, and obesity. Int J Mol Sci. 2011;12(5):3117-32.
311. Macut D, Bjekic-Macut J, Savic-Radojevic A. Dyslipidemia and oxidative stress in PCOS. Front Horm Res. 2013;40:51-63.
312. Glintborg D. Endocrine and metabolic characteristics in polycystic ovary syndrome. Dan Med J. 2016;63(4).
313. Boulman N, Levy Y, Leiba R, Shachar S, Linn R, Zinder O, et al. Increased C-reactive protein levels in the polycystic ovary syndrome: a marker of cardiovascular disease. J Clin Endocrinol Metab. 2004;89(5):2160-5.
314. Gao L, Gu Y, Yin X. High serum tumor necrosis factor-alpha levels in women with polycystic ovary syndrome: a meta-analysis. PLoS One. 2016;11(10):e0164021.
315. Escobar-Morreale HF, Botella-Carretero JI, Villuendas G, Sancho J, San Millan JL. Serum interleukin-18 concentrations are increased in the polycystic ovary syndrome: relationship to insulin resistance and to obesity. J Clin Endocrinol Metab. 2004;89(2):806-11.
316. Marciniak A, Nawrocka Rutkowska J, Brodowska A, Wisniewska B, Starczewski A. Cardiovascular system diseases in patients with polycystic ovary syndrome - the role of inflammation process in this pathology and possibility of early diagnosis and prevention. Ann Agric Environ Med. 2016;23(4):537-41.
317. Verit FF. High sensitive serum C-reactive protein and its relationship with other cardiovascular risk factors in normoinsulinemic polycystic ovary patients without metabolic syndrome. Arch Gynecol Obstet. 2010;281(6):1009-14.
318. Oh JY, Lee JA, Lee H, Oh JY, Sung YA, Chung H. Serum C-reactive protein levels in normal-weight polycystic ovary syndrome. Korean J Intern Med. 2009;24(4):350-5.
319. Kalyan S, Goshtesabi A, Sarray S, Joannou A, Almawi WY. Assessing C reactive protein/albumin ratio as a new biomarker for polycystic ovary syndrome: a case-control study of women from Bahraini medical clinics. BMJ Open. 2018;8(10):e021860.
320. Sathyapalan T, Atkin SL. Recent advances in cardiovascular aspects of polycystic ovary syndrome. Eur J Endocrinol. 2012;166(4):575-83.

321. Meun C, Gunning MN, Louwers YV, Peters H, Roos-Hesselink J, Roeters van Lennep J, et al. The cardiovascular risk profile of middle-aged women with polycystic ovary syndrome. Clin Endocrinol (Oxf). 2020;92(2):150-8.
322. Dokras A. Cardiovascular disease risk factors in polycystic ovary syndrome. Semin Reprod Med. 2008;26(1):39-44.
323. Toulis KA, Goulis DG, Mintziori G, Kintiraki E, Eukarpidis E, Mouratoglou SA, et al. Meta-analysis of cardiovascular disease risk markers in women with polycystic ovary syndrome. Hum Reprod Update. 2011;17(6):741-60.
324. Turkcuoglu I, Engin-Ustun Y, Turan F, Kali Z, Karabulut AB, Meydanli M, et al. Evaluation of asymmetric dimethylarginine, nitric oxide levels and associated independent variables in obese and lean patients with polycystic ovarian syndrome. Gynecol Endocrinol. 2011;27(9):609-14.
325. Heutling D, Schulz H, Nickel I, Kleinstein J, Kaltwasser P, Westphal S, et al. Asymmetrical dimethylarginine, inflammatory and metabolic parameters in women with polycystic ovary syndrome before and after metformin treatment. J Clin Endocrinol Metab. 2008;93(1):82-90.
326. Sylus AM, Nandeesha H, Chitra T. Matrix metalloproteinase-9 increases and Interleukin-10 reduces with increase in body mass index in polycystic ovary syndrome: A cross-sectional study. Int J Reprod Biomed. 2020;18(8):605-10.
327. Jabbour R, Ott J, Eppel W, Frigo P. Carotid intima-media thickness in polycystic ovary syndrome and its association with hormone and lipid profiles. PLoS One. 2020;15(4):e0232299.
328. Bicer M, Guler A, Unal Kocabas G, Imamoglu C, Baloglu A, Bilgir O, et al. Endocan is a predictor of increased cardiovascular risk in women with polycystic ovary syndrome. Endocr Res. 2017;42(2):145-53.
329. Calan M, Guler A, Unal Kocabas G, Alarslan P, Bicer M, Imamoglu C, et al. Association of kallistatin with carotid intima-media thickness in women with polycystic ovary syndrome. Minerva Endocrinol. 2018;43(3):236-45.
330. Karbek B, Ozbek M, Karakose M, Topaloglu O, Bozkurt NC, Cakir E, et al. Copeptin, a surrogate marker for arginine vasopressin, is associated with cardiovascular risk in patients with polycystic ovary syndrome. J Ovarian Res. 2014;7:31.
331. Taskin MI, Bulbul E, Adali E, Hismiogullari AA, Inceboz U. Circulating levels of obestatin and copeptin in obese and nonobese women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol. 2015;189:19-23.
332. Schindler AE. Progestogen deficiency and endometrial cancer risk. Maturitas. 2009;62(4):334-7.
333. Cheung AP. Ultrasound and menstrual history in predicting endometrial hyperplasia in polycystic ovary syndrome. Obstet Gynecol. 2001;98(2):325-31.
334. Savaris RF, Groll JM, Young SL, DeMayo FJ, Jeong JW, Hamilton AE, et al. Progesterone resistance in PCOS endometrium: a microarray analysis in clomiphene citrate-treated and artificial menstrual cycles. J Clin Endocrinol Metab. 2011;96(6):1737-46.
335. Eagleson CA, Gingrich MB, Pastor CL, Arora TK, Burt CM, Evans WS, et al. Polycystic ovarian syndrome: evidence that flutamide restores sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone. J Clin Endocrinol Metab. 2000;85(11):4047-52.
336. Apparao KB, Lovely LP, Gui Y, Lininger RA, Lessey BA. Elevated endometrial androgen receptor expression in women with polycystic ovarian syndrome. Biol Reprod. 2002;66(2):297-304.
337. Lin J, Lei ZM, Lojun S, Rao CV, Satyaswaroop PG, Day TG. Increased expression of luteinizing hormone/human chorionic gonadotropin receptor gene in human endometrial carcinomas. J Clin Endocrinol Metab. 1994;79(5):1483-91.
338. Matalliotakis I, Kourtis A, Koukoura O, Panidis D. Polycystic ovary syndrome: etiology and pathogenesis. Arch Gynecol Obstet. 2006;274(4):187-97.
339. Schildkraut JM, Schwingl PJ, Bastos E, Evanoff A, Hughes C. Epithelial ovarian cancer risk among women with polycystic ovary syndrome. Obstetrics & Gynecology. 1996;88(4, Part 1):554-9.

340. Moran LJ, Hutchison SK, Norman RJ, Teede HJ. Lifestyle changes in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2011(2):CD007506.
341. Domecq JP, Prutsky G, Mullan RJ, Hazem A, Sundaresh V, Elamin MB, et al. Lifestyle modification programs in polycystic ovary syndrome: systematic review and meta-analysis. The Journal of Clinical Endocrinology and Metabolism. 2013;98(12):4655-63.
342. Kiddy DS, Sharp PS, White DM, Scanlon MF, Mason HD, Bray CS, et al. Differences in clinical and endocrine features between obese and non-obese subjects with polycystic ovary syndrome: an analysis of 263 consecutive cases. Clin Endocrinol (Oxf). 1990;32(2):213-20.
343. Yildirim B, Sabir N, Kaleli B. Relation of intra-abdominal fat distribution to metabolic disorders in nonobese patients with polycystic ovary syndrome. Fertil Steril. 2003;79(6):1358-64.
344. Ndefo UA, Eaton A, Green MR. Polycystic ovary syndrome: a review of treatment options with a focus on pharmacological approaches. P T. 2013;38(6):336-55.
345. Kiddy DS, Hamilton-Fairley D, Bush A, Short F, Anyaoku V, Reed MJ, et al. Improvement in endocrine and ovarian function during dietary treatment of obese women with polycystic ovary syndrome. Clin Endocrinol (Oxf). 1992;36(1):105-11.
346. Lee S, Kuk JL, Davidson LE, Hudson R, Kilpatrick K, Graham TE, et al. Exercise without weight loss is an effective strategy for obesity reduction in obese individuals with and without type 2 diabetes. J Appl Physiol (1985). 2005;99(3):1220-5.
347. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Human Reproduction. 2018;33(9):1602-18.
348. Moran LJ, Pasquali R, Teede HJ, Hoeger KM, Norman RJ. Treatment of obesity in polycystic ovary syndrome: a position statement of the androgen excess and polycystic ovary syndrome society. Fertility and Sterility. 2009;92(6):1966-82.
349. Mavropoulos JC, Yancy WS, Hepburn J, Westman EC. The effects of a low-carbohydrate, ketogenic diet on the polycystic ovary syndrome: a pilot study. Nutr Metab (Lond). 2005;2:35.
350. Gower BA, Chandler-Laney PC, Ovalle F, Goree LL, Azziz R, Desmond RA, et al. Favourable metabolic effects of a eucaloric lower-carbohydrate diet in women with PCOS. Clin Endocrinol (Oxf). 2013;79(4):550-7.
351. Goss AM, Chandler-Laney PC, Ovalle F, Goree LL, Azziz R, Desmond RA, et al. Effects of a eucaloric reduced-carbohydrate diet on body composition and fat distribution in women with PCOS. Metabolism. 2014;63(10):1257-64.
352. McGrice M, Porter J. The effect of low carbohydrate diets on fertility hormones and outcomes in overweight and obese women: a systematic review. Nutrients. 2017;9(3).
353. Paoli A, Mancin L, Giacona MC, Bianco A, Caprio M. Effects of a ketogenic diet in overweight women with polycystic ovary syndrome. J Transl Med. 2020;18(1):104.
354. Douglas CC, Gower BA, Darnell BE, Ovalle F, Oster RA, Azziz R. Role of diet in the treatment of polycystic ovary syndrome. Fertil Steril. 2006;85(3):679-88.
355. Moran LJ, Ko H, Misso M, Marsh K, Noakes M, Talbot M, et al. Dietary composition in the treatment of polycystic ovary syndrome: a systematic review to inform evidence-based guidelines. J Acad Nutr Diet. 2013;113(4):520-45.
356. Harrison CL, Lombard CB, Moran LJ, Teede HJ. Exercise therapy in polycystic ovary syndrome: a systematic review. Hum Reprod Update. 2011;17(2):171-83.
357. Kite C, Lahart IM, Afzal I, Broom DR, Randeva H, Kyrou I, et al. Exercise, or exercise and diet for the management of polycystic ovary syndrome: a systematic review and meta-analysis. Systematic Reviews. 2019;8(1):51.
358. Mani H, Chudasama Y, Hadjiconstantinou M, Bodicoat DH, Edwardson C, Levy MJ, et al. Structured education programme for women with polycystic ovary syndrome: a randomised controlled trial. Endocr Connect. 2018;7(1):26-35.

359. Heller S, Lawton J, Amiel S, Cooke D, Mansell P, Brennan A, et al. Improving management of type 1 diabetes in the UK: the Dose Adjustment For Normal Eating (DAFNE) programme as a research test-bed A mixed-method analysis of the barriers to and facilitators of successful diabetes self-management, a health economic analysis, a cluster randomised controlled trial of different models of delivery of an educational intervention and the potential of insulin pumps and additional educator input to improve outcomes. Programme Grants for Applied Research. Southampton (UK)2014.
360. Khunti K, Gray LJ, Skinner T, Carey ME, Realf K, Dallosso H, et al. Effectiveness of a diabetes education and self management programme (DESMOND) for people with newly diagnosed type 2 diabetes mellitus: three year follow-up of a cluster randomised controlled trial in primary care. BMJ. 2012;344:e2333.
361. Guzick D. Polycystic ovary syndrome: symptomatology, pathophysiology, and epidemiology. Am J Obstet Gynecol. 1998;179(6 Pt 2):S89-S93.
362. Yildiz BO. Recent advances in the treatment of polycystic ovary syndrome. Expert Opin Investig Drugs. 2004;13(10):1295-305.
363. Jensterle M, Kravos NA, Ferjan S, Goricar K, Dolzan V, Janez A. Long-term efficacy of metformin in overweight-obese PCOS: longitudinal follow-up of retrospective cohort. Endocr Connect. 2020;9(1):44-54.
364. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest. 2001;108(8):1167-74.
365. Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. Clin Sci (Lond). 2012;122(6):253-70.
366. Wheaton WW, Weinberg SE, Hamanaka RB, Soberanes S, Sullivan LB, Anso E, et al. Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis. Elife. 2014;3:e02242.
367. Miller RA, Chu Q, Xie J, Foretz M, Viollet B, Birnbaum MJ. Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. Nature. 2013;494(7436):256-60.
368. Woo SL, Xu H, Li H, Zhao Y, Hu X, Zhao J, et al. Metformin ameliorates hepatic steatosis and inflammation without altering adipose phenotype in diet-induced obesity. PLoS One. 2014;9(3):e91111.
369. Geerling JJ, Boon MR, van der Zon GC, van den Berg SA, van den Hoek AM, Lombes M, et al. Metformin lowers plasma triglycerides by promoting VLDL-triglyceride clearance by brown adipose tissue in mice. Diabetes. 2014;63(3):880-91.
370. Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: an update. Ann Intern Med. 2002;137(1):25-33.
371. Pasquali R. Metformin in women with PCOS, pros. Endocrine. 2015;48(2):422-6.
372. Curi DD, Fonseca AM, Marcondes JA, Almeida JA, Bagnoli VR, Soares JM, Jr., et al. Metformin versus lifestyle changes in treating women with polycystic ovary syndrome. Gynecol Endocrinol. 2012;28(3):182-5.
373. Harborne LR, Sattar N, Norman JE, Fleming R. Metformin and weight loss in obese women with polycystic ovary syndrome: comparison of doses. The Journal of Clinical Endocrinology and Metabolism. 2005;90(8):4593-8.
374. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002;346(6):393-403.
375. Palomba S, Falbo A, Russo T, Manguso F, Tolino A, Zullo F, et al. Insulin sensitivity after metformin suspension in normal-weight women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2007;92(8):3128-35.
376. Akram T, Hasan S, Imran M, Karim A, Arslan M. Association of polycystic ovary syndrome with cardiovascular risk factors. Gynecol Endocrinol. 2010;26(1):47-53.
377. Kaya MG, Yildirim S, Calapkorur B, Akpek M, Unluhizarci K, Kelestimur F. Metformin improves endothelial function and carotid intima media thickness in patients with PCOS. Gynecol Endocrinol. 2015;31(5):401-5.
378. Mancini F, Cianciosi A, Reggiani GM, Facchinetti F, Battaglia C, de Aloysio D. Endothelial function and its relationship to leptin, homocysteine, and insulin resistance in lean and overweight eumenorrheic women and PCOS patients: a pilot study. Fertility and Sterility. 2009;91(6):2537-44.
379. Mather KJ, Kwan F, Corenblum B. Hyperinsulinemia in polycystic ovary syndrome correlates with increased cardiovascular risk independent of obesity. Fertil Steril. 2000;73(1):150-6.

380. Kim JJ, Choi YM. Dyslipidemia in women with polycystic ovary syndrome. Obstet Gynecol Sci. 2013;56(3):137-42.
381. Wild RA. Dyslipidemia in PCOS. Steroids. 2012;77(4):295-9.
382. Sin HY, Kim JY, Jung KH. Total cholesterol, high density lipoprotein and triglyceride for cardiovascular disease in elderly patients treated with metformin. Arch Pharm Res. 2011;34(1):99-107.
383. Fleming R, Hopkinson ZE, Wallace AM, Greer IA, Sattar N. Ovarian function and metabolic factors in women with oligomenorrhea treated with metformin in a randomized double blind placebo-controlled trial. The Journal of Clinical Endocrinology and Metabolism. 2002;87(2):569-74.
384. Ng EH, Wat NM, Ho PC. Effects of metformin on ovulation rate, hormonal and metabolic profiles in women with clomiphene-resistant polycystic ovaries: a randomized, double-blinded placebo-controlled trial. Hum Reprod. 2001;16(8):1625-31.
385. Sharpe A, Morley LC, Tang T, Norman RJ, Balen AH. Metformin for ovulation induction (excluding gonadotrophins) in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2019;12:CD013505.
386. Duleba AJ. Medical management of metabolic dysfunction in PCOS. Steroids. 2012;77(4):306-11.
387. Herbert L, Ribar A, Mitchell S, Phillips C. Discovering metformin-induced vitamin B12 deficiency in patients with type 2 diabetes in primary care. J Am Assoc Nurse Pract. 2019.
388. Longo SL, Ryan JM, Sheehan KB, Reid DJ, Conley MP, Bouwmeester CJ. Evaluation of vitamin B12 monitoring in patients on metformin in urban ambulatory care settings. Pharm Pract (Granada). 2019;17(3):1499.
389. Alvarez M, Sierra OR, Saavedra G, Moreno S. Vitamin B12 deficiency and diabetic neuropathy in patients taking metformin: a cross-sectional study. Endocr Connect. 2019;8(10):1324-9.
390. Alshammari AN, Iqbal R, Baksh IP. Vitamin B12 deficiency and the knowledge and practice of physicians regarding screening for vitamin B12 deficiency among type 2 diabetic patients on metformin in selected hospitals in Riyadh, Saudi Arabia. J Family Med Prim Care. 2019;8(7):2306-11.
391. Spiegelman BM. PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. Diabetes. 1998;47(4):507-14.
392. Brettenthaler N, De Geyter C, Huber PR, Keller U. Effect of the insulin sensitizer pioglitazone on insulin resistance, hyperandrogenism, and ovulatory dysfunction in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2004;89(8):3835-40.
393. Xu Y, Wu Y, Huang Q. Comparison of the effect between pioglitazone and metformin in treating patients with PCOS:a meta-analysis. Arch Gynecol Obstet. 2017;296(4):661-77.
394. Cho LW, Kilpatrick ES, Keevil BG, Coady AM, Atkin SL. Effect of metformin, orlistat and pioglitazone treatment on mean insulin resistance and its biological variability in polycystic ovary syndrome. Clin Endocrinol (Oxf). 2009;70(2):233-7.
395. Jearath V, Vashisht R, Rustagi V, Raina S, Sharma R. Pioglitazone-induced congestive heart failure and pulmonary edema in a patient with preserved ejection fraction. J Pharmacol Pharmacother. 2016;7(1):41-3.
396. Zeymer U. Cardiovascular benefits of acarbose in impaired glucose tolerance and type 2 diabetes. Int J Cardiol. 2006;107(1):11-20.
397. Hanefeld M, Schaper F. Acarbose: oral anti-diabetes drug with additional cardiovascular benefits. Expert Rev Cardiovasc Ther. 2008;6(2):153-63.
398. Rezai M, Jamshidi M, Mohammadbeigi R, Seyedoshohadaei F, Mohammadipour S, Moradi G. Comparing the effect of metformin and acarbose accompanying clomiphene on the successful ovulation induction in infertile women with polycystic ovary syndrome. Glob J Health Sci. 2016;8(9):54516.
399. Rosen MP, Breitkopf DM, Nagamani M. A randomized controlled trial of second- versus third-generation oral contraceptives in the treatment of acne vulgaris. Am J Obstet Gynecol. 2003;188(5):1158-60.
400. Wiegratz I, Jung-Hoffmann C, Kuhl H. Effect of two oral contraceptives containing ethinylestradiol and gestodene or norgestimate upon androgen parameters and serum binding proteins. Contraception. 1995;51(6):341-6.

401. Porcile A, Gallardo E. Long-term treatment of hirsutism: desogestrel compared with cyproterone acetate in oral contraceptives. Fertil Steril. 1991;55(5):877-81.
402. Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes. 1989;38(9):1165-74.
403. Nader S, Riad-Gabriel MG, Saad MF. The effect of a desogestrel-containing oral contraceptive on glucose tolerance and leptin concentrations in hyperandrogenic women. J Clin Endocrinol Metab. 1997;82(9):3074-7.
404. Morin-Papunen LC, Vauhkonen I, Koivunen RM, Ruokonen A, Martikainen HK, Tapanainen JS. Endocrine and metabolic effects of metformin versus ethinyl estradiol-cyproterone acetate in obese women with polycystic ovary syndrome: a randomized study. J Clin Endocrinol Metab. 2000;85(9):3161-8.
405. Morin-Papunen L, Vauhkonen I, Koivunen R, Ruokonen A, Martikainen H, Tapanainen JS. Metformin versus ethinyl estradiol-cyproterone acetate in the treatment of nonobese women with polycystic ovary syndrome: a randomized study. J Clin Endocrinol Metab. 2003;88(1):148-56.
406. Cibula D. Is insulin resistance an essential component of PCOS?: The influence of confounding factors. Hum Reprod. 2004;19(4):757-9.
407. Cullberg G, Hamberger L, Mattsson LA, Mobacken H, Samsioe G. Lipid metabolic studies in women with a polycystic ovary syndrome during treatment with a low-dose desogestrel-ethinylestradiol combination. Acta Obstet Gynecol Scand. 1985;64(3):203-7.
408. Hoeger K, Davidson K, Kochman L, Cherry T, Kopin L, Guzick DS. The impact of metformin, oral contraceptives, and lifestyle modification on polycystic ovary syndrome in obese adolescent women in two randomized, placebo-controlled clinical trials. J Clin Endocrinol Metab. 2008;93(11):4299-306.
409. Villaseca P, Hormaza P, Cardenas I, Oestreicher E, Arteaga E. Ethinylestradiol/cyproterone acetate in polycystic ovary syndrome: lipid and carbohydrate changes. Eur J Contracept Reprod Health Care. 2004;9(3):155-65.
410. Guido M, Romualdi D, Giuliani M, Suriano R, Selvaggi L, Apa R, et al. Drospirenone for the treatment of hirsute women with polycystic ovary syndrome: a clinical, endocrinological, metabolic pilot study. J Clin Endocrinol Metab. 2004;89(6):2817-23.
411. Mastorakos G, Koliopoulos C, Creatsas G. Androgen and lipid profiles in adolescents with polycystic ovary syndrome who were treated with two forms of combined oral contraceptives. Fertil Steril. 2002;77(5):919-27.
412. Vrbikova J, Stanicka S, Dvorakova K, Hill M, Vondra K, Bendlova B, et al. Metabolic and endocrine effects of treatment with peroral or transdermal oestrogens in conjunction with peroral cyproterone acetate in women with polycystic ovary syndrome. Eur J Endocrinol. 2004;150(2):215-23.
413. Luque-Ramirez M, Alvarez-Blasco F, Botella-Carretero JI, Martinez-Bermejo E, Lasuncion MA, Escobar-Morreale HF. Comparison of ethinyl-estradiol plus cyproterone acetate versus metformin effects on classic metabolic cardiovascular risk factors in women with the polycystic ovary syndrome. J Clin Endocrinol Metab. 2007;92(7):2453-61.
414. Sanam M, Ziba O. Desogestrel+ethinylestradiol versus levonorgestrel+ethinylestradiol. Which one has better affect on acne, hirsutism, and weight change. Saudi Med J. 2011;32(1):23-6.
415. Guido M, Romualdi D, Campagna G, Ricciardi L, Bompiani A, Lanzone A. Ethinylestradiol-chlormadinone acetate combination for the treatment of hirsutism and hormonal alterations of normal-weight women with polycystic ovary syndrome: evaluation of the metabolic impact. Reprod Sci. 2010;17(8):767-75.
416. Menshawy A, Ismail A, Abdel-Maboud M, El-Din AA, Elgebaly A, Gadelkarim M, et al. Effect of chlormadinone acetate versus drospirenone-containing oral contraceptives on the endocrinal features of women with polycystic ovary syndrome: Systematic review and meta-analysis of randomized clinical trials. J Gynecol Obstet Hum Reprod. 2019;48(9):763-70.
417. Amiri M, Kabir A, Nahidi F, Shekofteh M, Ramezani Tehrani F. Effects of combined oral contraceptives on the clinical and biochemical parameters of hyperandrogenism in patients with polycystic ovary syndrome: a systematic review and meta-analysis. Eur J Contracept Reprod Health Care. 2018;23(1):64-77.

418. Amiri M, Ramezani Tehrani F, Nahidi F, Kabir A, Azizi F. Comparing the effects of combined oral contraceptives containing progestins with low androgenic and antiandrogenic activities on the hypothalamic-pituitary-gonadal axis in patients with polycystic ovary syndrome: Systematic Review and Meta-Analysis. JMIR Res Protoc. 2018;7(4):e113.
419. Karagiannis A, Athyros VG, Mikhailidis DP. A comparison of the aldosterone-blocking agents eplerenone and spironolactone. Clin Cardiol. 2009;32(4):230.
420. Struthers A, Krum H, Williams GH. A comparison of the aldosterone-blocking agents eplerenone and spironolactone. Clin Cardiol. 2008;31(4):153-8.
421. Serafini PC, Catalino J, Lobo RA. The effect of spironolactone on genital skin 5α-reductase activity. Journal of Steroid Biochemistry. 1985;23(2):191-4.
422. Barrionuevo P, Nabhan M, Altayar O, Wang Z, Erwin PJ, Asi N, et al. Treatment options for hirsutism: a systematic review and network meta-analysis. J Clin Endocrinol Metab. 2018;103(4):1258-64.
423. Swiglo BA, Cosma M, Flynn DN, Kurtz DM, Labella ML, Mullan RJ, et al. Clinical review: antiandrogens for the treatment of hirsutism: a systematic review and metaanalyses of randomized controlled trials. J Clin Endocrinol Metab. 2008;93(4):1153-60.
424. Zillich AJ, Carter BL. Eplerenone--a novel selective aldosterone blocker. Ann Pharmacother. 2002;36(10):1567-76.
425. De Leo V, Lanzetta D, D'Antona D, la Marca A, Morgante G. Hormonal effects of flutamide in young women with polycystic ovary syndrome. J Clin Endocrinol Metab. 1998;83(1):99-102.
426. Paradisi R, Fabbri R, Battaglia C, Venturoli S. Ovulatory effects of flutamide in the polycystic ovary syndrome. Gynecol Endocrinol. 2013;29(4):391-5.
427. Mysore V, Shashikumar BM. Guidelines on the use of finasteride in androgenetic alopecia. Indian J Dermatol Venereol Leprol. 2016;82(2):128-34.
428. Eicheler W, Dreher M, Hoffmann R, Happle R, Aumuller G. Immunohistochemical evidence for differential distribution of 5 alpha-reductase isoenzymes in human skin. Br J Dermatol. 1995;133(3):371-6.
429. Bayne EK, Flanagan J, Einstein M, Ayala J, Chang B, Azzolina B, et al. Immunohistochemical localization of types 1 and 2 5alpha-reductase in human scalp. Br J Dermatol. 1999;141(3):481-91.
430. Thiboutot D, Bayne E, Thorne J, Gilliland K, Flanagan J, Shao Q, et al. Immunolocalization of 5alpha-reductase isozymes in acne lesions and normal skin. Arch Dermatol. 2000;136(9):1125-9.
431. Iamsumang W, Leerunyakul K, Suchonwanit P. Finasteride and its potential for the treatment of female pattern hair loss: Evidence to date. Drug Des Devel Ther. 2020;14:951-9.
432. Ruan X, Kubba A, Aguilar A, Mueck AO. Use of cyproterone acetate/ethinylestradiol in polycystic ovary syndrome: rationale and practical aspects. Eur J Contracept Reprod Health Care. 2017;22(3):183-90.
433. Reed M, Franks S. 4 Anti-androgens in gynaecological practice. Baillière's Clinical Obstetrics and Gynaecology. 1988;2(3):581-95.
434. Von Hofe J, Bates GW. Ovulation induction. Obstet Gynecol Clin North Am. 2015;42(1):27-37.
435. Moskovic DJ, Katz DJ, Akhavan A, Park K, Mulhall JP. Clomiphene citrate is safe and effective for long-term management of hypogonadism. BJU Int. 2012;110(10):1524-8.
436. Costello MF, Misso ML, Balen A, Boyle J, Devoto L, Garad RM, et al. Evidence summaries and recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome: assessment and treatment of infertility. Hum Reprod Open. 2019;2019(1):hoy021.
437. Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA, et al. Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. N Engl J Med. 2007;356(6):551-66.
438. Fertility: assessment and treatment for people with fertility problems. National Institute for Health and Clinical Excellence: Guidance. London2013.
439. Elkhateeb RR, Mahran AE, Kamel HH. Long-term use of clomiphene citrate in induction of ovulation in PCO patients with clomiphene citrate resistance. J Gynecol Obstet Hum Reprod. 2017;46(7):575-7.

440. Kivrak MB, Corum O, Alkan H, Atik O, Aydin I, Uney K. The pharmacokinetics of letrozole and its effect on gonadotropins in anestrous ewes. Theriogenology. 2021;176:225-32.
441. Bulun SE, Lin Z, Imir G, Amin S, Demura M, Yilmaz B, et al. Regulation of aromatase expression in estrogen-responsive breast and uterine disease: from bench to treatment. Pharmacol Rev. 2005;57(3):359-83.
442. Amer SA, Smith J, Mahran A, Fox P, Fakis A. Double-blind randomized controlled trial of letrozole versus clomiphene citrate in subfertile women with polycystic ovarian syndrome. Hum Reprod. 2017;32(8):1631-8.
443. Sakar MN, Oglak SC. Letrozole is superior to clomiphene citrate in ovulation induction in patients with polycystic ovary syndrome. Pak J Med Sci. 2020;36(7):1460-5.
444. Baird DT. A model for follicular selection and ovulation: lessons from superovulation. J Steroid Biochem. 1987;27(1-3):15-23.
445. Cunha A, Povoa AM. Infertility management in women with polycystic ovary syndrome: a review. Porto Biomed J. 2021;6(1):e116.
446. D'Amato G, Caroppo E, Pasquadibisceglie A, Carone D, Vitti A, Vizziello GM. A novel protocol of ovulation induction with delayed gonadotropin-releasing hormone antagonist administration combined with high-dose recombinant follicle-stimulating hormone and clomiphene citrate for poor responders and women over 35 years. Fertil Steril. 2004;81(6):1572-7.
447. Weiss NS, Kostova E, Nahuis M, Mol BWJ, van der Veen F, van Wely M. Gonadotrophins for ovulation induction in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2019;1:CD010290.
448. Bordewijk EM, Nahuis M, Costello MF, Van der Veen F, Tso LO, Mol BW, et al. Metformin during ovulation induction with gonadotrophins followed by timed intercourse or intrauterine insemination for subfertility associated with polycystic ovary syndrome. Cochrane Database Syst Rev. 2017;1:CD009090.
449. Sun B, Ma Y, Li L, Hu L, Wang F, Zhang Y, et al. Factors associated with ovarian hyperstimulation syndrome (OHSS) severity in women with polycystic ovary syndrome undergoing IVF/ICSI. Front Endocrinol (Lausanne). 2020;11:615957.
450. Franik S, Eltrop SM, Kremer JA, Kiesel L, Farquhar C. Aromatase inhibitors (letrozole) for subfertile women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2018;5:CD010287.
451. Ratts VS, Pauls RN, Pinto AB, Kraja A, Williams DB, Odem RR. Risk of multiple gestation after ovulation induction in polycystic ovary syndrome. J Reprod Med. 2007;52(10):896-900.
452. Delcour C, Robin G, Young J, Dewailly D. PCOS and Hyperprolactinemia: what do we know in 2019? Clin Med Insights Reprod Health. 2019;13:1179558119871921.
453. Davoudi Z, Araghi F, Vahedi M, Mokhtari N, Gheisari M. Prolactin level in polycystic ovary syndrome (PCOS): An approach to the diagnosis and management. Acta Biomed. 2021;92(5):e2021291.
454. Tripathy S, Mohapatra S, M M, Chandrasekhar A. Induction of ovulation with clomiphene citrate versus clomiphene with bromocriptine in PCOS patients with normal prolactin: a comparative study. J Clin Diagn Res. 2013;7(11):2541-3.
455. Vanky E, Salvesen KA, Carlsen SM. Six-month treatment with low-dose dexamethasone further reduces androgen levels in PCOS women treated with diet and lifestyle advice, and metformin. Hum Reprod. 2004;19(3):529-33.
456. Basirat Z, Zeinalzadeh M, Mahouti T, Golsorkhtabaramiri M. Impact of dexamethasone on pregnancy outcome in PCOs women candidate for IVF/ICSI, a single-blind randomized clinical trial study. Middle East Fertility Society Journal. 2016;21(3):184-8.
457. Parsanezhad ME, Alborzi S, Motazedian S, Omrani G. Use of dexamethasone and clomiphene citrate in the treatment of clomiphene citrate-resistant patients with polycystic ovary syndrome and normal dehydroepiandrosterone sulfate levels: a prospective, double-blind, placebo-controlled trial. Fertil Steril. 2002;78(5):1001-4.
458. Kodaman PH, Duleba AJ. HMG-CoA reductase inhibitors: do they have potential in the treatment of polycystic ovary syndrome? Drugs. 2008;68(13):1771-85.

459. Sathyapalan T, Atkin SL. Evidence for statin therapy in polycystic ovary syndrome. Ther Adv Endocrinol Metab. 2010;1(1):15-22.
460. Kodaman PH, Duleba AJ. Statins in the treatment of polycystic ovary syndrome. Semin Reprod Med. 2008;26(1):127-38.
461. Sathyapalan T, Kilpatrick ES, Coady AM, Atkin SL. The effect of atorvastatin in patients with polycystic ovary syndrome: a randomized double-blind placebo-controlled study. J Clin Endocrinol Metab. 2009;94(1):103-8.
462. Sathyapalan T, Kilpatrick ES, Coady AM, Atkin SL. Atorvastatin pretreatment augments the effect of metformin in patients with polycystic ovary syndrome (PCOS). Clin Endocrinol (Oxf). 2010;72(4):566-8.
463. Sathyapalan T, Hobkirk JP, Javed Z, Carroll S, Coady AM, Pemberton P, et al. The Effect of atorvastatin (and subsequent metformin) on adipose tissue acylation-stimulatory-protein concentration and inflammatory biomarkers in overweight/obese women with polycystic ovary syndrome. Front Endocrinol (Lausanne). 2019;10:394.
464. Sathyapalan T, Coady AM, Kilpatrick ES, Atkin SL. The effect of atorvastatin on pancreatic beta cell requirement in women with polycystic ovary syndrome. Endocrine Connections. 2017;6(8):811-6.
465. Sathyapalan T, Shepherd J, Coady AM, Kilpatrick ES, Atkin SL. Atorvastatin reduces malondialdehyde concentrations in patients with polycystic ovary syndrome. The Journal of Clinical Endocrinology and Metabolism. 2012;97(11):3951-5.
466. Sathyapalan T, Smith KA, Coady AM, Kilpatrick ES, Atkin SL. Atorvastatin therapy decreases androstenedione and dehydroepiandrosterone sulphate concentrations in patients with polycystic ovary syndrome: randomized controlled study. Ann Clin Biochem. 2012;49(Pt 1):80-5.
467. Johnson S, Schwartz SM. Pharmacologic and pharmacodynamic equivalence of 2 formulations of orlistat. Clin Pharmacol Drug Dev. 2018;7(7):773-80.
468. Jayagopal V, Kilpatrick ES, Holding S, Jennings PE, Atkin SL. Orlistat is as beneficial as metformin in the treatment of polycystic ovarian syndrome. J Clin Endocrinol Metab. 2005;90(2):729-33.
469. Kumar P, Arora S. Orlistat in polycystic ovarian syndrome reduces weight with improvement in lipid profile and pregnancy rates. J Hum Reprod Sci. 2014;7(4):255-61.
470. Panda SR, Jain M, Jain S, Saxena R, Hota S. Effect of orlistat versus metformin in various aspects of polycystic ovarian syndrome: a systematic review of randomized control trials. J Obstet Gynaecol India. 2018;68(5):336-43.
471. Salehpour S, Hosseini S, Nazari L, Saharkhiz N, Zademodarres S. Effects of orlistat on serum androgen levels among iranian obese women with polycystic ovarian syndrome. JBRA Assist Reprod. 2018;22(3):180-4.
472. Gillies CL, Abrams KR, Lambert PC, Cooper NJ, Sutton AJ, Hsu RT, et al. Pharmacological and lifestyle interventions to prevent or delay type 2 diabetes in people with impaired glucose tolerance: systematic review and meta-analysis. BMJ. 2007;334(7588):299.
473. Kujawska-Luczak M, Szulinska M, Skrypnik D, Musialik K, Swora-Cwynar E, Kregielska-Narozna M, et al. The influence of orlistat, metformin and diet on serum levels of insulin-like growth factor-1 in obeses women with and without insulin resistance. J Physiol Pharmacol. 2018;69(5).
474. McDuffie JR, Calis KA, Booth SL, Uwaifo GI, Yanovski JA. Effects of orlistat on fat-soluble vitamins in obese adolescents. Pharmacotherapy. 2002;22(7):814-22.
475. Elfhag K, Rossner S, Carlsson AM, Barkeling B. Sibutramine treatment in obesity: predictors of weight loss including rorschach personality data. Obes Res. 2003;11(11):1391-9.
476. Rolls BJ, Shide DJ, Thorwart ML, Ulbrecht JS. Sibutramine reduces food intake in non-dieting women with obesity. Obes Res. 1998;6(1):1-11.
477. Lindholm Å, Bixo M, Björn I, Wölner-Hanssen P, Eliasson M, Larsson A, et al. Effect of sibutramine on weight reduction in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Fertility and Sterility. 2008;89(5):1221-8.
478. Florakis D, Diamanti-Kandarakis E, Katsikis I, Nassis G, Karkanaki A, Georgopoulos N, et al. Effect of hypocaloric diet plus sibutramine treatment on hormonal and metabolic features in overweight and obese

women with polycystic ovary syndrome: a randomized, 24-week study. International Journal of Obesity. 2008;32(4):692-9.
479. James WP, Caterson ID, Coutinho W, Finer N, Van Gaal LF, Maggioni AP, et al. Effect of sibutramine on cardiovascular outcomes in overweight and obese subjects. N Engl J Med. 2010;363(10):905-17.
480. Dawson AJ, Kilpatrick ES, Coady AM, Elshewehy AMM, Dakroury Y, Ahmed L, et al. Endocannabinoid receptor blockade reduces alanine aminotransferase in polycystic ovary syndrome independent of weight loss. BMC Endocrine Disorders. 2017;17(1):41.
481. Sathyapalan T, Cho L, Kilpatrick ES, Le Roux CW, Coady AM, Atkin SL. Effect of rimonabant and metformin on glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 in obese women with polycystic ovary syndrome. Clin Endocrinol (Oxf). 2010;72(3):423-5.
482. Sathyapalan T, Cho LW, Kilpatrick ES, Coady AM, Atkin SL. Metformin maintains the weight loss and metabolic benefits following rimonabant treatment in obese women with polycystic ovary syndrome (PCOS). Clin Endocrinol (Oxf). 2009;70(1):124-8.
483. Sathyapalan T, Cho LW, Kilpatrick ES, Coady AM, Atkin SL. A comparison between rimonabant and metformin in reducing biochemical hyperandrogenaemia and insulin resistance in patients with polycystic ovary syndrome (PCOS): a randomized open-label parallel study. Clin Endocrinol (Oxf). 2008;69(6):931-5.
484. Moreira FA, Crippa JA. The psychiatric side-effects of rimonabant. Braz J Psychiatry. 2009;31(2):145-53.
485. Tek C. Naltrexone HCl/bupropion HCl for chronic weight management in obese adults: patient selection and perspectives. Patient Prefer Adherence. 2016;10:751-9.
486. Patel K, Allen S, Haque MN, Angelescu I, Baumeister D, Tracy DK. Bupropion: a systematic review and meta-analysis of effectiveness as an antidepressant. Ther Adv Psychopharmacol. 2016;6(2):99-144.
487. Greenway FL, Fujioka K, Plodkowski RA, Mudaliar S, Guttadauria M, Erickson J, et al. Effect of naltrexone plus bupropion on weight loss in overweight and obese adults (COR-I): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2010;376(9741):595-605.
488. Apovian CM, Aronne L, Rubino D, Still C, Wyatt H, Burns C, et al. A randomized, phase 3 trial of naltrexone SR/bupropion SR on weight and obesity-related risk factors (COR-II). Obesity (Silver Spring). 2013;21(5):935-43.
489. Wadden TA, Foreyt JP, Foster GD, Hill JO, Klein S, O'Neil PM, et al. Weight loss with naltrexone SR/bupropion SR combination therapy as an adjunct to behavior modification: the COR-BMOD trial. Obesity (Silver Spring). 2011;19(1):110-20.
490. Hollander P, Gupta AK, Plodkowski R, Greenway F, Bays H, Burns C, et al. Effects of naltrexone sustained-release/bupropion sustained-release combination therapy on body weight and glycemic parameters in overweight and obese patients with type 2 diabetes. Diabetes Care. 2013;36(12):4022-9.
491. Ahmed MI, Duleba AJ, El Shahat O, Ibrahim ME, Salem A. Naltrexone treatment in clomiphene resistant women with polycystic ovary syndrome. Hum Reprod. 2008;23(11):2564-9.
492. Fulghesu AM, Ciampelli M, Belosi C, Apa R, Guido M, Caruso A, et al. Naltrexone effect on pulsatile GnRH therapy for ovulation induction in polycystic ovary syndrome: a pilot prospective study. J Endocrinol Invest. 2001;24(7):483-90.
493. Creutzfeldt W. The incretin concept today. Diabetologia. 1979;16(2):75-85.
494. Papaetis GS. Incretin-based therapies in prediabetes: Current evidence and future perspectives. World J Diabetes. 2014;5(6):817-34.
495. Tzotzas T, Karras SN, Katsiki N. Glucagon-Like Peptide-1 (GLP-1) receptor agonists in the treatment of obese women with polycystic ovary syndrome. Curr Vasc Pharmacol. 2017;15(3):218-29.
496. Madsbad S. The role of glucagon-like peptide-1 impairment in obesity and potential therapeutic implications. Diabetes Obes Metab. 2014;16(1):9-21.
497. Ferjan S, Jensterle M, Oblak T, Zitnik IP, Marc J, Goricar K, et al. An impaired glucagon-like peptide-1 response is associated with prediabetes in polycystic ovary syndrome with obesity. J Int Med Res. 2019;47(10):4691-700.

498. Vrbikova J, Hill M, Bendlova B, Grimmichova T, Dvorakova K, Vondra K, et al. Incretin levels in polycystic ovary syndrome. Eur J Endocrinol. 2008;159(2):121-7.
499. Zhang F, Tang X, Cao H, Lu Q, Li N, Liu Y, et al. Impaired secretion of total glucagon-like peptide-1 in people with impaired fasting glucose combined impaired glucose tolerance. Int J Med Sci. 2012;9(7):574-81.
500. Garber AJ. Incretin effects on beta-cell function, replication, and mass: the human perspective. Diabetes Care. 2011;34 Suppl 2:S258-63.
501. Byrne MM, Gliem K, Wank U, Arnold R, Katschinski M, Polonsky KS, et al. Glucagon-like peptide 1 improves the ability of the beta-cell to sense and respond to glucose in subjects with impaired glucose tolerance. Diabetes. 1998;47(8):1259-65.
502. Yabe D, Seino Y, Seino Y. Incretin concept revised: The origin of the insulinotropic function of glucagon-like peptide-1 - the gut, the islets or both? J Diabetes Investig. 2018;9(1):21-4.
503. Crepaldi G, Carruba M, Comaschi M, Del Prato S, Frajese G, Paolisso G. Dipeptidyl peptidase 4 (DPP-4) inhibitors and their role in type 2 diabetes management. J Endocrinol Invest. 2007;30(7):610-4.
504. Hjerpsted JB, Flint A, Brooks A, Axelsen MB, Kvist T, Blundell J. Semaglutide improves postprandial glucose and lipid metabolism, and delays first-hour gastric emptying in subjects with obesity. Diabetes Obes Metab. 2018;20(3):610-9.
505. Triplitt C, Solis-Herrera C. GLP-1 Receptor agonists: practical considerations for clinical practice. Diabetes Educ. 2015;41(1 Suppl):32S-46S.
506. Abdalla MA, Deshmukh H, Atkin S, Sathyapalan T. A review of therapeutic options for managing the metabolic aspects of polycystic ovary syndrome. Ther Adv Endocrinol Metab. 2020;11:2042018820938305.
507. Alvarez E, Martinez MD, Roncero I, Chowen JA, Garcia-Cuartero B, Gispert JD, et al. The expression of GLP-1 receptor mRNA and protein allows the effect of GLP-1 on glucose metabolism in the human hypothalamus and brainstem. J Neurochem. 2005;92(4):798-806.
508. Farkas I, Vastagh C, Farkas E, Balint F, Skrapits K, Hrabovszky E, et al. Glucagon-like peptide-1 excites firing and increases GABAergic miniature postsynaptic currents (mPSCs) in gonadotropin-releasing hormone (GnRH) neurons of the male mice via activation of nitric oxide (NO) and suppression of endocannabinoid signaling pathways. Front Cell Neurosci. 2016;10:214.
509. Ma X, Bruning J, Ashcroft FM. Glucagon-like peptide 1 stimulates hypothalamic proopiomelanocortin neurons. J Neurosci. 2007;27(27):7125-9.
510. Lopez-Ferreras L, Richard JE, Noble EE, Eerola K, Anderberg RH, Olandersson K, et al. Lateral hypothalamic GLP-1 receptors are critical for the control of food reinforcement, ingestive behavior and body weight. Mol Psychiatry. 2018;23(5):1157-68.
511. Beiroa D, Imbernon M, Gallego R, Senra A, Herranz D, Villarroya F, et al. GLP-1 agonism stimulates brown adipose tissue thermogenesis and browning through hypothalamic AMPK. Diabetes. 2014;63(10):3346-58.
512. Heppner KM, Baquero AF, Bennett CM, Lindsley SR, Kirigiti MA, Bennett B, et al. GLP-1R signaling directly activates arcuate nucleus kisspeptin action in brain slices but does not rescue luteinizing hormone inhibition in ovariectomized mice during negative energy balance. eNeuro. 2017;4(1).
513. Outeirino-Iglesias V, Romani-Perez M, Gonzalez-Matias LC, Vigo E, Mallo F. GLP-1 Increases preovulatory LH source and the number of mature follicles, as well as synchronizing the onset of puberty in female rats. Endocrinology. 2015;156(11):4226-37.
514. Nishiyama Y, Hasegawa T, Fujita S, Iwata N, Nagao S, Hosoya T, et al. Incretins modulate progesterone biosynthesis by regulating bone morphogenetic protein activity in rat granulosa cells. J Steroid Biochem Mol Biol. 2018;178:82-8.
515. Jensterle M, Salamun V, Kocjan T, Vrtacnik Bokal E, Janez A. Short term monotherapy with GLP-1 receptor agonist liraglutide or PDE 4 inhibitor roflumilast is superior to metformin in weight loss in obese PCOS women: a pilot randomized study. J Ovarian Res. 2015;8:32.
516. McClenaghan NH. Physiological regulation of the pancreatic {beta}-cell: functional insights for understanding and therapy of diabetes. Exp Physiol. 2007;92(3):481-96.

517. Drucker DJ. Minireview: the glucagon-like peptides. Endocrinology. 2001;142(2):521-7.
518. Guo C, Huang T, Chen A, Chen X, Wang L, Shen F, et al. Glucagon-like peptide 1 improves insulin resistance in vitro through anti-inflammation of macrophages. Braz J Med Biol Res. 2016;49(12):e5826.
519. Jiang Y, Wang Z, Ma B, Fan L, Yi N, Lu B, et al. GLP-1 Improves adipocyte insulin sensitivity following induction of endoplasmic reticulum stress. Front Pharmacol. 2018;9:1168.
520. Parlevliet ET, de Leeuw van Weenen JE, Romijn JA, Pijl H. GLP-1 treatment reduces endogenous insulin resistance via activation of central GLP-1 receptors in mice fed a high-fat diet. Am J Physiol Endocrinol Metab. 2010;299(2):E318-24.
521. MacDonald PE, El-Kholy W, Riedel MJ, Salapatek AM, Light PE, Wheeler MB. The multiple actions of GLP-1 on the process of glucose-stimulated insulin secretion. Diabetes. 2002;51 Suppl 3:S434-42.
522. Smits MM, Tonneijck L, Muskiet MH, Kramer MH, Cahen DL, van Raalte DH. Gastrointestinal actions of glucagon-like peptide-1-based therapies: glycaemic control beyond the pancreas. Diabetes Obes Metab. 2016;18(3):224-35.
523. Meloni AR, DeYoung MB, Lowe C, Parkes DG. GLP-1 receptor activated insulin secretion from pancreatic beta-cells: mechanism and glucose dependence. Diabetes Obes Metab. 2013;15(1):15-27.
524. Buteau J, Roduit R, Susini S, Prentki M. Glucagon-like peptide-1 promotes DNA synthesis, activates phosphatidylinositol 3-kinase and increases transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) DNA binding activity in beta (INS-1)-cells. Diabetologia. 1999;42(7):856-64.
525. Buteau J, Foisy S, Rhodes CJ, Carpenter L, Biden TJ, Prentki M. Protein kinase Czeta activation mediates glucagon-like peptide-1-induced pancreatic beta-cell proliferation. Diabetes. 2001;50(10):2237-43.
526. Buteau J, Foisy S, Joly E, Prentki M. Glucagon-like peptide 1 induces pancreatic beta-cell proliferation via transactivation of the epidermal growth factor receptor. Diabetes. 2003;52(1):124-32.
527. He J, Cai L, Li J, Li Y, Tao X, Zhang Y. Exenatide Improves Endometrial Glands in PCOS Rats through AMPKα-SIRT1. 2020.
528. Tao X, Cai L, Chen L, Ge S, Deng X. Effects of metformin and Exenatide on insulin resistance and AMPKα-SIRT1 molecular pathway in PCOS rats. Journal of Ovarian Research. 2019;12(1):86.
529. Sun L, Ji C, Jin L, Bi Y, Feng W, Li P, et al. Effects of exenatide on metabolic changes, sexual hormones, inflammatory cytokines, adipokines, and weight change in a DHEA-treated rat model. Reprod Sci. 2016;23(9):1242-9.
530. Sekar R, Singh K, Arokiaraj AW, Chow BK. Pharmacological actions of glucagon-like peptide-1, gastric inhibitory polypeptide, and glucagon. Int Rev Cell Mol Biol. 2016;326:279-341.
531. Sekar R, Singh K, Arokiaraj A, Chow B. Pharmacological actions of glucagon-like peptide-1, gastric inhibitory polypeptide, and glucagon. International review of cell and molecular biology. 326: Elsevier; 2016. p. 279-341.
532. Ladenheim EE. Liraglutide and obesity: a review of the data so far. Drug Des Devel Ther. 2015;9:1867-75.
533. Papaetis GS, Filippou PK, Constantinidou KG, Stylianou CS. Liraglutide: new perspectives for the treatment of polycystic ovary syndrome. Clin Drug Investig. 2020;40(8):695-713.
534. Sun Z, Li P, Wang X, Lai S, Qiu H, Chen Z, et al. GLP-1/GLP-1R signaling regulates ovarian PCOS-associated granulosa cells proliferation and antiapoptosis by modification of forkhead box protein O1 phosphorylation sites. Int J Endocrinol. 2020;2020:1484321.
535. Singh A, Fernandes JRD, Chhabra G, Krishna A, Banerjee A. Liraglutide modulates adipokine expression during adipogenesis, ameliorating obesity, and polycystic ovary syndrome in mice. Endocrine. 2019;64(2):349-66.
536. Hughes S, Neumiller JJ. Oral semaglutide. Clin Diabetes. 2020;38(1):109-11.
537. Chen B, Moore A, Escobedo LV, Koletsky MS, Hou D, Koletsky RJ, et al. Sitagliptin lowers glucagon and improves glucose tolerance in prediabetic obese SHROB rats. Exp Biol Med (Maywood). 2011;236(3):309-14.
538. Dobrian AD, Ma Q, Lindsay JW, Leone KA, Ma K, Coben J, et al. Dipeptidyl peptidase IV inhibitor sitagliptin reduces local inflammation in adipose tissue and in pancreatic islets of obese mice. Am J Physiol Endocrinol Metab. 2011;300(2):E410-21.

539. Mu J, Woods J, Zhou YP, Roy RS, Li Z, Zycband E, et al. Chronic inhibition of dipeptidyl peptidase-4 with a sitagliptin analog preserves pancreatic beta-cell mass and function in a rodent model of type 2 diabetes. Diabetes. 2006;55(6):1695-704.
540. Wang Q, Shang J, Zhang Y, Zhou W. Metformin and sitagliptin combination therapy ameliorates polycystic ovary syndrome with insulin resistance through upregulation of lncRNA H19. Cell Cycle. 2019;18(19):2538-49.
541. Wang F, Zhang ZF, He YR, Wu HY, Wei SS. Effects of dipeptidyl peptidase-4 inhibitors on transforming growth factor-beta1 signal transduction pathways in the ovarian fibrosis of polycystic ovary syndrome rats. J Obstet Gynaecol Res. 2019;45(3):600-8.
542. Xu N, Kwon S, Abbott DH, Geller DH, Dumesic DA, Azziz R, et al. Epigenetic mechanism underlying the development of polycystic ovary syndrome (PCOS)-like phenotypes in prenatally androgenized rhesus monkeys. PLoS One. 2011;6(11):e27286.
543. Dineen L, Law C, Scher R, Pyon E. Alogliptin (nesina) for adults with type-2 diabetes. Pharmacy and Therapeutics. 2014;39(3):186.
544. Jensterle M, Goricar K, Janez A. Add on DPP-4 inhibitor alogliptin alone or in combination with pioglitazone improved beta-cell function and insulin sensitivity in metformin treated PCOS. Endocr Res. 2017;42(4):261-8.
545. Elkind-Hirsch KE, Paterson MS, Seidemann EL, Gutowski HC. Short-term therapy with combination dipeptidyl peptidase-4 inhibitor saxagliptin/metformin extended release (XR) is superior to saxagliptin or metformin XR monotherapy in prediabetic women with polycystic ovary syndrome: a single-blind, randomized, pilot study. Fertil Steril. 2017;107(1):253-60 e1.
546. Tao T, Wu P, Wang Y, Liu W. Comparison of glycemic control and beta-cell function in new onset T2DM patients with PCOS of metformin and saxagliptin monotherapy or combination treatment. BMC Endocr Disord. 2018;18(1):14.
547. El-Halwagy AS, Al-Gergawy AA, Eleslam ES, Abd Elbar ES. Clinical and biochemical changes in polycystic ovarian syndrome patients in response to 3 different oral hypoglycemic drugs: a double blind randomized controlled study. Open Journal of Obstetrics and Gynecology. 2016;7(1):117-28.
548. Chao EC. SGLT-2 Inhibitors: a new mechanism for glycemic control. Clin Diabetes. 2014;32(1):4-11.
549. Scheen AJ, Paquot N. Metabolic effects of SGLT-2 inhibitors beyond increased glucosuria: a review of the clinical evidence. Diabetes Metab. 2014;40(6 Suppl 1):S4-S11.
550. Pereira MJ, Eriksson JW. Emerging role of SGLT-2 inhibitors for the treatment of obesity. Drugs. 2019;79(3):219-30.
551. Yaribeygi H, Sathyapalan T, Maleki M, Jamialahmadi T, Sahebkar A. Molecular mechanisms by which SGLT2 inhibitors can induce insulin sensitivity in diabetic milieu: a mechanistic review. Life Sci. 2020;240:117090.
552. Roden M, Weng J, Eilbracht J, Delafont B, Kim G, Woerle HJ, et al. Empagliflozin monotherapy with sitagliptin as an active comparator in patients with type 2 diabetes: a randomised, double-blind, placebo-controlled, phase 3 trial. The Lancet Diabetes & Endocrinology. 2013;1(3):208-19.
553. Kovacs CS, Seshiah V, Swallow R, Jones R, Rattunde H, Woerle HJ, et al. Empagliflozin improves glycaemic and weight control as add-on therapy to pioglitazone or pioglitazone plus metformin in patients with type 2 diabetes: a 24-week, randomized, placebo-controlled trial. Diabetes Obes Metab. 2014;16(2):147-58.
554. Vasilakou D, Karagiannis T, Athanasiadou E, Mainou M, Liakos A, Bekiari E, et al. Sodium-glucose cotransporter 2 inhibitors for type 2 diabetes: a systematic review and meta-analysis. Ann Intern Med. 2013;159(4):262-74.
555. Bolinder J, Ljunggren O, Kullberg J, Johansson L, Wilding J, Langkilde AM, et al. Effects of dapagliflozin on body weight, total fat mass, and regional adipose tissue distribution in patients with type 2 diabetes mellitus with inadequate glycemic control on metformin. J Clin Endocrinol Metab. 2012;97(3):1020-31.
556. Fadini GP, Bonora BM, Zatti G, Vitturi N, Iori E, Marescotti MC, et al. Effects of the SGLT2 inhibitor dapagliflozin on HDL cholesterol, particle size, and cholesterol efflux capacity in patients with type 2 diabetes: a randomized placebo-controlled trial. Cardiovasc Diabetol. 2017;16(1):42.

557. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2015;373(22):2117-28.
558. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. N Engl J Med. 2017;377(7):644-57.
559. Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, et al. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. N Engl J Med. 2019;380(4):347-57.
560. Perkovic V, Jardine MJ, Neal B, Bompoint S, Heerspink HJL, Charytan DM, et al. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. N Engl J Med. 2019;380(24):2295-306.
561. Javed Z, Papageorgiou M, Deshmukh H, Rigby AS, Qamar U, Abbas J, et al. Effects of empagliflozin on metabolic parameters in polycystic ovary syndrome: a randomized controlled study. Clin Endocrinol (Oxf). 2019;90(6):805-13.
562. Coskun T, Sloop KW, Loghin C, Alsina-Fernandez J, Urva S, Bokvist KB, et al. LY3298176, a novel dual GIP and GLP-1 receptor agonist for the treatment of type 2 diabetes mellitus: From discovery to clinical proof of concept. Mol Metab. 2018;18:3-14.
563. Frias JP, Bastyr EJ, 3rd, Vignati L, Tschop MH, Schmitt C, Owen K, et al. The sustained effects of a dual GIP/GLP-1 receptor agonist, NNC0090-2746, in patients with type 2 diabetes. Cell Metab. 2017;26(2):343-52 e2.
564. Henderson SJ, Konkar A, Hornigold DC, Trevaskis JL, Jackson R, Fritsch Fredin M, et al. Robust anti-obesity and metabolic effects of a dual GLP-1/glucagon receptor peptide agonist in rodents and non-human primates. Diabetes Obes Metab. 2016;18(12):1176-90.
565. Zhou J, Cai X, Huang X, Dai Y, Sun L, Zhang B, et al. A novel glucagon-like peptide-1/glucagon receptor dual agonist exhibits weight-lowering and diabetes-protective effects. Eur J Med Chem. 2017;138:1158-69.
566. Finan B, Yang B, Ottaway N, Smiley DL, Ma T, Clemmensen C, et al. A rationally designed monomeric peptide triagonist corrects obesity and diabetes in rodents. Nat Med. 2015;21(1):27-36.
567. Muller TD, Finan B, Clemmensen C, DiMarchi RD, Tschop MH. The new biology and pharmacology of glucagon. Physiol Rev. 2017;97(2):721-66.
568. Choi I, Lee J, Kim J, Park Y, Jung S, Kim Y, et al. American diabetes association's 77th scientific session. 2017.
569. Adeva-Andany MM, Funcasta-Calderon R, Fernandez-Fernandez C, Castro-Quintela E, Carneiro-Freire N. Metabolic effects of glucagon in humans. J Clin Transl Endocrinol. 2019;15:45-53.
570. Haedersdal S, Lund A, Knop FK, Vilsboll T. The role of glucagon in the pathophysiology and treatment of type 2 diabetes. Mayo Clin Proc. 2018;93(2):217-39.
571. Vuylsteke V, Chastain LM, Maggu GA, Brown C. Imeglimin: A potential new multi-target drug for type 2 diabetes. Drugs R D. 2015;15(3):227-32.
572. Pirags V, Lebovitz H, Fouqueray P. Imeglimin, a novel glimin oral antidiabetic, exhibits a good efficacy and safety profile in type 2 diabetic patients. Diabetes Obes Metab. 2012;14(9):852-8.
573. Yaribeygi H, Maleki M, Sathyapalan T, Jamialahmadi T, Sahebkar A. Molecular mechanisms by which imeglimin improves glucose homeostasis. J Diabetes Res. 2020;2020:8768954.
574. Vial G, Chauvin MA, Bendridi N, Durand A, Meugnier E, Madec AM, et al. Imeglimin normalizes glucose tolerance and insulin sensitivity and improves mitochondrial function in liver of a high-fat, high-sucrose diet mice model. Diabetes. 2015;64(6):2254-64.
575. Ambros V. microRNAs: tiny regulators with great potential. Cell. 2001;107(7):823-6.
576. Flynt AS, Lai EC. Biological principles of microRNA-mediated regulation: shared themes amid diversity. Nat Rev Genet. 2008;9(11):831-42.
577. Lawrie CH, Saunders NJ, Soneji S, Palazzo S, Dunlop HM, Cooper CD, et al. MicroRNA expression in lymphocyte development and malignancy. Leukemia. 2008;22(7):1440-6.
578. Bar M, Wyman SK, Fritz BR, Qi J, Garg KS, Parkin RK, et al. MicroRNA discovery and profiling in human embryonic stem cells by deep sequencing of small RNA libraries. Stem Cells. 2008;26(10):2496-505.

579. Gallo A, Tandon M, Alevizos I, Illei GG. The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. PLoS One. 2012;7(3):e30679.
580. Hanson EK, Lubenow H, Ballantyne J. Identification of forensically relevant body fluids using a panel of differentially expressed microRNAs. Anal Biochem. 2009;387(2):303-14.
581. Ludwig N, Leidinger P, Becker K, Backes C, Fehlmann T, Pallasch C, et al. Distribution of miRNA expression across human tissues. Nucleic Acids Res. 2016;44(8):3865-77.
582. Xue Y, Lv J, Xu P, Gu L, Cao J, Xu L, et al. Identification of microRNAs and genes associated with hyperandrogenism in the follicular fluid of women with polycystic ovary syndrome. J Cell Biochem. 2018;119(5):3913-21.
583. Cortez MA, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA. MicroRNAs in body fluids--the mix of hormones and biomarkers. Nat Rev Clin Oncol. 2011;8(8):467-77.
584. Mari-Alexandre J, Barcelo-Molina M, Belmonte-Lopez E, Garcia-Oms J, Estelles A, Braza-Boils A, et al. Micro-RNA profile and proteins in peritoneal fluid from women with endometriosis: their relationship with sterility. Fertil Steril. 2018;109(4):675-84 e2.
585. Romakina VV, Zhirov IV, Nasonova SN, Zaseeva AV, Kochetov AG, Liang OV, et al. [MicroRNAs as biomarkers of cardiovascular diseases]. Kardiologiia. 2018(1):66-71.
586. Guay C, Regazzi R. Circulating microRNAs as novel biomarkers for diabetes mellitus. Nat Rev Endocrinol. 2013;9(9):513-21.
587. Long W, Zhao C, Ji C, Ding H, Cui Y, Guo X, et al. Characterization of serum microRNAs profile of PCOS and identification of novel non-invasive biomarkers. Cell Physiol Biochem. 2014;33(5):1304-15.
588. Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-sponsored 3rd PCOS consensus workshop group. Fertil Steril. 2012;97(1):28-38 e25.
589. Rosenzweig JL, Ferrannini E, Grundy SM, Haffner SM, Heine RJ, Horton ES, et al. Primary prevention of cardiovascular disease and type 2 diabetes in patients at metabolic risk: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2008;93(10):3671-89.
590. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71.
591. Babineau J. Product review: covidence (systematic review software). Journal of the Canadian Health Libraries Association/Journal de l'Association des bibliothèques de la santé du Canada. 2014;35(2):68-71.
592. Hupe M. EndNote X9. Journal of Electronic Resources in Medical Libraries. 2019;16(3-4):117-9.
593. Das S, Chatterjee SS. Cabell's blacklist: A new way to tackle predatory journals. Indian journal of Psychological Medicine. 2018;40(2):197-8.
594. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The cochrane collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343:d5928.
595. Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al. Cochrane handbook for systematic reviews of interventions: John Wiley & Sons; 2019.
596. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ. 2008;336(7650):924-6.
597. Wang R, Mol BW. The Rotterdam criteria for polycystic ovary syndrome: evidence-based criteria? Hum Reprod. 2017;32(2):261-4.
598. Wong L. Data analysis in qualitative research: a brief guide to using nvivo. Malays Fam Physician. 2008;3(1):14-20.
599. Ritchie J, Spencer L, O'Connor W. Carrying out qualitative analysis. Qualitative research practice: a guide for social science students and researchers. 2003;2003:219-62.
600. Balen A. The pathophysiology of polycystic ovary syndrome: trying to understand PCOS and its endocrinology. Best Pract Res Clin Obstet Gynaecol. 2004;18(5):685-706.

601. Le MT, Nguyen VQH, Truong QV, Le DD, Le VNS, Cao NT. Metabolic syndrome and insulin resistance syndrome among infertile women with polycystic ovary syndrome: a cross-sectional study from central Vietnam. Endocrinol Metab (Seoul). 2018;33(4):447-58.
602. Gobl CS, Ott J, Bozkurt L, Feichtinger M, Rehmann V, Cserjan A, et al. To assess the association between glucose metabolism and ectopic lipid content in different clinical classifications of PCOS. PLoS One. 2016;11(8):e0160571.
603. Rizzo M, Berneis K, Hersberger M, Pepe I, Di Fede G, Rini GB, et al. Milder forms of atherogenic dyslipidemia in ovulatory versus anovulatory polycystic ovary syndrome phenotype. Hum Reprod. 2009;24(9):2286-92.
604. Ferri N, Corsini A. Clinical evidence of statin therapy in non-dyslipidemic disorders. Pharmacol Res. 2014;88:20-30.
605. Gao L, Zhao FL, Li SC. Statin is a reasonable treatment option for patients with polycystic ovary Syndrome: a meta-analysis of randomized controlled trials. Exp Clin Endocrinol Diabetes. 2012;120(6):367-75.
606. Banaszewska B, Pawelczyk L, Spaczynski RZ, Duleba AJ. Effects of simvastatin and metformin on polycystic ovary syndrome after six months of treatment. J Clin Endocrinol Metab. 2011;96(11):3493-501.
607. Amiri M, Golsorkhtabaramiri M, Esmaeilzadeh S, Ghofrani F, Bijani A, Ghorbani L, et al. Effect of metformin and flutamide on anthropometric indices and laboratory tests in obese/overweight PCOS women under hypocaloric diet. Journal of Reproduction & Infertility. 2014;15(4):205-13.
608. Brettenthaler N, De Geyter C, Huber PR, Keller U. Effect of the insulin sensitizer pioglitazone on insulin resistance, hyperandrogenism, and ovulatory dysfunction in women with polycystic ovary syndrome. The Journal of Clinical Endocrinology and Metabolism. 2004;89(8):3835-40.
609. Elkind-Hirsch KE, Paterson MS, Seidemann EL, Gutowski HC. Short-term therapy with combination dipeptidyl peptidase-4 inhibitor saxagliptin/metformin extended release (XR) is superior to saxagliptin or metformin XR monotherapy in prediabetic women with polycystic ovary syndrome: a single-blind, randomized, pilot. Fertility and Sterility. 2017;107(1):253-60.e1.
610. Heidari B, Lerman A, Lalia AZ, Lerman LO, Chang AY. Effect of metformin on microvascular endothelial function in polycystic ovary syndrome. Mayo Clinic Proceedings. 2019;94(12):2455-66.
611. Liu X, Zhang Y, Zheng SY, Lin R, Xie YJ, Chen H, et al. Efficacy of exenatide on weight loss, metabolic parameters and pregnancy in overweight/obese polycystic ovary syndrome. Clinical Endocrinology. 2017;87(6):767-74.
612. Mohiyiddeen L, Watson AJ, Apostolopoulos NV, Berry R, Alexandraki KI, Jude EB. Effects of low-dose metformin and rosiglitazone on biochemical, clinical, metabolic and biophysical outcomes in polycystic ovary syndrome. Journal of obstetrics and gynaecology : The Journal of The Institute of Obstetrics and Gynaecology. 2013;33(2):165-70.
613. Sathyapalan T, Kilpatrick ES, Coady AM, Atkin SL. The effect of atorvastatin in patients with polycystic ovary syndrome: a randomized double-blind placebo-controlled study. The Journal of Clinical Endocrinology and Metabolism. 2009;94(1):103-8.
614. Shahebrahimi K, Jalilian N, Bazgir N, Rezaei M. Comparison clinical and metabolic effects of metformin and pioglitazone in polycystic ovary syndrome. Indian Journal of Endocrinology and Metabolism.20(6):805-9.
615. Sohrevardi SM, Nosouhi F, Hossein Khalilzade S, Kafaie P, Karimi-Zarchi M, Halvaei I, et al. Evaluating the effect of insulin sensitizers metformin and pioglitazone alone and in combination on women with polycystic ovary syndrome: An RCT. International Journal of Reproductive Biomedicine (Yazd, Iran). 2016;14(12):743-54.
616. Sova H, Puistola U, Morin-Papunen L, Karihtala P. Metformin decreases serum 8-hydroxy-2'-deoxyguanosine levels in polycystic ovary syndrome. Fertility and Sterility. 2013;99(2):593-8.
617. Tao T, Wu P, Wang Y, Liu W. Comparison of glycemic control and β-cell function in new onset T2DM patients with PCOS of metformin and saxagliptin monotherapy or combination treatment. BMC Endocrine Disorders. 2018;18(1):14.

618. Underdal MO, Stridsklev S, Oppen IH, Høgetveit K, Andersen MS, Vanky E. Does metformin treatment during pregnancy modify the future metabolic profile in women with PCOS? The Journal of Clinical Endocrinology and Metabolism. 2018;103(6):2408-13.
619. Zheng S, Zhang Y, Long T, Lu J, Liu X, Yan J, et al. Short term monotherapy with exenatide is superior to metformin in weight loss, improving insulin resistance and inflammation in Chinese overweight/obese PCOS women. Obesity Medicine. 2017;7((Wang F.) Department of Obstetrics and Gynecology, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong, China):15-20.
620. Ziaee A, Oveisi S, Abedini A, Hashemipour S, Karimzadeh T, Ghorbani A. Effect of metformin and pioglitazone treatment on cardiovascular risk profile in polycystic ovary syndrome. Acta Medica Indonesiana. 2012;44(1):16-22.
621. Jensterle M, Kravos NA, Goričar K, Janez A. Short-term effectiveness of low dose liraglutide in combination with metformin versus high dose liraglutide alone in treatment of obese PCOS: randomized trial. BMC Endocrine Disorders. 2017;17(1):5.
622. Jensterle M, Sebestjen M, Janez A, Prezelj J, Kocjan T, Keber I, et al. Improvement of endothelial function with metformin and rosiglitazone treatment in women with polycystic ovary syndrome. European Journal of Endocrinology. 2008;159(4):399-406.
623. Jensterle Sever M, Kocjan T, Pfeifer M, Kravos NA, Janez A. Short-term combined treatment with liraglutide and metformin leads to significant weight loss in obese women with polycystic ovary syndrome and previous poor response to metformin. European Journal of Endocrinology. 2014;170(3):451-9.
624. Mehrabian F, Ghasemi-Tehrani H, Mohamadkhani M, Moeinoddini M, Karimzadeh P. Comparison of the effects of metformin, flutamide plus oral contraceptives, and simvastatin on the metabolic consequences of polycystic ovary syndrome. Journal of Research in Medical Sciences : The Official Journal of Isfahan University of Medical Sciences. 2016;21:7.
625. Moghetti P, Castello R, Negri C, Tosi F, Perrone F, Caputo M, et al. Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. The Journal of Clinical Endocrinology and Metabolism. 2000;85(1):139-46.
626. Anaforoglu I, Algun E, Incecayir O, Ersoy K. Higher metabolic risk with National Institutes of Health versus Rotterdam diagnostic criteria for polycystic ovarian syndrome in Turkish women. Metab Syndr Relat Disord. 2011;9(5):375-80.
627. Gambineri A, Pelusi C, Genghini S, Morselli-Labate AM, Cacciari M, Pagotto U, et al. Effect of flutamide and metformin administered alone or in combination in dieting obese women with polycystic ovary syndrome. Clinical Endocrinology. 2004;60(2):241-9.
628. Lord J, Thomas R, Fox B, Acharya U, Wilkin T. The effect of metformin on fat distribution and the metabolic syndrome in women with polycystic ovary syndrome--a randomised, double-blind, placebo-controlled trial. BJOG : an International Journal of Obstetrics and Gynaecology. 2006;113(7):817-24.
629. Ng EH, Wat NM, Ho PC. Effects of metformin on ovulation rate, hormonal and metabolic profiles in women with clomiphene-resistant polycystic ovaries: a randomized, double-blinded placebo-controlled trial. Human Reproduction (Oxford, England). 2001;16(8):1625-31.
630. Trolle B, Lauszus FF, Frystyk J, Flyvbjerg A. Adiponectin levels in women with polycystic ovary syndrome: impact of metformin treatment in a randomized controlled study. Fertility and Sterility. 2010;94(6):2234-8.
631. Naka KK, Kalantaridou SN, Kravariti M, Bechlioulis A, Kazakos N, Calis KA, et al. Effect of the insulin sensitizers metformin and pioglitazone on endothelial function in young women with polycystic ovary syndrome: a prospective randomized study. Fertility and Sterility. 2011;95(1):203-9.
632. Ortega-González C, Luna S, Hernández L, Crespo G, Aguayo P, Arteaga-Troncoso G, et al. Responses of serum androgen and insulin resistance to metformin and pioglitazone in obese, insulin-resistant women with polycystic ovary syndrome. The Journal of Clinical Endocrinology and Metabolism. 2005;90(3):1360-5.

633. Glintborg D, Hermann AP, Andersen M, Hagen C, Beck-Nielsen H, Veldhuis JD, et al. Effect of pioglitazone on glucose metabolism and luteinizing hormone secretion in women with polycystic ovary syndrome. Fertility and Sterility. 2006;86(2):385-97.
634. Navali N, Pourabolghasem S, Fouladi RF, Nikpour MA. Therapeutic effects of biguanide vs. statin in polycystic ovary syndrome: a randomized clinical trial. Pakistan Journal of Biological Sciences : PJBS. 2011;14(11):658-63.
635. Akbari M, Almasi A, Naderi Z, Kouhpayezadeh J, Pourali R, Hossinzadeh Z. The effect of atrovastatin on the ovarian arterial blood flow and serum androgen level in PCOS patient. Biomed Pharmacol J. 2016;9(3):1041-8.
636. Puurunen J, Piltonen T, Puukka K, Ruokonen A, Savolainen MJ, Bloigu R, et al. Statin therapy worsens insulin sensitivity in women with polycystic ovary syndrome (PCOS): a prospective, randomized, double-blind, placebo-controlled study. The Journal of Clinical Endocrinology and Metabolism. 2013;98(12):4798-807.
637. Glintborg D, Støving RK, Hagen C, Hermann AP, Frystyk J, Veldhuis JD, et al. Pioglitazone treatment increases spontaneous growth hormone (GH) secretion and stimulated GH levels in polycystic ovary syndrome. The Journal of Clinical Endocrinology and Metabolism. 2005;90(10):5605-12.
638. Guan Y, Wang D, Bu H, Zhao T, Wang H. The Effect of Metformin on polycystic ovary syndrome in overweight women: a systematic review and meta-analysis of randomized controlled trials. Int J Endocrinol. 2020;2020:5150684.
639. Chou KH, von Eye Corleta H, Capp E, Spritzer PM. Clinical, metabolic and endocrine parameters in response to metformin in obese women with polycystic ovary syndrome: a randomized, double-blind and placebo-controlled trial. Horm Metab Res. 2003;35(2):86-91.
640. Trolle B, Flyvbjerg A, Kesmodel U, Lauszus FF. Efficacy of metformin in obese and non-obese women with polycystic ovary syndrome: a randomized, double-blinded, placebo-controlled cross-over trial. Hum Reprod. 2007;22(11):2967-73.
641. Kim HS, Ren G, Kim T, Bhatnagar S, Yang Q, Bahk YY, et al. Metformin reduces saturated fatty acid-induced lipid accumulation and inflammatory response by restoration of autophagic flux in endothelial cells. Sci Rep. 2020;10(1):13523.
642. McFarland AJ, Anoopkumar-Dukie S, Arora DS, Grant GD, McDermott CM, Perkins AV, et al. Molecular mechanisms underlying the effects of statins in the central nervous system. Int J Mol Sci. 2014;15(11):20607-37.
643. Wang J, Zhu L, Hu K, Tang Y, Zeng X, Liu J, et al. Effects of metformin treatment on serum levels of C-reactive protein and interleukin-6 in women with polycystic ovary syndrome: a meta-analysis: A PRISMA-compliant article. Medicine (Baltimore). 2017;96(39):e8183.
644. Dawson AJ, Sathyapalan T, Vince R, Coady AM, Ajjan RA, Kilpatrick ES, et al. The effect of exenatide on cardiovascular risk markers in women with polycystic ovary syndrome. Front Endocrinol (Lausanne). 2019;10:189.
645. Tarkun I, Cetinarslan B, Turemen E, Sahin T, Canturk Z, Komsuoglu B. Effect of rosiglitazone on insulin resistance, C-reactive protein and endothelial function in non-obese young women with polycystic ovary syndrome. Eur J Endocrinol. 2005;153(1):115-21.
646. Strowitzki T. Advanced diagnosis of polycystic ovary syndrome-new prediction models with standard parameters. Fertil Steril. 2021;115(1):92-3.
647. Teede H, Deeks A, Moran L. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. BMC Med. 2010;8:41.
648. Baptiste CG, Battista MC, Trottier A, Baillargeon JP. Insulin and hyperandrogenism in women with polycystic ovary syndrome. J Steroid Biochem Mol Biol. 2010;122(1-3):42-52.
649. Nestler JE. Role of hyperinsulinemia in the pathogenesis of the polycystic ovary syndrome, and its clinical implications. Semin Reprod Endocrinol. 1997;15(2):111-22.
650. Chantrapanichkul P, Indhavivadhana S, Wongwananuruk T, Techatraisak K, Dangrat C, Sa-Nga-Areekul N. Prevalence of type 2 diabetes mellitus compared between lean and overweight/obese patients with polycystic ovarian syndrome: a 5-year follow-up study. Arch Gynecol Obstet. 2020;301(3):809-16.

651. Lin HV, Accili D. Hormonal regulation of hepatic glucose production in health and disease. Cell Metab. 2011;14(1):9-19.
652. Brown MS, Goldstein JL. Selective versus total insulin resistance: a pathogenic paradox. Cell Metab. 2008;7(2):95-6.
653. Santoleri D, Titchenell PM. Resolving the paradox of hepatic insulin resistance. Cell Mol Gastroenterol Hepatol. 2019;7(2):447-56.
654. Polak K, Czyzyk A, Simoncini T, Meczekalski B. New markers of insulin resistance in polycystic ovary syndrome. J Endocrinol Invest. 2017;40(1):1-8.
655. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia. 2017;60(9):1577-85.
656. Du Q, Wang YJ, Yang S, Wu B, Han P, Zhao YY. A systematic review and meta-analysis of randomized controlled trials comparing pioglitazone versus metformin in the treatment of polycystic ovary syndrome. Curr Med Res Opin. 2012;28(5):723-30.
657. Hauner H. The mode of action of thiazolidinediones. Diabetes Metab Res Rev. 2002;18 Suppl 2:S10-5.
658. Mokhtari V, Afsharian P, Shahhoseini M, Kalantar SM, Moini A. A review on various uses of N-Acetyl Cysteine. Cell J. 2017;19(1):11-7.
659. Cetinkalp S, Karadeniz M, Erdogan M, Ozgen G, Saygl F, Ylmaz C. The effects of rosiglitazone, metformin, and estradiol-cyproterone acetate on lean patients with polycystic ovary syndrome. Endocrinologist. 2009;19(3):94-7.
660. Cheng F, Zhao L, Wu Y, Huang T, Yang G, Zhang Z, et al. Serum vascular endothelial growth factor B is elevated in women with polycystic ovary syndrome and can be decreased with metformin treatment. Clinical Endocrinology. 2016;84(3):386-93.
661. Devin JK, Nian H, Celedonio JE, Wright P, Brown NJ. Sitagliptin decreases visceral fat and blood glucose in women with polycystic ovarian syndrome. J Clin Endocrinol Metab. 2020;105(1).
662. Diamanti-Kandarakis E, Katsikis I, Piperi C, Alexandraki K, Panidis D. Effect of long-term orlistat treatment on serum levels of advanced glycation end-products in women with polycystic ovary syndrome. Clinical Endocrinology. 2007;66(1):103-9.
663. Eisenhardt S, Schwarzmann N, Henschel V, Germeyer A, von Wolff M, Hamann A, et al. Early effects of metformin in women with polycystic ovary syndrome: a prospective randomized, double-blind, placebo-controlled trial. The Journal of Clinical Endocrinology and Metabolism. 2006;91(3):946-52.
664. Elkind-Hirsch KE, Paterson MS, Seidemann EL, Gutowski HC. Short-term therapy with combination dipeptidyl peptidase-4 inhibitor saxagliptin/metformin extended release (XR) is superior to saxagliptin or metformin XR monotherapy in prediabetic women with polycystic ovary syndrome: a single-blind, randomized, pilot study. Fertility and Sterility. 2017;107(1):253-+.
665. Ferjan S, Janez A, Jensterle M. Dipeptidyl peptidase-4 inhibitor sitagliptin prevented weight regain in obese women with polycystic ovary syndrome previously treated with liraglutide: a pilot randomized study. Metabolic Syndrome and Related Disorders. 2017;15(10):515-20.
666. Ferjan S, Janez A, Jensterle M. DPP-4 inhibitor sitagliptin as a potential treatment option in metformin-intolerant obese women with polycystic ovary syndrome: a pilot randomized study. Endocrine Practice : Official Journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists. 2018;24(1):69-77.
667. Javanmanesh F, Kashanian M, Rahimi M, Sheikhansari N. A comparison between the effects of metformin and N-acetyl cysteine (NAC) on some metabolic and endocrine characteristics of women with polycystic ovary syndrome. Gynecological Endocrinology : the official Journal of the International Society of Gynecological Endocrinology. 2016;32(4):285-9.
668. Jensterle M, Kravos N, Janež A. Short-term effectiveness of low dose liraglutide in combination with metformin vs. high dose liraglutide alone in treatment of obesity. Diabetes. 2016;65((Jensterle M.; Kravos N.; Janež A.) Ljubljana, Slovenia):A70.

669. Jensterle M, Kravos NA, Goricar K, Janez A. Short-term effectiveness of low dose liraglutide in combination with metformin versus high dose liraglutide alone in treatment of obese PCOS: randomized trial. BMC Endocr Disord. 2017;17(1):5.
670. Kazerooni T, Shojaei-Baghini A, Dehbashi S, Asadi N, Ghaffarpasand F, Kazerooni Y. Effects of metformin plus simvastatin on polycystic ovary syndrome: a prospective, randomized, double-blind, placebo-controlled study. Fertility and Sterility. 2010;94(6):2208-13.
671. Kocak M, Caliskan E, Simsir C, Haberal A. Metformin therapy improves ovulatory rates, cervical scores, and pregnancy rates in clomiphene citrate-resistant women with polycystic ovary syndrome. Fertility and Sterility. 2002;77(1):101-6.
672. Li Y, Tan J, Wang Q, Duan C, Hu Y, Huang W. Comparing the individual effects of metformin and rosiglitazone and their combination in obese women with polycystic ovary syndrome: a randomized controlled trial. Fertility and Sterility. 2020;113(1):197-204.
673. Lingaiah S, Morin-Papunen L, Risteli J, Tapanainen JS. Metformin decreases bone turnover markers in polycystic ovary syndrome: a post hoc study. Fertility and Sterility. 2019;112(2):362-70.
674. Moini A, Kanani M, Kashani L, Hosseini R, Hosseini L. Effect of orlistat on weight loss, hormonal and metabolic profiles in women with polycystic ovarian syndrome: a randomized double-blind placebo-controlled trial. Endocrine. 2015;49(1):286-9.
675. Nemati M, Nemati S, Taheri AM, Heidari B. Comparison of metformin and N-acetyl cysteine, as an adjuvant to clomiphene citrate, in clomiphene-resistant women with polycystic ovary syndrome. Journal of Gynecology Obstetrics and Human Reproduction. 2017;46(7):579-85.
676. Rezai M, Jamshidi M, Mohammadbeigi R, Seyedoshohadaei F, Mohammadipour S, Moradi G. Comparing the effect of metformin and acarbose accompanying clomiphene on the successful ovulation induction in infertile women with polycystic ovary syndrome. Global Journal of Health Science. 2016;8(9):54516.
677. Shahebrahimi K, Jalilian N, Bazgir N, Rezaei M. Comparison clinical and metabolic effects of metformin and pioglitazone in polycystic ovary syndrome. Indian J Endocrinol Metab. 2016;20(6):805-9.
678. Yilmaz M, Karakoç A, Törüner FB, Cakir N, Tiras B, Ayvaz G, et al. The effects of rosiglitazone and metformin on menstrual cyclicity and hirsutism in polycystic ovary syndrome. Gynecological Endocrinology : the official Journal of the International Society of Gynecological Endocrinology. 2005;21(3):154-60.
679. Aroda VR, Ciaraldi TP, Burke P, Mudaliar S, Clopton P, Phillips S, et al. Metabolic and hormonal changes induced by pioglitazone in polycystic ovary syndrome: a randomized, placebo-controlled clinical trial. The Journal of Clinical Endocrinology and Metabolism. 2009;94(2):469-76.
680. Hanjalic-Beck A, Gabriel B, Schaefer W, Zahradnik HP, Schories M, Tempfer C, et al. Metformin versus acarbose therapy in patients with polycystic ovary syndrome (PCOS): a prospective randomised double-blind study. Gynecological Endocrinology : the official Journal of the International Society of Gynecological Endocrinology. 2010;26(9):690-7.
681. Jensterle M, Janez A, Mlinar B, Marc J, Prezelj J, Pfeifer M. Impact of metformin and rosiglitazone treatment on glucose transporter 4 mRNA expression in women with polycystic ovary syndrome. European Journal of Endocrinology. 2008;158(6):793-801.
682. Ladson G, Dodson WC, Sweet SD, Archibong AE, Kunselman AR, Demers LM, et al. The effects of metformin with lifestyle therapy on polycystic ovary syndrome: a randomized double blind study. Endocrine Reviews. 2010;31(3).
683. Mehrabian F, Ghasemi-Tehrani H, Mohamadkhani M, Moeinoddini M, Karimzadeh P. Comparison of the effects of metformin, flutamide plus oral contraceptives, and simvastatin on the metabolic consequences of polycystic ovary syndrome. J Res Med Sci. 2016;21:7.
684. Sönmez AS, Yasar L, Savan K, Koç S, Ozcan J, Toklar A, et al. Comparison of the effects of acarbose and metformin use on ovulation rates in clomiphene citrate-resistant polycystic ovary syndrome. Human Reproduction (Oxford, England). 2005;20(1):175-9.

685. Steiner CA, Janez A, Jensterle M, Reisinger K, Forst T, Pfützner A. Impact of treatment with rosiglitazone or metformin on biomarkers for insulin resistance and metabolic syndrome in patients with polycystic ovary syndrome. Journal of Diabetes Science and Technology. 2007;1(2):211-7.
686. Cho LW, Kilpatrick ES, Keevil BG, Coady AM, Atkin SL. Effect of metformin, orlistat and pioglitazone treatment on mean insulin resistance and its biological variability in polycystic ovary syndrome. Clinical Endocrinology. 2009;70(2):233-7.
687. Yarali H, Yildiz BO, Demirol A, Zeyneloğlu HB, Yiğit N, Bükülmez O, et al. Co-administration of metformin during rFSH treatment in patients with clomiphene citrate-resistant polycystic ovarian syndrome: a prospective randomized trial. Human Reproduction (Oxford, England). 2002;17(2):289-94.
688. Vandermolen DT, Ratts VS, Evans WS, Stovall DW, Kauma SW, Nestler JE. Metformin increases the ovulatory rate and pregnancy rate from clomiphene citrate in patients with polycystic ovary syndrome who are resistant to clomiphene citrate alone. Fertility and Sterility. 2001;75(2):310-5.
689. Chou KH, von Eye Corleta H, Capp E, Spritzer PM. Clinical, metabolic and endocrine parameters in response to metformin in obese women with polycystic ovary syndrome: a randomized, double-blind and placebo-controlled trial. Hormone and Metabolic Research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 2003;35(2):86-91.
690. Naka KK, Kalantaridou SN, Kravariti M, Bechlioulis A, Kazakos N, Calis KA, et al. Effect of the insulin sensitizers metformin and pioglitazone on endothelial function in young women with polycystic ovary syndrome: a prospective randomized study. Fertil Steril. 2011;95(1):203-9.
691. Kilicdag EB, Bagis T, Zeyneloglu HB, Tarim E, Aslan E, Haydardedeoglu B, et al. Homocysteine levels in women with polycystic ovary syndrome treated with metformin versus rosiglitazone: a randomized study. Human Reproduction (Oxford, England). 2005;20(4):894-9.
692. Paredes Palma JC, López Byhen E, Ibáñez L, Balladares Macedo L, Paredes Palma C, Ramírez Velázquez C. Comparative treatment between sitagliptin vs. metformin, alone or in combination, in patients with polycystic ovary syndrome. A clinical entity at high risk for developing diabetes mellitus and gestational diabetes: a pilot study. Revista Medica del Hospital General de Mexico. 2018;81(1):15-26.
693. Ciotta L, Calogero AE, Farina M, De Leo V, La Marca A, Cianci A. Clinical, endocrine and metabolic effects of acarbose, an alpha-glucosidase inhibitor, in PCOS patients with increased insulin response and normal glucose tolerance. Human Reproduction (Oxford, England). 2001;16(10):2066-72.
694. Penna IA, Canella PR, Reis RM, Silva de Sá MF, Ferriani RA. Acarbose in obese patients with polycystic ovarian syndrome: a double-blind, randomized, placebo-controlled study. Human Reproduction (Oxford, England). 2005;20(9):2396-401.
695. Jayagopal V, Kilpatrick ES, Holding S, Jennings PE, Atkin SL. Orlistat is as beneficial as metformin in the treatment of polycystic ovarian syndrome. The Journal of Clinical Endocrinology and Metabolism. 2005;90(2):729-33.
696. Elkind-Hirsch K, Marrioneaux O, Bhushan M, Vernor D, Bhushan R. Comparison of single and combined treatment with exenatide and metformin on menstrual cyclicity in overweight women with polycystic ovary syndrome. The Journal of Clinical Endocrinology and Metabolism. 2008;93(7):2670-8.
697. Pernicova I, Korbonits M. Metformin--mode of action and clinical implications for diabetes and cancer. Nat Rev Endocrinol. 2014;10(3):143-56.
698. Morgante G, Massaro MG, Scolaro V, Cappelli V, Luddi A, Troia L, et al. Metformin doses and body mass index: clinical outcomes in insulin resistant polycystic ovary syndrome women. Eur Rev Med Pharmacol Sci. 2020;24(15):8136-42.
699. Patel R, Shah G. Effect of metformin on clinical, metabolic and endocrine outcomes in women with polycystic ovary syndrome: a meta-analysis of randomized controlled trials. Curr Med Res Opin. 2017;33(9):1545-57.

700. Li XJ, Yu YX, Liu CQ, Zhang W, Zhang HJ, Yan B, et al. Metformin vs thiazolidinediones for treatment of clinical, hormonal and metabolic characteristics of polycystic ovary syndrome: a meta-analysis. Clin Endocrinol (Oxf). 2011;74(3):332-9.
701. Song Y, Wang H, Huang H, Zhu Z. Comparison of the efficacy between NAC and metformin in treating PCOS patients: a meta-analysis. Gynecol Endocrinol. 2020;36(3):204-10.
702. Hoeger KM, Dokras A, Piltonen T. Update on PCOS: consequences, challenges and guiding treatment. J Clin Endocrinol Metab. 2020.
703. Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. Nat Rev Endocrinol. 2011;7(4):219-31.
704. Mu N, Zhu Y, Wang Y, Zhang H, Xue F. Insulin resistance: a significant risk factor of endometrial cancer. Gynecol Oncol. 2012;125(3):751-7.
705. Shao Y, Cheng S, Hou J, Zuo Y, Zheng W, Xia M, et al. Insulin is an important risk factor of endometrial cancer among premenopausal women: a case-control study in China. Tumour Biol. 2016;37(4):4721-6.
706. Gambineri A, Pelusi C, Vicennati V, Pagotto U, Pasquali R. Obesity and the polycystic ovary syndrome. Int J Obes Relat Metab Disord. 2002;26(7):883-96.
707. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod. 2018;33(9):1602-18.
708. Ghandi S, Aflatoonian A, Tabibnejad N, Moghaddam MH. The effects of metformin or orlistat on obese women with polycystic ovary syndrome: a prospective randomized open-label study. Journal of Assisted Reproduction and Genetics. 2011;28(7):591-6.
709. Karimzadeh MA, Eftekhar M, Taheripanah R, Tayebi N, Sakhavat L, Zare F. The effect of administration of metformin on lipid profile changes and insulin resistance in patients with polycystic ovary syndrome. Middle East Fertil Soc J. 2007;12(3):174-8.
710. Lam PM, Tam WH, Ma RC, Cheung LP, Tsui MH, Tong PC, et al. The reproductive and metabolic effect of rosiglitazone on Chinese women with polycystic ovarian syndrome--a double-blind randomized placebo-controlled study. Fertility and Sterility. 2011;96(2):445-51.e1.
711. Morin-Papunen L, Rantala AS, Unkila-Kallio L, Tiitinen A, Hippeläinen M, Perheentupa A, et al. Metformin improves pregnancy and live-birth rates in women with polycystic ovary syndrome (PCOS): a multicenter, double-blind, placebo-controlled randomized trial. The Journal of Clinical Endocrinology and Metabolism. 2012;97(5):1492-500.
712. Morteza Taghavi S, Rokni H, Fatemi S. Metformin decreases thyrotropin in overweight women with polycystic ovarian syndrome and hypothyroidism. Diabetes & Vascular Disease Research. 2011;8(1):47-8.
713. Muneyyirci-Delale O, Kaplan J, Joulak I, Yang L, Von Gizycki H, Nacharaju VL. Serum free fatty acid levels in PCOS patients treated with glucophage, magnesium oxide and spironolactone. Gynecological Endocrinology : the official Journal of the International Society of Gynecological Endocrinology. 2013;29(5):474-7.
714. Romualdi D, Giuliani M, Cristello F, Fulghesu AM, Selvaggi L, Lanzone A, et al. Metformin effects on ovarian ultrasound appearance and steroidogenic function in normal-weight normoinsulinemic women with polycystic ovary syndrome: a randomized double-blind placebo-controlled clinical trial. Fertility and Sterility. 2010;93(7):2303-10.
715. Vanky E, Salvesen KA, Heimstad R, Fougner KJ, Romundstad P, Carlsen SM. Metformin reduces pregnancy complications without affecting androgen levels in pregnant polycystic ovary syndrome women: results of a randomized study. Human Reproduction (Oxford, England). 2004;19(8):1734-40.
716. Zahra M, Shah M, Ali A, Rahim R. Effects of metformin on endocrine and metabolic parameters in patients with polycystic ovary syndrome. Hormone and Metabolic Research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 2017;49(2):103-8.
717. Ashraf Ganie M, Khurana ML, Eunice M, Gulati M, Dwivedi SN, Ammini AC. Erratum: Comparison of efficacy of spironolactone with metformin in the management of polycystic ovary syndrome: an open-labeled

study (The Journal of Clinical Endocrinology & Metabolism (2004) 89 (2756-2762)). J Clin Endocrinol Metab. 2004;89(9):4655.
718. Ganie MA, Khurana ML, Nisar S, Shah PA, Shah ZA, Kulshrestha B, et al. Improved efficacy of low-dose spironolactone and metformin combination than either drug alone in the management of women with polycystic ovary syndrome (PCOS): a six-month, open-label randomized study. The Journal of Clinical Endocrinology and Metabolism. 2013;98(9):3599-607.
719. Jensterle M, Salamun V, Kocjan T, Bokal EV, Janez A. Short term monotherapy with GLP-1 receptor agonist liraglutide or PDE 4 inhibitor roflumilast is superior to metformin in weight loss in obese PCOS women: a pilot randomized study. Endocrine Reviews. 2015;36((Janez A.) University Medical Center Ljubljana, Ljubljana, Slovenia).
720. Batista JG, Soares JM, Maganhin CC, Simões RS, Tomaz G, Baracat EC. Assessing the benefits of rosiglitazone in women with polycystic ovary syndrome through its effects on insulin-like growth factor 1, insulin-like growth factor-binding protein-3 and insulin resistance: a pilot study. Clinics (Sao Paulo, Brazil). 2012;67(3):283-7.
721. Chen Z, Zhang M, Qiao Y, Yang J. Effects of letrozole in combination with low-dose intramuscular injection of human menopausal gonadotropin on ovulation and pregnancy of 156 patients with polycystic ovary syndrome. Pakistan Journal of Medical Sciences. 2016;32(6):1434-8.
722. Palomba S, Falbo A, Russo T, Manguso F, Tolino A, Zullo F, et al. Insulin sensitivity after metformin suspension in normal-weight women with polycystic ovary syndrome. The Journal of Clinical Endocrinology and Metabolism. 2007;92(8):3128-35.
723. Cheng F, Zhao L, Wu Y, Huang T, Yang G, Zhang Z, et al. Serum vascular endothelial growth factor B is elevated in women with polycystic ovary syndrome and can be decreased with metformin treatment. Clin Endocrinol (Oxf). 2016;84(3):386-93.
724. Jensterle M, Kravos NA, Pfeifer M, Kocjan T, Janez A. A 12-week treatment with the long-acting glucagon-like peptide 1 receptor agonist liraglutide leads to significant weight loss in a subset of obese women with newly diagnosed polycystic ovary syndrome. Hormones (Athens). 2015;14(1):81-90.
725. Glintborg D, Højlund K, Andersen M, Henriksen JE, Beck-Nielsen H, Handberg A. Soluble CD36 and risk markers of insulin resistance and atherosclerosis are elevated in polycystic ovary syndrome and significantly reduced during pioglitazone treatment. Diabetes Care. 2008;31(2):328-34.
726. Legro RS, Zaino RJ, Demers LM, Kunselman AR, Gnatuk CL, Williams NI, et al. The effects of metformin and rosiglitazone, alone and in combination, on the ovary and endometrium in polycystic ovary syndrome. American Journal of Obstetrics and Gynecology. 2007;196(4):402.e1-10; discussion .e10.
727. Ahmad J, Shukla N, Khan AR, Ahmed F, Siddiqui MA. Comparison of metabolic effects of metformin and rosiglitazone in the management of polycystic ovary syndrome (PCOS): a prospective, parallel, randomized, open-label study. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2008;2(1):37-46.
728. Cataldo NA, Abbasi F, McLaughlin TL, Basina M, Fechner PY, Giudice LC, et al. Metabolic and ovarian effects of rosiglitazone treatment for 12 weeks in insulin-resistant women with polycystic ovary syndrome. Hum Reprod. 2006;21(1):109-20.
729. Dereli D, Dereli T, Bayraktar F, Ozgen AG, Yilmaz C. Endocrine and metabolic effects of rosiglitazone in non-obese women with polycystic ovary disease. Endocr J. 2005;52(3):299-308.
730. Rautio K, Tapanainen JS, Ruokonen A, Morin-Papunen LC. Endocrine and metabolic effects of rosiglitazone in overweight women with PCOS: a randomized placebo-controlled study. Hum Reprod. 2006;21(6):1400-7.
731. Naderpoor N, Shorakae S, de Courten B, Misso ML, Moran LJ, Teede HJ. Metformin and lifestyle modification in polycystic ovary syndrome: systematic review and meta-analysis. Hum Reprod Update. 2015;21(5):560-74.
732. Graff SK, Mario FM, Ziegelmann P, Spritzer PM. Effects of orlistat vs. metformin on weight loss-related clinical variables in women with PCOS: systematic review and meta-analysis. Int J Clin Pract. 2016;70(6):450-61.

733. Aghili R, Malek M, Valojerdi AE, Banazadeh Z, Najafi L, Khamseh ME. Body composition in adults with newly diagnosed type 2 diabetes: effects of metformin. J Diabetes Metab Disord. 2014;13(1):88.
734. Maruthur NM, Tseng E, Hutfless S, Wilson LM, Suarez-Cuervo C, Berger Z, et al. Diabetes medications as monotherapy or metformin-based combination therapy for type 2 diabetes: a systematic review and meta-analysis. Ann Intern Med. 2016;164(11):740-51.
735. Filipova E, Uzunova K, Kalinov K, Vekov T. Effects of pioglitazone therapy on blood parameters, weight and BMI: a meta-analysis. Diabetol Metab Syndr. 2017;9:90.
736. Song LL, Wang X, Yang ZJ, Kong XM, Chen XP, Zhang B, et al. Factors associated with improvement in waist-to-height ratio among newly diagnosed type 2 diabetes patients treated with acarbose or metformin: A randomized clinical trial study. World J Diabetes. 2020;11(11):514-26.
737. Abdalla M, Deshmukh H, Atkin SL, Sathyapalan T. miRNAs as a novel clinical biomarker and therapeutic targets in polycystic ovary syndrome (PCOS): A review. Life Sci. 2020;259:118174.
738. Abdalla MA, Deshmukh H, Mohammed I, Atkin S, Reid M, Sathyapalan T. The effect of free androgen index on the quality of life of women with polycystic ovary syndrome: a cross-sectional study. Front Physiol. 2021;12:652559.
739. Badawy A, Elnashar A. Treatment options for polycystic ovary syndrome. Int J Womens Health. 2011;3:25-35.
740. Roehrborn CG, Lee M, Meehan A, Waldstreicher J, Group PS. Effects of finasteride on serum testosterone and body mass index in men with benign prostatic hyperplasia. Urology. 2003;62(5):894-9.
741. Ajossa S, Guerriero S, Paoletti AM, Orrù M, Melis GB. The antiandrogenic effect of flutamide improves uterine perfusion in women with polycystic ovary syndrome. Fertility and Sterility. 2002;77(6):1136-40.
742. Banaszewska B, Pawelczyk L, Spaczynski RZ, Dziura J, Duleba AJ. Effects of simvastatin and oral contraceptive agent on polycystic ovary syndrome: prospective, randomized, crossover trial. The Journal of Clinical Endocrinology and Metabolism. 2007;92(2):456-61.
743. Bhattacharya SM, Jha A, DasMukhopadhyay L. Comparison of two contraceptive pills containing drospirenone and 20 µg or 30 µg ethinyl estradiol for polycystic ovary syndrome. International journal of gynaecology and obstetrics: the official Jornal of the International Federation of Gynaecology and Obstetrics. 2016;132(2):210-3.
744. Bilgir O, Kebapcilar L, Taner C, Bilgir F, Kebapcilar A, Bozkaya G, et al. The effect of ethinylestradiol (EE)/cyproterone acetate (CA) and EE/CA plus metformin treatment on adhesion molecules in cases with polycystic ovary syndrome (PCOS). Internal Medicine (Tokyo, Japan). 2009;48(14):1193-9.
745. Carlsen SM, Vanky E, Fleming R. Anti-Müllerian hormone concentrations in androgen-suppressed women with polycystic ovary syndrome. Human Reproduction (Oxford, England). 2009;24(7):1732-8.
746. Elsersy MAM. Efficacy of combined cabergoline and metformin compared to metformin alone on cycle regularity in patients with polycystic ovarian disease with hyperprolactinemia: a randomized clinical trial. Journal of Obstetrics and Gynaecology of India. 2017;67(5):363-9.
747. Gambineri A, Patton L, Vaccina A, Cacciari M, Morselli-Labate AM, Cavazza C, et al. Treatment with flutamide, metformin, and their combination added to a hypocaloric diet in overweight-obese women with polycystic ovary syndrome: a randomized, 12-month, placebo-controlled study. J Clin Endocrinol Metab. 2006;91(10):3970-80.
748. Glintborg D, Altinok ML, Mumm H, Hermann AP, Ravn P, Andersen M. Body composition is improved during 12 months' treatment with metformin alone or combined with oral contraceptives compared with treatment with oral contraceptives in polycystic ovary syndrome. The Journal of Clinical Endocrinology and Metabolism. 2014;99(7):2584-91.
749. Glintborg D, Mumm H, Holst JJ, Andersen M. Effect of oral contraceptives and/or metformin on GLP-1 secretion and reactive hypoglycaemia in polycystic ovary syndrome. Endocrine Connections. 2017;6(4):267-77.

750. Jensterle M, Goricar K, Janez A. Metformin as an initial adjunct to low-dose liraglutide enhances the weight-decreasing potential of liraglutide in obese polycystic ovary syndrome: randomized control study. Experimental and Therapeutic Medicine. 2016;11(4):1194-200.
751. Kebapcilar L, Bilgir O, Taner CE, Kebapcilar AG, Kozaci DL, Alacacioglu A, et al. Oral contraceptives alone and with spironolactone increase sCD40 ligand in PCOS patients. Archives of Gynecology and Obstetrics. 2010;281(3):539-43.
752. Kebapcilar L, Taner CE, Kebapcilar AG, Alacacioglu A, Sari I. Comparison of four different treatment regimens on coagulation parameters, hormonal and metabolic changes in women with polycystic ovary syndrome. Archives of Gynecology and Obstetrics. 2010;281(1):35-42.
753. Leelaphiwat S, Jongwutiwes T, Lertvikool S, Tabcharoen C, Sukprasert M, Rattanasiri S, et al. Comparison of desogestrel/ethinyl estradiol plus spironolactone versus cyproterone acetate/ethinyl estradiol in the treatment of polycystic ovary syndrome: a randomized controlled trial. The Journal of Obstetrics and Gynaecology Research. 2015;41(3):402-10.
754. Romualdi D, De Cicco S, Busacca M, Gagliano D, Lanzone A, Guido M. Clinical efficacy and metabolic impact of two different dosages of ethinyl-estradiol in association with drospirenone in normal-weight women with polycystic ovary syndrome: a randomized study. Journal of Endocrinological Investigation. 2013;36(8):636-41.
755. Sahu A, Tripathy P, Mohanty J, Nagy A. Doppler analysis of ovarian stromal blood flow changes after treatment with metformin versus ethinyl estradiol-cyproterone acetate in women with polycystic ovarian syndrome: a randomized controlled trial. 2018.
756. Vanky E, Salvesen KA, Carlsen SM. Six-month treatment with low-dose dexamethasone further reduces androgen levels in PCOS women treated with diet and lifestyle advice, and metformin. Human Reproduction (Oxford, England). 2004;19(3):529-33.
757. Wu J, Zhu Y, Jiang Y, Cao Y. Effects of metformin and ethinyl estradiol-cyproterone acetate on clinical, endocrine and metabolic factors in women with polycystic ovary syndrome. Gynecological Endocrinology : the official Journal of the International Society of Gynecological Endocrinology. 2008;24(7):392-8.
758. Zain MM, Jamaluddin R, Ibrahim A, Norman RJ. Comparison of clomiphene citrate, metformin, or the combination of both for first-line ovulation induction, achievement of pregnancy, and live birth in Asian women with polycystic ovary syndrome: a randomized controlled trial. Fertility and Sterility. 2009;91(2):514-21.
759. Amiri M, Nahidi F, Bidhendi-Yarandi R, Khalili D, Tohidi M, Ramezani Tehrani F. A comparison of the effects of oral contraceptives on the clinical and biochemical manifestations of polycystic ovary syndrome: a crossover randomized controlled trial. Human Reproduction (Oxford, England). 2020;35(1):175-86.
760. Ghaneei A, Jowkar A, Hasani Ghavam MR, Ghaneei ME. Cabergoline plus metformin therapy effects on menstrual irregularity and androgen system in polycystic ovary syndrome women with hyperprolactinemia. Iranian Journal of Reproductive Medicine. 2015;13(2):93-100.
761. Ahmad J, Shukla N, Khan AR, Ahmed F, Siddiqui MA. Comparison of metabolic effects of metformin and rosiglitazone in the management of polycystic ovary syndrome (PCOS): a prospective, parallel, randomized, open-label study. Diabetes Metab Syndr Clin Res Rev. 2008;2(1):37-46.
762. Panidis D, Georgopoulos NA, Piouka A, Katsikis I, Saltamavros AD, Decavalas G, et al. The impact of oral contraceptives and metformin on anti-Müllerian hormone serum levels in women with polycystic ovary syndrome and biochemical hyperandrogenemia. Gynecological Endocrinology : the official journal of the International Society of Gynecological Endocrinology. 2011;27(8):587-92.
763. Teede HJ, Meyer C, Hutchison SK, Zoungas S, McGrath BP, Moran LJ. Endothelial function and insulin resistance in polycystic ovary syndrome: the effects of medical therapy. Fertility and Sterility. 2010;93(1):184-91.
764. Buvat J, Buvat-Herbaut M, Marcolin G, Racadot A, Fourlinnie JC, Beuscart R, et al. A double blind controlled study of the hormonal and clinical effects of bromocriptine in the polycystic ovary syndrome. The Journal of Clinical Endocrinology and Metabolism. 1986;63(1):119-24.

765. Murdoch AP, McClean KG, Watson MJ, Dunlop W, Kendall Taylor P. Treatment of hirsutism in polycystic ovary syndrome with bromocriptine. British Journal of Obstetrics and Gynaecology. 1987;94(4):358-65.
766. Rautio K, Tapanainen JS, Ruokonen A, Morin-Papunen LC. Endocrine and metabolic effects of rosiglitazone in overweight women with PCOS: a randomized placebo-controlled study. Human Reproduction (Oxford, England). 2006;21(6):1400-7.
767. Jensterle M, Salamun V, Kocjan T, Vrtacnik Bokal E, Janez A. Short term monotherapy with GLP-1 receptor agonist liraglutide or PDE 4 inhibitor roflumilast is superior to metformin in weight loss in obese PCOS women: a pilot randomized study. Journal of Ovarian Research. 2015;8:32.
768. Glintborg D, Mumm H, Altinok ML, Richelsen B, Bruun JM, Andersen M. Adiponectin, interleukin-6, monocyte chemoattractant protein-1, and regional fat mass during 12-month randomized treatment with metformin and/or oral contraceptives in polycystic ovary syndrome. Journal of Endocrinological Investigation. 2014;37(8):757-64.
769. Glintborg D, Sidelmann JJ, Altinok ML, Mumm H, Andersen M. Increased thrombin generation in women with polycystic ovary syndrome: a pilot study on the effect of metformin and oral contraceptives. Metabolism: Clinical and Experimental. 2015;64(10):1272-8.
770. Harborne L, Fleming R, Lyall H, Sattar N, Norman J. Metformin or antiandrogen in the treatment of hirsutism in polycystic ovary syndrome. The Journal of Clinical Endocrinology and Metabolism. 2003;88(9):4116-23.
771. Kumar Y, Kotwal N, Singh Y, Upreti V, Somani S, Hari Kumar KVS. A randomized, controlled trial comparing the metformin, oral contraceptive pills and their combination in patients with polycystic ovarian syndrome. Journal of Family Medicine and Primary Care.7(3):551-6.
772. Morin-Papunen L, Vauhkonen I, Koivunen R, Ruokonen A, Martikainen H, Tapanainen JS. Metformin versus ethinyl estradiol-cyproterone acetate in the treatment of nonobese women with polycystic ovary syndrome: a randomized study. The Journal of Clinical Endocrinology and Metabolism. 2003;88(1):148-56.
773. Morin-Papunen LC, Vauhkonen I, Koivunen RM, Ruokonen A, Martikainen HK, Tapanainen JS. Endocrine and metabolic effects of metformin versus ethinyl estradiol-cyproterone acetate in obese women with polycystic ovary syndrome: a randomized study. The Journal of Clinical Endocrinology and Metabolism. 2000;85(9):3161-8.
774. Cibula D, Fanta M, Vrbikova J, Stanicka S, Dvorakova K, Hill M, et al. The effect of combination therapy with metformin and combined oral contraceptives (COC) versus COC alone on insulin sensitivity, hyperandrogenaemia, SHBG and lipids in PCOS patients. Human Reproduction (Oxford, England). 2005;20(1):180-4.
775. Elter K, Imir G, Durmusoglu F. Clinical, endocrine and metabolic effects of metformin added to ethinyl estradiol-cyproterone acetate in non-obese women with polycystic ovarian syndrome: a randomized controlled study. Human Reproduction (Oxford, England). 2002;17(7):1729-37.
776. Malkawi HY, Qublan HS. The effect of metformin plus clomiphene citrate on ovulation and pregnancy rates in clomiphene-resistant women with polycystic ovary syndrome. Saudi Medical Journal. 2002;23(6):663-6.
777. Kahraman K, Sükür YE, Atabekoğlu CS, Ateş C, Taşkın S, Cetinkaya SE, et al. Comparison of two oral contraceptive forms containing cyproterone acetate and drospirenone in the treatment of patients with polycystic ovary syndrome: a randomized clinical trial. Archives of Gynecology and Obstetrics. 2014;290(2):321-8.
778. Duleba AJ, Banaszewska B, Spaczynski RZ, Pawelczyk L. Simvastatin improves biochemical parameters in women with polycystic ovary syndrome: results of a prospective, randomized trial. Fertility and Sterility. 2006;85(4):996-1001.
779. Falsetti L, De Fusco D, Eleftheriou G, Rosina B. Treatment of hirsutism by finasteride and flutamide in women with polycystic ovary syndrome. Gynecological Endocrinology : the official journal of the International Society of Gynecological Endocrinology. 1997;11(4):251-7.
780. Falsetti L, Gambera A, Legrenzi L, Iacobello C, Bugari G. Comparison of finasteride versus flutamide in the treatment of hirsutism. European Journal of Endocrinology. 1999;141(4):361-7.

781. Teede H, Tassone EC, Piltonen T, Malhotra J, Mol BW, Pena A, et al. Effect of the combined oral contraceptive pill and/or metformin in the management of polycystic ovary syndrome: a systematic review with meta-analyses. Clin Endocrinol (Oxf). 2019;91(4):479-89.
782. Fraison E, Kostova E, Moran LJ, Bilal S, Ee CC, Venetis C, et al. Metformin versus the combined oral contraceptive pill for hirsutism, acne, and menstrual pattern in polycystic ovary syndrome. Cochrane Database Syst Rev. 2020;8:CD005552.
783. Al Khalifah RA, Florez ID, Zoratti MJ, Dennis B, Thabane L, Bassilious E. Efficacy of treatments for polycystic ovarian syndrome management in adolescents. J Endocr Soc. 2021;5(1):bvaa155.
784. Teede HJ, Misso ML, Deeks AA, Moran LJ, Stuckey BG, Wong JL, et al. Assessment and management of polycystic ovary syndrome: summary of an evidence-based guideline. Med J Aust. 2011;195(6):S65-112.
785. Wu S, Divall S, Wondisford F, Wolfe A. Reproductive tissues maintain insulin sensitivity in diet-induced obesity. Diabetes. 2012;61(1):114-23.
786. Wang R, Li W, Bordewijk EM, Legro RS, Zhang H, Wu X, et al. First-line ovulation induction for polycystic ovary syndrome: an individual participant data meta-analysis. Hum Reprod Update. 2019;25(6):717-32.
787. Homburg R. Clomiphene citrate--end of an era? a mini-review. Hum Reprod. 2005;20(8):2043-51.
788. Casper RF. Aromatase inhibitors in ovarian stimulation. J Steroid Biochem Mol Biol. 2007;106(1-5):71-5.
789. Amer SA, Smith J, Mahran A, Fox P, Fakis A. Double-blind randomized controlled trial of letrozole versus clomiphene citrate in subfertile women with polycystic ovarian syndrome. Human Reproduction (Oxford, England). 2017;32(8):1631-8.
790. Banerjee Ray P, Ray A, Chakraborti PS. Comparison of efficacy of letrozole and clomiphene citrate in ovulation induction in Indian women with polycystic ovarian syndrome. Archives of Gynecology and Obstetrics. 2012;285(3):873-7.
791. Baruah J, Roy KK, Rahman SM, Kumar S, Sharma JB, Karmakar D. Endometrial effects of letrozole and clomiphene citrate in women with polycystic ovary syndrome using spiral artery doppler. Archives of Gynecology and Obstetrics. 2009;279(3):311-4.
792. Basirat Z, Kashifard M, Amiri MG. Enhanced ovarian folliclular development by metformin does not correlate with pregnancy rate: a randomized trial. International Journal of Fertility & Sterility. 2012;6(1):31-6.
793. Bayar U, Basaran M, Kiran S, Coskun A, Gezer S. Use of an aromatase inhibitor in patients with polycystic ovary syndrome: a prospective randomized trial. Fertility and Sterility. 2006;86(5):1447-51.
794. Dasari P, Pranahita G. The efficacy of metformin and clomiphene citrate combination compared with clomiphene citrate alone for ovulation induction in infertile patients with PCOS. Journal of Human Reproductive sciences. 2009;2(1):18-22.
795. El-khayat W, Abdel Moety G, Al Mohammady M, Hamed D. A randomized controlled trial of clomifene citrate, metformin, and pioglitazone versus letrozole, metformin, and pioglitazone for clomifene-citrate-resistant polycystic ovary syndrome. International Journal of Gynaecology and Obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2016;132(2):206-9.
796. Ganesh A, Goswami SK, Chattopadhyay R, Chaudhury K, Chakravarty B. Comparison of letrozole with continuous gonadotropins and clomiphene-gonadotropin combination for ovulation induction in 1387 PCOS women after clomiphene citrate failure: a randomized prospective clinical trial. Journal of Assisted Reproduction and Genetics. 2009;26(1):19-24.
797. Ghahiri A, Mogharehabed N, Mamourian M. Letrozole as the first-line treatment of infertile women with poly cystic ovarian syndrome (PCOS) compared with clomiphene citrate: a clinical trial. Advanced Biomedical Research. 2016;5:6.
798. Kar S, Sanchita S. Clomiphene citrate, metformin or a combination of both as the first line ovulation induction drug for Asian Indian women with polycystic ovarian syndrome: a randomized controlled trial. Journal of Human Reproductive Sciences.8(4):197-201.
799. Liu C, Feng G, Huang W, Wang Q, Yang S, Tan J, et al. Comparison of clomiphene citrate and letrozole for ovulation induction in women with polycystic ovary syndrome: a prospective randomized trial. Gynecological

Endocrinology : the official journal of the International Society of Gynecological Endocrinology. 2017;33(11):872-6.
800. Chen Z, Zhang M, Qiao Y, Yang J. Effects of letrozole in combination with low-dose intramuscular injection of human menopausal gonadotropin on ovulation and pregnancy of 156 patients with polycystic ovary syndrome. Pak J Med Sci. 2016;32(6):1434-8.
801. Moll E, Korevaar JC, Bossuyt PM, van der Veen F. Does adding metformin to clomifene citrate lead to higher pregnancy rates in a subset of women with polycystic ovary syndrome? Human Reproduction (Oxford, England). 2008;23(8):1830-4.
802. Behnoud N, Farzaneh F, Ershadi S. The effect of clomiphene citrate versus letrozole on pregnancy rate in women with polycystic ovary syndrome: a randomized clinical trial. Crescent Journal of Medical and Biological Sciences. 2019;6(3):335-40.
803. Dehbashi S, Kazerooni T, Robati M, Alborzi S, Parsanezhad ME, Shadman A. Comparison of the effects of letrozole and clomiphene citrate on ovulation and pregnancy rate in patients with polycystic ovary syndrome. Iranian Journal of Medical Sciences. 2009;34(1):23-8.
804. Legro RS, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Casson P, et al. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. The New England Journal of Medicine. 2014;371(2):119-29.
805. Najafi PZ, Noghabi SP, Afzali N, Mohammadzadeh S. Comparing the effect of clomiphene citrate and letrozole on ovulation induction in infertile women with polycystic ovary syndrome. JPMA The Journal of the Pakistan Medical Association. 2020;70(2):268-71.
806. Ayaz A, Alwan Y, Farooq MU. Metformin-clomiphene citrate vs. clomiphene citrate alone: Polycystic ovarian syndrome. Journal of Human Reproductive Sciences. 2013;6(1):15-8.
807. Katica V, Rama A, Abadžić N. Efficacy of ovulation induction with clomiphene citrate and metformin in women with polycystic ovary syndrome - pilot study. Folia Medica - Facultatis Medicinae Universitatis Saraeviensis. 2014;49(1):37-42.
808. Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA, et al. Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. The New England Journal of Medicine. 2007;356(6):551-66.
809. Sahin Y, Yirmibeş U, Keleştimur F, Aygen E. The effects of metformin on insulin resistance, clomiphene-induced ovulation and pregnancy rates in women with polycystic ovary syndrome. European Journal of Obstetrics, Gynecology, and Reproductive Biology. 2004;113(2):214-20.
810. Nakamura Y, Takasaki A, Sugino N, Tamura H, Takiguchi S, Takayama H, et al. Studies on the effects of initial injection doses of follicle stimulating hormone on the pregnancy and the ovarian hyperstimulation syndrome incidence in polycystic ovarian syndrome patients. Reprod Med Biol. 2003;2(2):63-7.
811. Wang R, Kim BV, van Wely M, Johnson NP, Costello MF, Zhang H, et al. Treatment strategies for women with WHO group II anovulation: systematic review and network meta-analysis. BMJ. 2017;356:j138.
812. Palomba S, Orio F, Jr., Falbo A, Russo T, Tolino A, Zullo F. Clomiphene citrate versus metformin as first-line approach for the treatment of anovulation in infertile patients with polycystic ovary syndrome. J Clin Endocrinol Metab. 2007;92(9):3498-503.
813. Roque M, Tostes AC, Valle M, Sampaio M, Geber S. Letrozole versus clomiphene citrate in polycystic ovary syndrome: systematic review and meta-analysis. Gynecol Endocrinol. 2015;31(12):917-21.
814. Akinoso-Imran AQ, Adetunji H. Systematic review and meta-analysis of letrozole and clomiphene citrate in polycystic ovary syndrome. Middle East Fertility Society Journal. 2018;23(3):163-70.
815. He D, Jiang F. Meta-analysis of letrozole versus clomiphene citrate in polycystic ovary syndrome. Reprod Biomed Online. 2011;23(1):91-6.
816. Barnard L, Ferriday D, Guenther N, Strauss B, Balen AH, Dye L. Quality of life and psychological well being in polycystic ovary syndrome. Hum Reprod. 2007;22(8):2279-86.
817. Nisenblat V, Norman RJ. Androgens and polycystic ovary syndrome. Curr Opin Endocrinol Diabetes Obes. 2009;16(3):224-31.

818. Salley KE, Wickham EP, Cheang KI, Essah PA, Karjane NW, Nestler JE. Glucose intolerance in polycystic ovary syndrome--a position statement of the Androgen Excess Society. J Clin Endocrinol Metab. 2007;92(12):4546-56.
819. Garruti G, Depalo R, Vita MG, Lorusso F, Giampetruzzi F, Damato AB, et al. Adipose tissue, metabolic syndrome and polycystic ovary syndrome: from pathophysiology to treatment. Reprod Biomed Online. 2009;19(4):552-63.
820. Essah PA, Wickham EP, Nestler JE. The metabolic syndrome in polycystic ovary syndrome. Clin Obstet Gynecol. 2007;50(1):205-25.
821. Wadden TA, Berkowitz RI, Womble LG, Sarwer DB, Phelan S, Cato RK, et al. Randomized trial of lifestyle modification and pharmacotherapy for obesity. N Engl J Med. 2005;353(20):2111-20.
822. Wadden TA, Volger S, Sarwer DB, Vetter ML, Tsai AG, Berkowitz RI, et al. A two-year randomized trial of obesity treatment in primary care practice. N Engl J Med. 2011;365(21):1969-79.
823. Abdalla MA, Shah N, Deshmukh H, Sahebkar A, Ostlundh L, Al-Rifai RH, et al. Effect of pharmacological interventions on lipid profiles and C-reactive protein in polycystic ovary syndrome: A systematic review and meta-analysis. Clin Endocrinol (Oxf). 2021.
824. Kumar P, Khan K. Effects of metformin use in pregnant patients with polycystic ovary syndrome. J Hum Reprod Sci. 2012;5(2):166-9.
825. Jorquera G, Echiburu B, Crisosto N, Sotomayor-Zarate R, Maliqueo M, Cruz G. Metformin during pregnancy: effects on offspring development and metabolic function. Front Pharmacol. 2020;11:653.
826. Zain MM, Jamaluddin R, Ibrahim A, Norman RJ. Comparison of clomiphene citrate, metformin, or the combination of both for first-line ovulation induction, achievement of pregnancy, and live birth in Asian women with polycystic ovary syndrome: a randomized controlled trial. Fertil Steril. 2009;91(2):514-21.
827. Nestler JE. Metformin in the treatment of infertility in polycystic ovarian syndrome: an alternative perspective. Fertil Steril. 2008;90(1):14-6.
828. Abdalla MA, Deshmukh H, Atkin S, Sathyapalan T. The potential role of incretin-based therapies for polycystic ovary syndrome: a narrative review of the current evidence. Ther Adv Endocrinol Metab. 2021;12:2042018821989238.
829. Azziz R, Ehrmann DA, Legro RS, Fereshetian AG, O'Keefe M, Ghazzi MN. Troglitazone decreases adrenal androgen levels in women with polycystic ovary syndrome. Fertility and Sterility. 2003;79(4):932-7.
830. Psaty BM, Furberg CD. Rosiglitazone and cardiovascular risk. N Engl J Med. 2007;356(24):2522-4.
831. Ajjan RA, Grant PJ. The cardiovascular safety of rosiglitazone. Expert Opin Drug Saf. 2008;7(4):367-76.
832. Bailey CJ. Thiazolidinediones. xPharm: the comprehensive pharmacology reference: Elsevier; 2011.
833. Valsamakis G, Lois K, Kumar S, Mastorakos G. Metabolic and other effects of pioglitazone as an add-on therapy to metformin in the treatment of polycystic ovary syndrome (PCOS). Hormones (Athens). 2013;12(3):363-78.
834. Cataldo NA, Abbasi F, McLaughlin TL, Basina M, Fechner PY, Giudice LC, et al. Metabolic and ovarian effects of rosiglitazone treatment for 12 weeks in insulin-resistant women with polycystic ovary syndrome. Human Reproduction (Oxford, England). 2006;21(1):109-20.
835. Dereli D, Dereli T, Bayraktar F, Ozgen AG, Yilmaz C. Endocrine and metabolic effects of rosiglitazone in non-obese women with polycystic ovary disease. Endocrine Journal. 2005;52(3):299-308.
836. Glintborg D, Frystyk J, Højlund K, Andersen KK, Henriksen JE, Hermann AP, et al. Total and high molecular weight (HMW) adiponectin levels and measures of glucose and lipid metabolism following pioglitazone treatment in a randomized placebo-controlled study in polycystic ovary syndrome. Clinical Endocrinology. 2008;68(2):165-74.
837. Huang R, Zhao PF, Xu JH, Liu DD, Luo FD, Dai YH. Effects of placebo-controlled insulin-sensitizing drugs on hormonal parameters in polycystic ovary syndrome patients: a network meta-analysis. J Cell Biochem. 2018;119(3):2501-11.

838. Zhao H, Xing C, Zhang J, He B. Comparative efficacy of oral insulin sensitizers metformin, thiazolidinediones, inositol, and berberine in improving endocrine and metabolic profiles in women with PCOS: a network meta-analysis. Reprod Health. 2021;18(1):171.
839. Xing C, Li C, He B. Insulin sensitizers for improving the endocrine and metabolic profile in overweight women with PCOS. J Clin Endocrinol Metab. 2020;105(9).
840. Juurlink DN, Gomes T, Lipscombe LL, Austin PC, Hux JE, Mamdani MM. Adverse cardiovascular events during treatment with pioglitazone and rosiglitazone: population based cohort study. BMJ. 2009;339:b2942.
841. Froment P, Touraine P. Thiazolidinediones and fertility in polycystic ovary syndrome (PCOS). PPAR Res. 2006;2006:73986.
842. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Fertil Steril. 2018;110(3):364-79.
843. Gibson-Helm M, Teede H, Dunaif A, Dokras A. Delayed diagnosis and a lack of information associated with dissatisfaction in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2017;102(2):604-12.
844. Dokras A, Saini S, Gibson-Helm M, Schulkin J, Cooney L, Teede H. Gaps in knowledge among physicians regarding diagnostic criteria and management of polycystic ovary syndrome. Fertil Steril. 2017;107(6):1380-6 e1.
845. Crete J, Adamshick P. Managing polycystic ovary syndrome: what our patients are telling us. J Holist Nurs. 2011;29(4):256-66.
846. Fernandez RC, Moore VM, Rumbold AR, Whitrow MJ, Avery JC, Davies MJ. Diagnosis delayed: health profile differences between women with undiagnosed polycystic ovary syndrome and those with a clinical diagnosis by age 35 years. Hum Reprod. 2021;36(8):2275-84.
847. Deakin TA, Cade JE, Williams R, Greenwood DC. Structured patient education: the diabetes X-PERT Programme makes a difference. Diabet Med. 2006;23(9):944-54.
848. Christie D, Thompson R, Sawtell M, Allen E, Cairns J, Smith F, et al. Structured, intensive education maximising engagement, motivation and long-term change for children and young people with diabetes: a cluster randomised controlled trial with integral process and economic evaluation - the CASCADE study. Health Technol Assess. 2014;18(20):1-202.
849. Hadjiconstantinou M, Dunkley AJ, Eborall H, Robertson N, Khunti K, Davies M. Perceptions of healthcare professionals and people with type 2 diabetes on emotional support: a qualitative study. BJGP Open. 2020;4(1).
850. Lim SS, Hutchison SK, Van Ryswyk E, Norman RJ, Teede HJ, Moran LJ. Lifestyle changes in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2019;3:CD007506.
851. Randeva HS, Lewandowski KC, Drzewoski J, Brooke-Wavell K, O'Callaghan C, Czupryniak L, et al. Exercise decreases plasma total homocysteine in overweight young women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2002;87(10):4496-501.
852. Arentz S, Smith CA, Abbott J, Bensoussan A. Perceptions and experiences of lifestyle interventions in women with polycystic ovary syndrome (PCOS), as a management strategy for symptoms of PCOS. BMC Womens Health. 2021;21(1):107.
853. Davies MJ, Heller S, Skinner TC, Campbell MJ, Carey ME, Cradock S, et al. Effectiveness of the diabetes education and self management for ongoing and newly diagnosed (DESMOND) programme for people with newly diagnosed type 2 diabetes: cluster randomised controlled trial. BMJ. 2008;336(7642):491-5.
854. Schlenk EA, Lias JL, Sereika SM, Dunbar-Jacob J, Kwoh CK. Improving physical activity and function in overweight and obese older adults with osteoarthritis of the knee: a feasibility study. Rehabil Nurs. 2011;36(1):32-42.
855. Brennan L, Teede H, Skouteris H, Linardon J, Hill B, Moran L. Lifestyle and behavioral management of polycystic ovary syndrome. J Womens Health (Larchmt). 2017;26(8):836-48.

13 Chapter 13: Appendix

13.1 Participants' consent form for the living with PCOS study-pilot study

PARTICIPANT CONSENT FORM – Pilot study

Full Title: Living with polycystic ovary syndrome (LW-PCOS), piloting the structured educational programme.

Name of Researchers: Prof T Sathyapalan Dr Maria Papageorgiou Dr Mohammed Abdalla Mrs Lisa Baldwin

Participant identification number: _____

Please read the following statements, and if you agree, place your initials in the box:

- I confirm that I have read and understood the Participant Information Sheet (LW-PCOS.V2. 5th February 2021) for the above research study. I have had the opportunity to ask questions, and I am satisfied with the answers I have received. ☐
- I understand that my participation will involve me taking part in the development and piloting of an educational programme for PCOS, and the questions asked will relate to PCOS. ☐
- I am aware that the data will be none identifiable and stored in a locked filing cabinet or on a password-protected computer. ☐
- I understand that data gathered from the results of the pilot study may be presented at a conference or published. ☐
- I agree to the storage of anonymised data for future research in PCOS and other long-term conditions for up to 5 years. ☐
- I freely agree to participate in the above study and understand that I can withdraw at any time without giving any reason, and without my medical care or legal rights being affected. ☐
- I understand that confidentiality will be maintained for any discussion that arises during these sessions. ☐

Name of Participant:	Signature:	Date and Time:
_____	_____	_____
Name of Researcher:	Signature:	Date and Time:
_____	_____	_____

Original document for the researcher, one copy for the participant, and one copy to be kept with source documents.

13.2 Ethical approval letter

Health Research Authority

London - Brent Research Ethics Committee
80 London Road Skipton House
London SE1 6LH

Please note: This is the favourable opinion of the REC only and does not allow

you to start your study at NHS sites in England until you receive HRA Approval

08 February 2021

Professor Thozhukat Sathyapalan
Chair in Academic Endocrinology, Diabetes and Metabolism / Honorary Consultant Physician
University of Hull
Michael White Diabetes Centre, Brocklehurst Building
220-236 Anlaby Road, Hull
HU3 2RW

Dear Professor, Sathyapalan

Study title: Living With Polycystic Ovary Syndrome (LW-PCOS) - a structured education programme
REC reference: 20/PR/0840
IRAS project ID: 287175

Thank you for your letter of 05 February 2021, responding to the Proportionate Review Sub-Committee's request for changes to the documentation for the above study.

The revised documentation has been reviewed and approved on behalf of the PR sub-committee.

Confirmation of ethical opinion
On behalf of the Research Ethics Committee (REC), I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.
Good practice principles and responsibilities
The UK Policy Framework for Health and Social Care Research sets out principles of good practice in the management and conduct of health and social care research. It also outlines the responsibilities of individuals and organisations, including those related to the four elements of research transparency:

1. registering research studies
2. reporting results
3. informing participants
4. sharing study data and tissue

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Confirmation of Capacity and Capability (in England, Northern Ireland and Wales) or NHS management permission (in Scotland) should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA and HCRW Approval (England and Wales)/ NHS permission for research is available in the Integrated Research Application System.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

All research should be registered in a publicly accessible database and we expect all researchers, research sponsors and others to meet this fundamental best practice standard.

It is a condition of the REC favourable opinion that **all clinical trials are registered** on a publicly accessible database within six weeks of recruiting the first research participant. For this purpose, 'clinical trials' are defined as the first four project categories in IRAS project filter question 2. Failure to register is a breach of these approval conditions, unless a deferral has been agreed by or on behalf of the Research Ethics Committee (see here for more information on requesting a deferral: https://www.hra.nhs.uk/planning-and-improving-research/research-planning/research-registration-research-project-identifiers/

If you have not already included registration details in your IRAS application form, you should notify the REC of the registration details as soon as possible.

Publication of Your Research Summary

We will publish your research summary for the above study on the research summaries section of our website, together with your contact details, no earlier than three months from the date of this favourable opinion letter.

Should you wish to provide a substitute contact point, make a request to defer, or require further information, please visit:

https://www.hra.nhs.uk/planning-and-improving-research/application-summaries/research-summaries/

N.B. If your study is related to COVID-19 we will aim to publish your research summary within 3 days rather than three months.

During this public health emergency, it is vital that everyone can promptly identify all relevant research related to COVID-19 that is taking place globally. If you haven't already done so, please register your study on a public registry as soon as possible and provide the REC with the registration detail, which will be posted alongside other information relating to your project. We are also asking sponsors not to request deferral of publication of research summary for any projects relating to COVID-19. In addition, to facilitate finding and extracting studies related to COVID-19 from public databases, please enter the WHO official acronym for the coronavirus disease (COVID-19) in the full title of your study. Approved COVID-19 studies can be found at: https://www.hra.nhs.uk/covid-19-research/approved-covid-19-research/

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

After ethical review: Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study, including early termination of the study
- Final report
- Reporting results

The latest guidance on these topics can be found at https://www.hra.nhs.uk/approvals-amendments/managing-your-approval/.

Ethical review of research sites

The favourable opinion applies to all NHS/HSC sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" above).

Approved documents

The documents reviewed and approved by the Committee are:

Document	*Version*	*Date*
Covering letter on headed paper		20 November 2020
Covering letter on headed paper [letter]	LW-PCOS.V 2.5th February 2021	05 February 2021

Initial Assessment for REC		27 November 2020
IRAS Application Form [IRAS_Form_05022021]		05 February 2021
Letter from funder		11 April 2019
Letters of invitation to participant	1	01 August 2020
Non-validated questionnaire	1	01 August 2020
Non-validated questionnaire	1	01 August 2020
Non-validated questionnaire	1	01 August 2020
Non-validated questionnaire	1	01 August 2020
Participant consent form	LW-PCOS.V 2.5th February 2021	05 February 2021
Participant information sheet (PIS)	LW-PCOS.V 2.5th February 2021	05 February 2021
Research protocol or project proposal	LW-PCOS.V 2.5th February 2021	05 February 2021
Summary CV for Chief Investigator (CI)		19 October 2020
Summary CV for student [CV]	Version 1.5th February 2021	05 February 2021

We are pleased to welcome researchers and research staff to our HRA Learning Events and online learning opportunities– see details at:
https://www.hra.nhs.uk/planning-and-improving-research/learning/

IRAS project ID: 287175 **Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely

pp

Dr Manish Saxena Chair

Email: brent.rec@hra.nhs.uk

Enclosures: "After ethical review – guidance for researchers" [SL-AR2] Copy to:

Mr Michael Murrey

Professor Thozhukat Sathyapalan, University of Hull
Lead Nation
England: approvals@hra.nhs.uk

13.3 HRA approval letter

Professor Thozhukat Sathyapalan

Chair in Academic Endocrinology, Diabetes and Metabolism / Honorary Consultant Physician University of Hull
Michael White Diabetes Centre, Brocklehurst Building
Academic diabetes, endocrinology and metabolism
220-236 Anlaby Road, Hull, HU3 2RW

Email: approvals@hra.nhs.uk

HCRW.approvals@wales.nhs.uk

08 February 2021
Dear Professor Sathyapalan

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title: **Living With Polycystic Ovary Syndrome (LW-PCOS) - a structured education programme**
IRAS project ID: **287175**
REC reference: **20/PR/0840**
Sponsor **Hull University Teaching Hospital NHS Trust**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.
Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?

HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.
If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?

HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The standard conditions document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

Who should I contact for further information?

Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **287175**. Please quote this on all correspondence.

Yours sincerely,

Natalie Wilson

Approvals Manager

Email: **brent.rec@hra.nhs.uk**

Copy to: *Mr Michael Murrey, Hull University Teaching Hospital NHS Trust, Sponsor contact*

List of Documents

The final document set assessed and approved by HRA and HCRW Approval is listed below.

Document	*Version*	*Date*
Covering letter on headed paper		20 November 2020
Covering letter on headed paper [letter]	LW-PCOS.V2.5th February 2021	05 February 2021
Initial Assessment for REC		27 November 2020
IRAS Application Form [IRAS_Form_05022021]		05 February 2021
Letter from funder		11 April 2019
Letters of invitation to participant	1	01 August 2020
Non-validated questionnaire	1	01 August 2020
Non-validated questionnaire	1	01 August 2020
Non-validated questionnaire	1	01 August 2020
Non-validated questionnaire	1	01 August 2020
Participant consent form	LW-PCOS.V2.5th February 2021	05 February 2021
Participant information sheet (PIS)	LW-PCOS.V2.5th February 2021	05 February 2021
Research protocol or project proposal	LW-PCOS.V2.5th February 2021	05 February 2021
Summary CV for Chief Investigator (CI)		19 October 2020
Summary CV for student [CV]	Version 1.5th February 2021	05 February 2021

13.4 Survey on patient's perspectives on the development of an educational programme for polycystic ovary syndrome (PCOS)

Personal Information

1. What is your age group? Please circle an option that best describes you:

a. Less than 19 **b**. 19-30 **c**. 31-40 **d**. above 40

2. What is your employment/education status? Please circle the option(s) that best describes you:

a. employed **b**. unemployed **c**. in full time education **d**. in training.

e. preferred not to say.

3. How old were you when you were diagnosed with PCOS ? Please state in years YY

4. Which of the following do you think might improve your confidence in managing your PCOS? Please tick all that apply:

Information about PCOS including causes and long-term consequences	
Information on diet and exercise	
Information on behavioural change techniques (e.g. goal setting, self-monitoring, peer support)	
Psychological support	
Medication and treatment options	
Long-term monitoring including check for diabetes, heart problems, infertility, and cancer	
Information on services available	
Other	

5. If you answered **"other"**, please state what would help you:

6. If an educational programme about living with PCOS was available, how useful would it be to you? Please circle your answer in the scale below, where 5 is very useful and 1 is not at all useful:

1	2	3	4	5
Not at all		somewhat		very much

Would you please state the reason(s) for your answer?

_____

_____

_____

Information about PCOS

7. Do you think you have enough knowledge about PCOS? Please circle the answer in the scale below

1	2	3	4	5
Not at all		somewhat		very much

8. Do you think there is need to increase your knowledge about PCOS? Please circle in the scale below

1	2	3	4	5
Not at all		somewhat		very much

9. Do you think you need to know more about the symptoms of PCOS ?

1	2	3	4	5
Not at all		somewhat		very much

10. Do you think you know about the current clinical management strategies for PCOS?

1	2	3	4	5
Not at all		somewhat		very much

11. Do you feel you need to know about the long-term complications associated with PCOS?

1	2	3	4	5
Not at all		somewhat		very much

12. Have you needed treatment for your condition?

Yes ☐ No ☐ If yes, please tick what the type (s) of treatment.

Lifestyle management ☐ Medication(s) ☐ Both ☐

13. What additional things would you like to know about PCOS? Please use the space below to give further details

Information about lifestyle modifications including diet and exercise

14. Would you like to learn more about the healthy food choices and a balanced diet?

1	2	3	4	5
Not at all		somewhat		very much

15. Would you like to know about the recommendations for physical activity?

1	2	3	4	5
Not at all		somewhat		very much

16. Would you like to learn ways to increase your physical activities?

1	2	3	4	5
Not at all		somewhat		very much

17. Would you like to know how to lose and maintain weight through changes in diet and exercise?

1	2	3	4	5
Not at all		somewhat		very much

18. Would you like to know about the popular diets including (very low-calorie diets, meal replacement products, low carb diets, ketogenic diets, intermittent fasting)?

1	2	3	4	5
Not at all		somewhat		very much

19. What additional things that you would like to know about lifestyle modifications? Please use the space below to give further details

20. The following behavioural change techniques have been shown to help individuals lose and maintain their weight. What is your current knowledge level about the following?

Setting goals relevant to exercise?

1	2	3	4	5
Not at all		somewhat		very much

Setting goals relevant to diet?

1	2	3	4	5
Not at all		somewhat		very much

Regular monitoring of your weight?

1	2	3	4	5
Not at all		somewhat		very much

Keeping a food record i.e. what you have been eating and drinking?

1	2	3	4	5
Not at all		somewhat		very much

Keeping activity records i.e. how many steps per day and distance?

1	2	3	4	5
Not at all		somewhat		very much

Group exercise/educational/weight loss sessions?

1	2	3	4	5
Not at all		somewhat		very much

Contact with an expert (GP, dietitian etc)?

1	2	3	4	5
Not at all		somewhat		very much

Overcoming barriers related to diet and exercise?

1	2	3	4	5
Not at all		somewhat		very much

Controlling your emotions that may lead you to overeating?

1	2	3	4	5
Not at all		somewhat		very much

21. Would you like to know more about the above techniques, that may help you lose/maintain your weight?

1	2	3	4	5
Not at all		somewhat		very much

Information about your symptoms

Below is a list of symptoms which may be associated with PCOS, please answer if you have experienced any of these and how concerned you feel that they affect you. Answer all questions which apply.

22. Irregular periods (oligomenorrhea), circle the most appropriate answer.

1	2	3	4	5
Never		Yes, but in the past		Yes, currently

If you have answered yes, please circle how severe you feel your symptoms are:

1	2	3	4	5
No concern		minor concern		major concern

23. Absence of periods (amenorrhea), circle the most appropriate answer.

24.

1	2	3	4	5
Never		Yes, but in the past		Yes, currently

If you have answered yes, please circle how severe you feel your symptoms are:

1	2	3	4	5
No concern		minor concern		major concern

25. Excess body or facial hair (hirsutism), circle the most appropriate answer.

26.

1	2	3	4	5
Never		Yes, but in the past		Yes, currently

If you have answered yes, please circle how severe you feel your symptoms are:

1	2	3	4	5
No concern		minor concern		major concern

27. Poor skin and spots (acne), circle the most appropriate answer.

1	2	3	4	5
Never		Yes, but in the past		Yes, currently

If you have answered yes, please circle how severe you feel your symptoms are:

1	2	3	4	5
No concern		minor concern		major concern

28. Difficulty with fertility/ falling pregnant (anovulation), circle the most appropriate answer.

1	2	3	4	5
Never		Yes, but in the past		Yes, currently

If you have answered yes, please circle how severe you feel your symptoms are:

1	2	3	4	5
No concern		minor concern		major concern

29. Weight gain / difficulty losing weight, circle the most appropriate answer.

1	2	3	4	5
Never		Yes, but in the past		Yes, currently

If you have answered yes, please circle how severe you feel your symptoms are:

1	2	3	4	5
No concern		minor concern		major concern

30. Mood-swings, circle the most appropriate answer.

1	2	3	4	5
Never		Yes, but in the past		Yes, currently

If you have answered yes, please mark on the line how severe you feel your symptoms are:

1	2	3	4	5
No concern		minor concern		major concern

31. Male-pattern baldness, circle the most appropriate answer.

1	2	3	4	5
Never		Yes, but in the past		Yes, currently

If you have answered yes, please circle how severe you feel your symptoms are:

1	2	3	4	5
No concern		minor concern		major concern

32. Altered eating patterns (binge eating), circle the most appropriate answer.

1	2	3	4	5
Never		Yes, but in the past		Yes, currently

If you have answered yes, please circle how severe you feel your symptoms are:

1	2	3	4	5
No concern		minor concern		major concern

33. Cravings for certain foods, especially high fat, or sugary foods, circle the most appropriate answer.

1	2	3	4	5
Never		Yes, but in the past		Yes, currently

If you have answered yes, please circle how severe you feel your symptoms are:

1	2	3	4	5
No concern		minor concern		major concern

<u>Information on medications</u>

33. Which medication (s) you are on? Please state below

34. How long have you been on this treatment (month/year)?

MM	YY

35. What do you feel about your knowledge of the treatment options available for PCOS?

1	2	3	4	5
Not at all		somewhat		very much

36. Would you like to know more about the available treatment options?

1	2	3	4	5
Not at all		somewhat		very much

37. Would you like to know more about anything else with regards to the treatment Please state below

Following being offered the treatment how do you feel this has affected each of your symptoms. Circle one of the responses which is closest to how you feel the period spent following the treatment has affected your symptoms.

38. Irregular periods (oligomenorrhea), please circle the most appropriate answer.

1	2	3	4	5
Not improved		somewhat improved		very much improved

39. Absence of periods (amenorrhea), please circle the most appropriate answer.

1	2	3	4	5
Not improved		somewhat improved		very much improved

40. Excess body or facial hair (hirsutism), please circle the most appropriate answer.

1	2	3	4	5
Not improved		somewhat improved		very much improved

41. Poor skin and spots (acne) please circle the most appropriate answer

1	2	3	4	5
Not improved		somewhat improved		very much improved

42. Difficulty with fertility/ falling pregnant (anovulation), please circle the most appropriate answer

1	2	3	4	5
Not improved		somewhat improved		very much improved

43. Weight gain / difficulty losing weight, please circle the most appropriate answer.

1	2	3	4	5
Not improved		somewhat improved		very much improved

44. Mood swings, please circle the most appropriate answer.

1	2	3	4	5

Not improved somewhat improved very much improved

45. Male pattern baldness, please circle the most appropriate answer.

1	2	3	4	5
Not improved		somewhat improved		very much improved

46. Altered eating patterns (binge eating), please circle the most appropriate answer.

1	2	3	4	5
Not improved		somewhat improved		very much improved

47. Cravings for certain foods, especially high fat, or sugary foods, please circle the most appropriate answer.

1	2	3	4	5
Not improved		somewhat improved		very much improved

Information on long-term monitoring

48. Would you like to know more about the long-term monitoring and health checks available for PCOS including check for diabetes, heart problems, infertility, and cancer?

1	2	3	4	5
Not at all		somewhat		very much

Is there anything else you would like to tell us about your experience living with PCOS?

End of Questionnaire

The research team would like to thank you for your time in completing this questionnaire.

Contact Details for Further Information

Any queries and feedback about the research study can be discussed with any member of the research study team, who can be contacted on 01482 675387.
Independent advice about the research study can be sought from the local Patient Advice and Liaison Service on 01482 623065 or pals@hey.nhs.uk.

13.5 Pre-pilot evaluation form

Thank you for taking part in this survey. The survey is designed to captures your expectations, knowledge, and perception from taking part in the educational session. It should take 5-10 minutes to complete. Please write your response to each question in the allocated area.

1) What would you expect to gain from the educational session ?

2) How much you would expect to benefit from this session ?

1	2	3	4	5
Not at all		somewhat		very much

3) How engaging do you expect this session will be ?

1	2	3	4	5
Not engaging		somewhat engaging		very much engaging

4) Do you expect this session will be informative ?

1	2	3	4	5
Not informative		somewhat informative		very informative

5) Do you know what PCOS is ?

1	2	3	4	5
Not at all		somewhat		very much

6) Do you think it is a virus or bacteria that caused your illness?

1	2	3	4	5
Not at all		somewhat		very much

7) Do you think this condition runs in your family?

1	2	3	4	5
Not at all		somewhat		very much

8) Do you believe that your condition is largely due to your own behaviour ?

1	2	3	4	5
Not at all		somewhat		very much

9) Do you believe that being under stress or pressure was a major reason for your condition ?

1 2 3 4 5

Not at all somewhat very much

10) Do you have any idea what are the common symptoms associated with PCOS?

1 2 3 4 5

Not at all somewhat very much

11) Do you think your symptoms are permanent and will last with you for the rest of your life?

1 2 3 4 5

Not at all somewhat very much

12) Do you believe your illness is a serious condition and it has major consequences in your life ?

1 2 3 4 5

Not at all somewhat very much

13) Do you know what are the long-term consequences associated with PCOS?

1 2 3 4 5

Not at all somewhat very much

14) Do you know the current treatment options available for PCOS?

1 2 3 4 5

Not at all somewhat very much

15) Do you think your current treatments are effective in curing your condition?

1 2 3 4 5

Not at all somewhat very much

16) Do you think it easier to live with your condition?

1 2 3 4 5

Not at all somewhat very much

17) Do you think there is a lot of what you can do to improve your condition ?

1 2 3 4 5

Not at all somewhat very much

18) Do you think what you can do will determine whether your symptoms get worse or better ?

1 2 3 4 5

Not at all somewhat very much

19) Do you know the behaviour change strategies that help you losing weight and maintain weight loss?

1	2	3	4	5
Not at all		somewhat		very much

20) Do you know what are healthy food choices and balanced diet that can help you lose weight?

1	2	3	4	5
Not at all		somewhat		very much

21) Do you have any idea about the recent recommendation for physical activity and how to increase your activity levels?

1	2	3	4	5
Not at all		somewhat		very much

22) Do you know what are the behaviour changes techniques that can help you losing weight and maintaining weight loss?

1	2	3	4	5
Not at all		somewhat		very much

23) Do you know what kind of support available for PCOS ?

1	2	3	4	5
Not at all		somewhat		very much

24) How much do you believe it is important to know about your condition?

1	2	3	4	5
Not at all		somewhat		very much

25) Do you think it is important to be conscious about your health?

1	2	3	4	5
Not at all		somewhat		very much

26) Do you believe it is important to gain knowledge about the long-term consequences related to your condition and ways of prevention available to you?

1	2	3	4	5
Not at all		somewhat		very much

27) Would you please list your other expectations, suggestions, and comments below?

13.6 Post-pilot evaluation form

Thank you for taking part in this survey. The survey is designed to captures your satisfactions and knowledge gained by taking part in the educational session. It should take 5-10 minutes to complete. Please write your response to each question in the allocated area.

1) How would you rate the feasibility of accessing the programme quickly?

1	2	3	4	5
Not easy		somehow easy		very easy

2) How satisfied are you with the delivery of the session ?

1	2	3	4	5
Not satisfied		somewhat satisfied		very satisfied

3) Have you had the opportunity to express/talk freely about PCOS-related symptoms and your feelings ?

Yes ☐ No ☐

4) How do you rate the time of the session ?

1	2	3	4	5
long-time		just right time		perfect time

5) How satisfied are you with quality of the presented educational materials ?

1	2	3	4	5
Not satisfied		somewhat satisfied		very satisfied

6) What do you think about the clarity of the messages being given ?

1	2	3	4	5
Not clear		somehow clear		very clear

7) How satisfied are you with information presented in the educational session?

1	2	3	4	5
Not satisfied		somewhat satisfied		very satisfied

8) How relevant do you think the knowledge / skills necessary for management of PCOS were ?

1	2	3	4	5
Not relevant		somehow relevant		very relevant

9) How would you rate your ability to keep up with the information provided ?

1	2	3	4	5
Not able		somehow able		very much able

10) How relevant were the information about your personal risk of developing PCOS-related complications ?

1	2	3	4	5
Not relevant		somehow relevant		very relevant

11) How do you rate the educator?

1	2	3	4	5
Not informative		somewhat informative		very informative

12) Rate your overall satisfaction with the session

1	2	3	4	5
Not satisfied		somewhat satisfied		very satisfied

13) Has your knowledge about PCOS and its associated symptoms improved?

1	2	3	4	5
Not at all		somewhat		very much

14) Are you able to remember what are the long-term consequences associated with your condition?

1	2	3	4	5
Not at all		somewhat		very much

15) How much do you know about the recommended physical activity?

1	2	3	4	5
Not at all		somewhat		very much

16) Do you remember what are the behavioural change strategies that help you losing weight and maintain weight loss?

1	2	3	4	5
Not at all		somewhat		very much

17) What part of the session did you like?

18) What part of the session did you dislike?

19) Which part of the session needs further improvement?

20) Please provide your suggestions on how the sessions could be further improved

21) Would you recommend this session to anyone else?

1	2	3	4	5
Not at all		somewhat recommended		very much

13.7 Participants knowledge, skills development, and illness perception evaluation form

Thank you for taking part in this survey. The survey is designed to captures your responses to knowledge gained, skills developed and your perceptions after the educational session. Please answer the question in the allocated area.

Knowledge evaluation

1) How much would you rate the knowledge you have gained about the anatomy and physiology of your reproductive organ ?

1	2	3	4	5
Not much		reasonable		very much

2) How much would you rate the knowledge you have gained about symptoms and signs of your condition ?

1	2	3	4	5
Not much		reasonable		very much

3) How much would you rate the knowledge you have gained about how PCOS could be diagnosed ?

1	2	3	4	5
Not much		reasonable		very much

4) How much would you rate the knowledge you have gained about PCOS management strategies ?

1	2	3	4	5
Not much		reasonable		very much

5) How much would you rate the knowledge you have gained about the PCOS-related complications ?

1	2	3	4	5
Not much		reasonable		very much

6) Please provide any further details in the designated area below

Skills development evaluation

1) How much your goals setting skills improved ?

1	2	3	4	5
Not much		reasonable		very much

2) How much your self-monitoring skills have improved ?

1	2	3	4	5
Not much		reasonable		very much

3) How much your self-efficacy skills have improved ?

1	2	3	4	5
Not much		reasonable		very much

4) How much your skills to overcome barriers surrounding physical activity and dietary changes have improved ?

1	2	3	4	5
Not much		reasonable		very much

5) Please provide any further details in the designated area below

Illness perception evaluation

1) How often do you think about your illness ?

1	2	3	4	5
Not much		occasionally		frequently

In your own personal view, we would like to know how you now see your illness

2) A virus or bacteria are the main cause of my illness ?

1	2	3	4	5
disagree		agree		strongly agree

3) The environment has played big role in my illness?

1	2	3	4	5
disagree		agree		strongly agree

4) Bad dietary habits have caused my illness ?

1	2	3	4	5
disagree		agree		strongly agree

5) My illness is running in my family and it is genetic ?

1	2	3	4	5
disagree		agree		strongly agree

6) My condition is due to my own behaviour?

1	2	3	4	5
disagree		agree		strongly agree

7) It was just by chance that I become ill ?

1	2	3	4	5
disagree		agree		strongly agree

8) Both stress and other people have played a major role in my illness ?

1	2	3	4	5
disagree		agree		strongly agree

9) This condition is a permanent rather than temporary and it will be with me for the rest of my life ?

1	2	3	4	5
disagree		agree		strongly agree

10) My illness have major health consequences in my life ?

1	2	3	4	5
disagree		agree		strongly agree

11) Now my conditions is manageable, and it is easy to live with ?

1	2	3	4	5
disagree		agree		strongly agree

12) My symptoms will improve with time ?

1	2	3	4	5
disagree		agree		strongly agree

13) Now there is a lot that I can do to improve my symptoms ?

1	2	3	4	5
disagree		agree		strongly agree

14) There is not much that can be done to improve my illness ?

1	2	3	4	5
disagree		agree		strongly agree

15) What I do in my daily life will determine whether my condition will get better or worse?

1	2	3	4	5
disagree		agree		strongly agree

16) My current treatment will be effective in curing my illness ?

1	2	3	4	5
disagree		agree		strongly agree

13.8 literature search

Literature search in PubMed, Scopus, Embase, Web of Science, Embase, Cochrane and grey sources plus the updated search in PubMed on 2021-03-20

Full search in all databases

Database	Search string	Results	Notes
Source: PubMed (NLM) **Coverage/search date:** from inception - 2020-04-14	((("PCOS"[Title/Abstract] OR "polycystic ovarian syndrome"[Title/Abstract] OR "polycystic ovary syndrome"[Title/Abstract] OR "polycystic ovary disease"[Title/Abstract] OR "Stein-Leventhal syndrome"[Title/Abstract] OR "Stein Leventhal syndrome"[Title/Abstract] OR "sclerocystic ovarian degeneration"[Title/Abstract] OR "sclerocystic ovary syndrome"[Title/Abstract] OR "sclerocystic ovaries"[Title/Abstract] OR "sclerocystic ovary"[Title/Abstract] OR "Polycystic Ovary Syndrome"[Mesh])) AND (medicine*[Title/Abstract] OR medication*[Title/Abstract] OR "Pharmaceutical Preparations"[Mesh] OR "pharmaceutical preparation*"[Title/Abstract] OR "pharmacological intervention*"[Title/Abstract] OR "drug intervention*"[Title/Abstract] OR "drug therapy"[Title/Abstract] OR "drug therapies"[Title/Abstract] OR "therapeutic agent*"[Title/Abstract] OR "drug treatment*"[Title/Abstract] OR "pharmacological agent*"[Title/Abstract] OR pharmacotherapy[Title/Abstract] OR pharmacotherapies[Title/Abstract] OR "Drug Therapy"[Mesh:NoExp] OR "insulin sensitizing drug*"[Title/Abstract] OR "insulin sensitizing agent*"[Title/Abstract] OR "insulin sensitising drug*"[Title/Abstract] OR "insulin sensitising agent*"[Title/Abstract] OR biguanide[Title/Abstract] OR metformin [Title/Abstract] OR "sustained release metformin "[Title/Abstract] OR "Buformin"[Mesh] OR pioglitazone [Title/Abstract] OR thiazolidinedione*[Title/Abstract] OR "Thiazolidinediones"[Mesh] OR rosiglitazone[Title/Abstract] OR glitazones[Title/Abstract] OR "GLP-1 agonist"[Title/Abstract] OR "GLP-1 agonists"[Title/Abstract] OR "GLP-1RA"[Title/Abstract] OR "GLP-1 R"[Title/Abstract] OR "GLP-1 agonist"[Title/Abstract] OR "dual GLP-1/GIP receptor agonist"[Title/Abstract] OR incretin* [Title/Abstract] OR "Incretins"[Mesh] OR liraglutide[Title/Abstract] OR "Liraglutide"[Mesh] OR semaglutide[Title/Abstract] OR exenatide[Title/Abstract] OR "Exenatide"[Mesh] OR "glucagon like peptide-1"[Title/Abstract] OR "Contraceptives, Oral"[Mesh] OR contraceptive* [Title/Abstract] OR "OCPs"[Title/Abstract] OR "COC"[Title/Abstract] OR "COCs"[Title/Abstract] OR ethinylestradiol[Title/Abstract] OR "Ethinyl Estradiol"[Mesh] OR “ethinyl estradiol"[Title/Abstract] OR "ethynyl estradiol"[Title/Abstract] OR drospirenone[Title/Abstract] OR "cyclical progesterone"[Title/Abstract] OR "medroxyprogesterone"[Title/Abstract] OR "Medroxyprogesterone"[Mesh] OR "levonorgestrel-releasing intrauterine system"[Title/Abstract] OR "LNG-IUS"[Title/Abstract] OR "IUCD"[Title/Abstract] OR	**1,273**	All search terms are searched in the search fields: “title” and “abstract” (here marked with TI/AB) and in MeSH (when available). A filter for English language is applied.

	"IUS"[Title/Abstract] OR "IUD"[Title/Abstract] OR "Clomiphene"[Mesh] OR clomiphene[Title/Abstract] OR letrozole[Title/Abstract] OR "Letrozole"[Mesh] OR "aromatase inhibitor*"[Title/Abstract] OR "ART"[Title/Abstract] OR clomid[Title/Abstract] OR "Aromatase Inhibitors"[Mesh] OR "aromatase inhibitor*"[Title/Abstract] OR "mineralocorticoid receptor antagonist*"[Title/Abstract] OR “mineralocorticoid antagonist*”[Title/Abstract] OR "Mineralocorticoid Receptor Antagonists"[Mesh] OR “receptor antagonist*”[Title/Abstract] OR spironolactone[Title/Abstract] OR "Spironolactone"[Mesh] OR "aldosterone antagonist*"[Title/Abstract] OR antiandrogen*[Title/Abstract] OR ”Androgen Antagonists"[Mesh] OR finasteride[Title/Abstract] OR "Finasteride"[Mesh] OR flutamide[Title/Abstract] OR "Flutamide"[Mesh] OR eplerenone[Title/Abstract] OR "Eplerenone"[Mesh] OR eflornithine[Title/Abstract] OR "Eflornithine"[Mesh] OR vaniqa[Title/Abstract] OR saroglitazar[Title/Abstract] OR "hydroxymethylglutaryl-CoA reductase inhibitors"[Title/Abstract] OR "Hydroxymethylglutaryl-CoA Reductase Inhibitors"[Mesh] OR statins[Title/Abstract] OR atorvastatin[Title/Abstract] OR "Atorvastatin"[Mesh] OR simvastatin[Title/Abstract] OR "Simvastatin"[Mesh] OR pravastatin[Title/Abstract] OR "Pravastatin"[Mesh] OR fluvastatin[Title/Abstract] OR "Fluvastatin"[Mesh] OR rosuvastatin[Title/Abstract] OR "Rosuvastatin Calcium"[Mesh] OR orlistat[Title/Abstract] OR "Orlistat"[Mesh] OR gliptins[Title/Abstract] OR "DDP-4 inhibitors"[Title/Abstract] OR "DDP-4 inhibitor"[Title/Abstract] OR "dipeptidyl peptidase - 4 inhibitors"[Title/Abstract] OR "dipeptidyl peptidase - 4 inhibitor"[Title/Abstract] OR "dipeptidyl-peptidase IV inhibitor"[Title/Abstract] OR "dipeptidyl-peptidase IV inhibitors"[Title/Abstract] OR "Dipeptidyl-Peptidase IV Inhibitors"[Mesh] OR Sitagliptin[Title/Abstract] OR "Sitagliptin Phosphate"[Mesh] OR sitagliptin[Title/Abstract] OR vildagliptin[Title/Abstract] OR "Vildagliptin"[Mesh] OR saxagliptin[Title/Abstract] OR linagliptin[Title/Abstract] OR "Linagliptin"[Mesh] OR alogliptin[Title/Abstract] OR dapagliflozin[Title/Abstract] OR "SGLT2 receptor antagonists"[Title/Abstract] OR "SGLT-2 receptor antagonist"[Title/Abstract] OR "SGLT2 receptor inhibitor"[Title/Abstract] OR "SGLT2 inhibitors"[Title/Abstract] OR "gliflozin*”[Title/Abstract] OR "sodium glucose co-transporter-2 inhibitors"[Title/Abstract] OR "sodium glucose co-transporter-2 inhibitor"[Title/Abstract] OR "Sodium-Glucose Transporter 2 Inhibitors"[Mesh] OR empagliflozin[Title/Abstract] OR inositol[Title/Abstract] OR "Inositol"[Mesh] OR "myo-inositol"[Title/Abstract] OR "MYO"[Title/Abstract] OR "myo-inositol"[Title/Abstract] OR rimonabant[Title/Abstract] OR "Rimonabant"[Mesh] OR "endocannabinoid receptor blocker"[Title/Abstract] OR "weight loss agent*”[Title/Abstract] OR "weight loss drugs*”[Title/Abstract] OR "weight loss medication*”[Title/Abstract] OR sibutramine[Title/Abstract] OR "triptorelin"[Title/Abstract] OR decapeptyl[Title/Abstract] OR "Triptorelin Pamoate"[Mesh] OR "GnRH antagonist"[Title/Abstract] OR triptorelin[Title/Abstract] OR gonadotropin[Title/Abstract] OR "Gonadotropins"[Mesh] OR "follitropin alpha"[Title/Abstract] OR "follicle stimulating hormone"[Title/Abstract] OR "follicle stimulating hormone"[Title/Abstract] OR "GnRH antagonists"[Title/Abstract] OR "GnRH-a"[Title/Abstract] OR "gonadotropin releasing hormone antagonist"[Title/Abstract] OR "gonadotropin releasing hormone antagonists"[Title/Abstract] OR "GnRH		

	receptor agonist"[Title/Abstract] OR triptorelin[Title/Abstract] OR elagolix[Title/Abstract] OR "corifollitropin alpha"[Title/Abstract] OR acarbose[Title/Abstract] OR "Acarbose"[Mesh] OR nimodipine[Title/Abstract] OR "Nimodipine"[Mesh] OR amlodipine[Title/Abstract] OR "Amlodipine"[Mesh] OR cabergoline[Title/Abstract] OR "Cabergoline"[Mesh] OR bromocriptine[Title/Abstract] OR "Bromocriptine"[Mesh] OR **"**dopamine receptor agonist"[Title/Abstract] OR "dopamine receptor agonists"[Title/Abstract] OR "Dopamine Agonists"[Mesh] OR "phosphodiesterase-4 inhibitors"[Title/Abstract] OR "phosphodiesterase-4 inhibitor"[Title/Abstract] OR "PDE-4 Inhibitor" [Title/Abstract] OR "PDE-4 Inhibitors" [Title/Abstract] OR "Phosphodiesterase 4 Inhibitors"[Mesh] OR roflumilast[Title/Abstract] OR "growth hormone*"[Title/Abstract] OR "GH"[Title/Abstract] OR "hGH"[Title/Abstract] OR "Human Growth Hormone"[Mesh] OR "phentermine"[Title/Abstract] OR topiramate[Title/Abstract] OR myoinosital[Title/Abstract] OR "Myo-Inositol-1-Phosphate Synthase"[Mesh])) AND (("Randomized Controlled Trial" [Publication Type] OR "RCT"[Title/Abstract] OR randomize*[Title/Abstract] OR randomis*[Title/Abstract] OR "double blind*"[Title/Abstract] OR "single blind*"[Title/Abstract] OR "double-blind*"[Title/Abstract] OR "single- blind*"[Title/Abstract] OR "Double-Blind Method"[Mesh] OR "Single-Blind Method"[Mesh] OR "Placebos"[Mesh] OR placebo*[Title/Abstract] OR "controlled clinical trial"[Title/Abstract] OR "cluster controlled trial"[Title/Abstract] OR "cluster controlled trials"[Title/Abstract] OR "crossover controlled trial"[Title/Abstract] OR "crossover controlled trials"[Title/Abstract] OR "open labeled"[Title/Abstract] OR "open labelled"[Title/Abstract] OR "open label"[Title/Abstract])) AND (English[lang])		
Source: Scopus (Elsevier) **Coverage/search date:** from inception - 2020-04-14	(((TITLE-ABS-KEY: "PCOS" OR "polycystic ovarian syndrome" OR "polycystic ovary syndrome" OR "polycystic ovary disease" OR "Stein-Leventhal syndrome" OR "Stein Leventhal syndrome" OR "sclerocystic ovarian degeneration" OR "sclerocystic ovary syndrome" OR "sclerocystic ovaries" OR "sclerocystic ovary") AND (TITLE-ABS-KEY: medicine* OR medication* OR "pharmaceutical preparation*"OR "pharmacological intervention*" OR "drug intervention*" OR "drug therapy" OR "drug therapies" OR "therapeutic agent*" OR "drug treatment*" OR "pharmacological agent*" OR pharmacotherapy OR pharmacotherapies OR "insulin sensitizing drug*" OR "insulin sensitizing agent*" OR "insulin sensitising drug*" OR "insulin sensitising agent*" OR biguanide OR metformin OR "sustained release metformin " OR pioglitazone OR thiazolidinedione* OR rosiglitazone OR glitazones OR "GLP-1 agonist" OR "GLP-1 agonists" OR "GLP-1RA" OR "GLP-1 R" OR "GLP-1 agonist" OR "dual GLP-1/GIP receptor agonist" OR incretin* OR liraglutide OR semaglutide OR exenatide OR "glucagon like peptide-1" OR contraceptive* OR "OCPs" OR "COC" OR "COCs" OR ethinylestradiol. OR "ethinyl estradiol" OR "ethynyl estradiol" OR drospirenone OR "cyclical progesterone" OR "medroxyprogesterone" OR "levonorgestrel-releasing intrauterine system" OR "LNG-IUS" OR "IUCD" OR "IUS" OR "IUD" OR clomiphene OR letrozole OR "aromatase inhibitor*" OR "ART" OR clomid OR "aromatase inhibitor*" OR "mineralocorticoid receptor antagonist*" OR "mineralocorticoid antagonist*" OR "receptor antagonist*" OR spironolactone OR "aldosterone antagonist*" OR antiandrogen*	**854**	All search terms are searched in the search fields: "title, "abstract" and "keywords" (here marked with: TITLE-ABS-KEY"). No thesaurus available. A filter for English language is applied.

	OR finasteride OR flutamide OR eplerenone OR eflornithine OR vaniqa OR saroglitazar OR "hydroxymethylglutaryl-CoA reductase inhibitors" OR statins OR atorvastatin OR simvastatin OR pravastatin OR fluvastatin OR rosuvastatin OR orlistat OR gliptins OR "DDP-4 inhibitors" OR "DDP-4 inhibitor" OR "dipeptidyl peptidase - 4 inhibitors" OR "dipeptidyl peptidase - 4 inhibitor" OR "dipeptidyl-peptidase IV inhibitor" OR "dipeptidyl-peptidase IV inhibitors" OR Sitagliptin OR sitagliptin OR vildagliptin OR saxagliptin OR linagliptin OR alogliptin OR dapagliflozin OR "SGLT2 receptor antagonists" OR "SGLT-2 receptor antagonist" OR "SGLT2 receptor inhibitor" OR "SGLT2 inhibitors" OR "gliflozin*" OR "sodium glucose co-transporter-2 inhibitors" OR "sodium glucose co-transporter-2 inhibitor" OR empagliflozin OR inositol OR "myo-inositol" OR "MYO" OR "myo-inositol" OR rimonabant OR "endocannabinoid receptor blocker" OR "weight loss agent*" OR "weight loss drugs*" OR "weight loss medication*" OR sibutramine OR "triptorelin" OR decapeptyl OR "GnRH antagonist" OR triptorelin OR gonadotropin OR "follitropin alpha" OR "follicle stimulating hormone" OR "follicle stimulating hormone" OR "GnRH antagonists" OR "GnRH-a" OR "gonadotropin releasing hormone antagonist" OR "gonadotropin releasing hormone antagonists" OR "GnRH receptor agonist" OR triptorelin OR elagolix OR "corifollitropin alpha" OR acarbose OR nimodipine OR amlodipine OR cabergoline OR bromocriptine OR **"**dopamine receptor agonist" OR "dopamine receptor agonists" OR "phosphodiesterase-4 inhibitors" OR "phosphodiesterase-4 inhibitor" OR "PDE-4 Inhibitor" OR "PDE-4 Inhibitors" OR roflumilast OR "growth hormone*" OR "GH" OR "hGH" OR "phentermine" OR topiramate OR myoinositol) AND (TITLE-ABS-KEY: "RCT" OR "double blind*" OR "single blind*" OR "double-blind*" OR "single- blind*" OR placebo OR "controlled clinical trial" OR randomize* OR randomis* OR "cluster controlled trial" OR "cluster controlled trials" OR "crossover controlled trial" OR "crossover controlled trials" OR "open labeled" OR "open labelled" OR "open label")))		
Source: Medline (Web of Science, Clarivate) **Coverage/search date:** from inception - 2020-04-14	(((MeSH HEADING:exp: Polycystic Ovary Syndrome) OR (TOPIC: "PCOS" OR "polycystic ovarian syndrome" OR "polycystic ovary syndrome" OR "polycystic ovary disease" OR "Stein-Leventhal syndrome" OR "Stein Leventhal syndrome" OR "sclerocystic ovarian degeneration" OR "sclerocystic ovary syndrome" OR "sclerocystic ovaries" OR "sclerocystic ovary") AND ((MeSH HEADING: Drug Therapy) OR (TOPIC: medicine* OR medication* OR "pharmaceutical preparation*"OR "pharmacological intervention*" OR "drug intervention*" OR "drug therapy" OR "drug therapies" OR "therapeutic agent*" OR "drug treatment*" OR "pharmacological agent*" OR pharmacotherapy OR pharmacotherapies OR "insulin sensitizing drug*" OR "insulin sensitizing agent*" OR "insulin sensitising drug*" OR "insulin sensitising agent*" OR biguanide OR metformin OR "sustained release metformin " OR pioglitazone OR thiazolidinedione* OR rosiglitazone OR glitazones OR "GLP-1 agonist" OR "GLP-1 agonists" OR "GLP-1RA" OR "GLP-1 R" OR "GLP-1 agonist" OR "dual GLP-1/GIP receptor agonist" OR incretin* OR liraglutide OR semaglutide OR exenatide OR "glucagon like peptide-1" OR contraceptive* OR "OCPs" OR "COC" OR "COCs" OR ethinylestradiol. OR "ethinyl estradiol" OR "ethynyl estradiol" OR drospirenone OR "cyclical progesterone" OR "medroxyprogesterone" OR "levonorgestrel-releasing intrauterine system" OR "LNG-IUS" OR "IUCD" OR "IUS" OR "IUD" OR clomiphene	**1,228**	All search terms are searched in the search field: "TOPIC" (including title, abstract and author supplied keywords).and in MeSH (when available). MeSH variations compared to PubMed's MeSH are applied as per

	OR letrozole OR "aromatase inhibitor*" OR "ART" OR clomid OR "aromatase inhibitor*" OR "mineralocorticoid receptor antagonist*" OR “mineralocorticoid antagonist*” OR “receptor antagonist*” OR spironolactone OR "aldosterone antagonist*" OR antiandrogen* OR finasteride OR flutamide OR eplerenone OR eflornithine OR vaniqa OR saroglitazar OR "hydroxymethylglutaryl-CoA reductase inhibitors" OR statins OR atorvastatin OR simvastatin OR pravastatin OR fluvastatin OR rosuvastatin OR orlistat OR gliptins OR "DDP-4 inhibitors" OR "DDP-4 inhibitor" OR "dipeptidyl peptidase - 4 inhibitors" OR "dipeptidyl peptidase - 4 inhibitor" OR "dipeptidyl-peptidase IV inhibitor" OR "dipeptidyl-peptidase IV inhibitors" OR Sitagliptin OR sitagliptin OR vildagliptin OR saxagliptin OR linagliptin OR alogliptin OR dapagliflozin OR "SGLT2 receptor antagonists" OR "SGLT-2 receptor antagonist" OR "SGLT2 receptor inhibitor" OR "SGLT2 inhibitors" OR "gliflozin*” OR "sodium glucose co-transporter-2 inhibitors" OR "sodium glucose co-transporter-2 inhibitor" OR empagliflozin OR inositol OR "myo-inositol" OR "MYO" OR "myo-inositol" OR rimonabant OR "endocannabinoid receptor blocker" OR "weight loss agent*” OR "weight loss drugs*” OR "weight loss medication*” OR sibutramine OR "triptorelin" OR decapeptyl OR "GnRH antagonist" OR triptorelin OR gonadotropin OR "follitropin alpha" OR "follicle stimulating hormone" OR "follicle stimulating hormone" OR "GnRH antagonists" OR "GnRH-a" OR "gonadotropin releasing hormone antagonist" OR "gonadotropin releasing hormone antagonists" OR "GnRH receptor agonist" OR triptorelin OR elagolix OR "corifollitropin alpha" OR acarbose OR nimodipine OR amlodipine OR cabergoline OR bromocriptine OR “dopamine receptor agonist” OR “dopamine receptor agonists” OR "phosphodiesterase-4 inhibitors" OR "phosphodiesterase-4 inhibitor" OR “PDE-4 Inhibitor” OR “PDE-4 Inhibitors” OR roflumilast OR "growth hormone*” OR "GH" OR “hGH” OR "phentermine" OR topiramate OR myoinositol) AND (MeSH HEADING:exp:“Randomized Controlled Trial” OR “Double-Blind Method” OR “Single-Blind Method”) OR TOPIC: "RCT" OR "double blind*" OR "single blind*" OR "double- blind*" OR "single- blind*"OR "controlled clinical trial" OR randomize* OR randomis* OR "cluster controlled trial" OR "cluster controlled trials" OR "crossover controlled trial" OR "crossover controlled trials" OR placebo* OR "open labeled" OR "open labelled" OR "open label")))		availability and recommendations Medline. A filter for English language is applied.
Source: Web of Science (Core Collection, Clarivate) **Coverage/search date:** from inception - 2020-04-14	(((TOPIC: "PCOS" OR "polycystic ovarian syndrome" OR "polycystic ovary syndrome" OR "polycystic ovary disease" OR "Stein-Leventhal syndrome" OR "Stein Leventhal syndrome" OR "sclerocystic ovarian degeneration" OR "sclerocystic ovary syndrome" OR "sclerocystic ovaries" OR "sclerocystic ovary") AND (TOPIC: medicine* OR medication* OR "pharmaceutical preparation*"OR "pharmacological intervention*" OR "drug intervention*" OR "drug therapy" OR "drug therapies" OR "therapeutic agent*" OR "drug treatment*" OR "pharmacological agent*" OR pharmacotherapy OR pharmacotherapies OR "insulin sensitizing drug*" OR "insulin sensitizing agent*" OR "insulin sensitising drug*" OR "insulin sensitising agent*" OR biguanide OR metformin OR "sustained release metformin " OR pioglitazone OR thiazolidinedione* OR rosiglitazone OR glitazones OR "GLP-1 agonist" OR "GLP-1 agonists" OR "GLP-1RA" OR "GLP-1 R" OR "GLP-1 agonist" OR "dual GLP-1/GIP receptor agonist" OR incretin* OR liraglutide OR	**1,095**	All search terms are searched in the field: “TOPIC” (including title, abstract and author supplied keywords). No thesaurus available.

	semaglutide OR exenatide OR "glucagon like peptide-1" OR contraceptive* OR "OCPs" OR "COC" OR "COCs" OR ethinylestradiol. OR “ethinyl estradiol" OR "ethynyl estradiol" OR drospirenone OR "cyclical progesterone" OR "medroxyprogesterone" OR "levonorgestrel-releasing intrauterine system" OR "LNG-IUS" OR "IUCD" OR "IUS" OR "IUD" OR clomiphene OR letrozole OR "aromatase inhibitor*" OR "ART" OR clomid OR "aromatase inhibitor*" OR "mineralocorticoid receptor antagonist*" OR “mineralocorticoid antagonist*” OR “receptor antagonist*” OR spironolactone OR "aldosterone antagonist*" OR antiandrogen* OR finasteride OR flutamide OR eplerenone OR eflornithine OR vaniqa OR saroglitazar OR "hydroxymethylglutaryl-CoA reductase inhibitors" OR statins OR atorvastatin OR simvastatin OR pravastatin OR fluvastatin OR rosuvastatin OR orlistat OR gliptins OR "DDP-4 inhibitors" OR "DDP-4 inhibitor" OR "dipeptidyl peptidase - 4 inhibitors" OR "dipeptidyl peptidase - 4 inhibitor" OR "dipeptidyl-peptidase IV inhibitor" OR "dipeptidyl-peptidase IV inhibitors "OR Sitagliptin OR sitagliptin OR vildagliptin OR saxagliptin OR linagliptin OR alogliptin OR dapagliflozin OR "SGLT2 receptor antagonists" OR "SGLT-2 receptor antagonist" OR "SGLT2 receptor inhibitor" OR "SGLT2 inhibitors" OR "gliflozin*” OR "sodium glucose co-transporter-2 inhibitors" OR "sodium glucose co-transporter-2 inhibitor" OR empagliflozin OR inositol OR "myo-inositol" OR "MYO" OR "myo-inositol" OR rimonabant OR "endocannabinoid receptor blocker" OR "weight loss agent*” OR "weight loss drugs*” OR "weight loss medication*” OR sibutramine OR "triptorelin" OR decapeptyl OR "GnRH antagonist" OR triptorelin OR gonadotropin OR "follitropin alpha" OR "follicle stimulating hormone" OR "follicle stimulating hormone" OR "GnRH antagonists" OR "GnRH-a" OR "gonadotropin releasing hormone antagonist" OR "gonadotropin releasing hormone antagonists" OR "GnRH receptor agonist" OR triptorelin OR elagolix OR "corifollitropin alpha" OR acarbose OR nimodipine OR amlodipine OR cabergoline OR bromocriptine OR “dopamine receptor agonist” OR “dopamine receptor agonists” OR "phosphodiesterase-4 inhibitors" OR "phosphodiesterase-4 inhibitor" OR “PDE-4 Inhibitor” OR “PDE-4 Inhibitors” OR roflumilast OR "growth hormone*” OR "GH" OR “hGH” OR "phentermine" OR topiramate OR myoinositol) AND (TOPIC: "RCT" OR "double blind*" OR "single blind*" OR "double- blind*" OR "single- blind*"OR "controlled clinical trial" OR randomize* OR randomis* OR "cluster controlled trial" OR "cluster controlled trials" OR "crossover controlled trial" OR "crossover controlled trials" OR placebo* OR "open labeled" OR "open labelled" OR "open label")))		A filter for English language is applied.
Source: Embase (Source: Embase only, Elsevier) **Coverage/search date:** from inception -	((("PCOS":ab,ti OR "polycystic ovarian syndrome":ab,ti OR "polycystic ovary syndrome:ab,ti " OR "polycystic ovary disease":ab,ti OR "Stein-Leventhal syndrome":ab,ti OR "Stein Leventhal syndrome":ab,ti OR "sclerocystic ovarian degeneration”:ab,ti OR "sclerocystic ovary syndrome":ab,ti OR "sclerocystic ovaries":ab,ti OR "sclerocystic ovary":ab,ti OR 'ovary polycystic disease'/exp) AND (medicine*:ab,ti OR medication*:ab,ti OR "pharmaceutical preparation*":ab,ti OR "pharmacological intervention*":ab,ti OR "drug intervention*":ab,ti OR "drug therapy":ab,ti OR "drug therapies":ab,ti OR "therapeutic agent*":ab,ti OR "drug treatment*":ab,ti OR "pharmacological agent*":ab,ti OR pharmacotherapy:ab,ti OR pharmacotherapies:ab,ti OR "insulin sensitizing drug*":ab,ti OR "insulin sensitizing agent*":ab,ti OR "insulin	**708**	All search terms are searched in the fields: “title” and “abstract” (here marked with “:ab,ti”) and in the “thesaurus” (here marked with

2020-04-14	sensitising drug*":ab,ti OR "insulin sensitising agent*":ab,ti OR biguanide:ab,ti OR metformin :ab,ti OR "sustained release metformin ":ab,ti OR pioglitazone :ab,ti OR thiazolidinedione*:ab,ti OR rosiglitazone:ab,ti OR glitazones:ab,ti OR "GLP-1 agonist":ab,ti OR "GLP-1 agonists":ab,ti OR "GLP-1RA":ab,ti OR "GLP-1 R":ab,ti OR "GLP-1 agonist":ab,ti OR "dual GLP-1/GIP receptor agonist":ab,ti OR incretin*:ab,ti OR liraglutide:ab,ti OR semaglutide:ab,ti OR exenatide:ab,ti OR "glucagon like peptide-1":ab,ti OR contraceptive*:ab,ti OR "OCPs":ab,ti OR "COC":ab,ti OR "COCs":ab,ti OR ethinylestradiol :ab,ti OR "ethinyl estradiol":ab,ti OR "ethynyl estradiol":ab,ti OR drospirenone:ab,ti OR "cyclical progesterone":ab,ti OR "medroxyprogesterone":ab,ti OR "levonorgestrel-releasing intrauterine system":ab,ti OR "LNG-IUS":ab,ti OR "IUCD":ab,ti OR "IUS":ab,ti OR "IUD":ab,ti OR clomiphene:ab,ti OR letrozole:ab,ti OR "aromatase inhibitor*":ab,ti OR "ART":ab,ti OR clomid:ab,ti OR "aromatase inhibitor*":ab,ti OR "mineralocorticoid receptor antagonist*":ab,ti OR "mineralocorticoid antagonist*":ab,ti OR "receptor antagonist*":ab,ti OR spironolactone:ab,ti OR "aldosterone antagonist*":ab,ti OR antiandrogen*:ab,ti OR finasteride OR flutamide:ab,ti OR eplerenone:ab,ti OR eflornithine:ab,ti OR vaniqa:ab,ti OR saroglitazar:ab,ti OR "hydroxymethylglutaryl-CoA reductase inhibitors":ab,ti OR statins:ab,ti OR atorvastatin:ab,ti OR simvastatin:ab,ti OR pravastatin:ab,ti OR fluvastatin:ab,ti OR rosuvastatin:ab,ti OR orlistat:ab,ti OR gliptins:ab,ti OR "DDP-4 inhibitors":ab,ti OR "DDP-4 inhibitor":ab,ti OR "dipeptidyl peptidase - 4 inhibitors":ab,ti OR "dipeptidyl peptidase - 4 inhibitor":ab,ti OR "dipeptidyl-peptidase IV inhibitor":ab,ti OR "dipeptidyl-peptidase IV inhibitors":ab,ti OR Sitagliptin:ab,ti OR sitagliptin:ab,ti OR vildagliptin:ab,ti OR saxagliptin:ab,ti OR linagliptin:ab,ti OR alogliptin:ab,ti OR dapagliflozin:ab,ti OR "SGLT2 receptor antagonists":ab,ti OR "SGLT-2 receptor antagonist":ab,ti OR "SGLT2 receptor inhibitor":ab,ti OR "SGLT2 inhibitors":ab,ti OR "gliflozin*":ab,ti OR "sodium glucose co-transporter-2 inhibitors":ab,ti OR "sodium glucose co-transporter-2 inhibitor":ab,ti OR empagliflozin:ab,ti OR inositol:ab,ti OR "myo-inositol":ab,ti OR "MYO":ab,ti OR "myo-inositol":ab,ti OR rimonabant:ab,ti OR "endocannabinoid receptor blocker":ab,ti OR "weight loss agent*":ab,ti OR "weight loss drugs*":ab,ti OR "weight loss medication*":ab,ti OR sibutramine:ab,ti OR "triptorelin":ab,ti OR decapeptyl:ab,ti OR "GnRH antagonist":ab,ti OR triptorelin:ab,ti OR gonadotropin:ab,ti OR "follitropin alpha":ab,ti OR "follicle stimulating hormone":ab,ti OR "follicle stimulating hormone":ab,ti OR "GnRH antagonists":ab,ti OR "GnRH-a":ab,ti OR "gonadotropin releasing hormone antagonist":ab,ti OR "gonadotropin releasing hormone antagonists":ab,ti OR "GnRH receptor agonist":ab,ti OR triptorelin OR elagolix:ab,ti OR "corifollitropin alpha":ab,ti OR acarbose:ab,ti OR nimodipine:ab,ti OR amlodipine:ab,ti OR cabergoline:ab,ti OR bromocriptine:ab,ti OR "dopamine receptor agonist" :ab,ti OR "dopamine receptor agonists":ab,ti OR "phosphodiesterase-4 inhibitors":ab,ti OR "phosphodiesterase-4 inhibitor":ab,ti OR "PDE-4 Inhibitor":ab,ti OR "PDE-4 Inhibitors":ab,ti OR roflumilast:ab,ti OR "growth hormone*":ab,ti OR "GH":ab,ti OR "hGH":ab,ti OR "phentermine":ab,ti OR topiramate:ab,ti OR myoinositol:ab,ti OR 'myoinositol 1 phosphate synthase'/de OR 'phosphodiesterase iv inhibitor'/de OR 'cabergoline'/de OR 'amlodipine'/de OR 'nimodipine'/de OR 'acarbose'/de OR 'gonadotropin'/de OR 'triptorelin'/de OR 'rimonabant'/de OR 'inositol'/de OR 'sodium glucose		"/de") when available. Thesaurus (Emtree) variations compared to PubMed's MeSH are applied as per availability and recommendations Embase. A filter for English language is applied.

	cotransporter 2 inhibitor'/de OR 'linagliptin'/de OR vildagliptin'/de OR 'sitagliptin'/de OR 'dipeptidyl peptidase iv inhibitor'/de OR 'tetrahydrolipstatin'/de OR _'rosuvastatin'/de OR 'fluindostatin'/de OR 'pravastatin'/de OR 'simvastatin'/de OR 'atorvastatin'/de OR 'hydroxymethylglutaryl coenzyme a reductase inhibitor'/de OR 'eflornithine'/de OR 'eplerenone'/de OR 'flutamide'/de OR 'finasteride'/de OR 'antiandrogen'/de OR 'spironolactone'/de OR 'mineralocorticoid antagonist'/de OR 'aromatase inhibitor'/de OR 'letrozole'/de OR 'clomifene'/exp OR 'medroxyprogesterone'/de OR 'ethinylestradiol'/de OR 'oral contraceptive agent'/de OR 'exendin 4'/exp OR 'liraglutide'/de OR 'incretin'/de OR 'buformin'/de OR 'drug'/de OR 'drug therapy'/de) AND ("RCT":ab,ti OR "double blind*":ab,ti OR "single blind*":ab,ti OR "double- blind*":ab,ti OR "single- blind*":ab,ti OR "controlled clinical trial":ab,ti OR randomize*:ab,ti OR randomis*:ab,ti OR "cluster controlled trial":ab,ti OR "cluster controlled trials":ab,ti OR "crossover controlled trial":ab,ti OR "crossover controlled trials":ab,ti OR placebo*:ab,ti OR "open labeled":ab,ti OR "open labelled":ab,ti OR "open label":ab,ti OR 'placebo'/de OR 'randomized controlled trial'/de OR 'double blind procedure'/exp OR 'single blind procedure'/exp)))		
Source: Cochrane Library (Cochrane Collaboration) **Coverage and search date:** from inception - 2020-04-14	((("PCOS"[Title/Abstract/Keywords] OR "polycystic ovarian syndrome"[Title/Abstract/Keywords] OR "polycystic ovary syndrome"[Title/Abstract/Keywords] OR "polycystic ovary disease"[Title/Abstract/Keywords] OR "Stein-Leventhal syndrome"[Title/Abstract/Keywords] OR "Stein Leventhal syndrome"[Title/Abstract/Keywords] OR "sclerocystic ovarian degeneration"[Title/Abstract/Keywords] OR "sclerocystic ovary syndrome"[Title/Abstract/Keywords] OR "sclerocystic ovaries"[Title/Abstract/Keywords] OR "sclerocystic ovary"[Title/Abstract/Keywords] OR "Polycystic Ovary Syndrome"[Mesh])) AND (medicine*[Title/Abstract/Keywords] OR medication*[Title/Abstract/Keywords] OR "Pharmaceutical Preparations"[Mesh] OR "pharmaceutical preparations"[Title/Abstract/Keywords] OR "pharmaceutical preparation"[Title/Abstract/Keywords] OR "pharmacological intervention"[Title/Abstract/Keywords] OR "pharmacological interventions"[Title/Abstract/Keywords] OR "drug interventions"[Title/Abstract/Keywords] OR "drug interventions"[Title/Abstract/Keywords] OR "drug therapy"[Title/Abstract/Keywords] OR "drug therapies"[Title/Abstract/Keywords] OR "therapeutic agents"[Title/Abstract/Keywords] OR "therapeutic agent"[Title/Abstract/Keywords] OR "drug treatment"[Title/Abstract/Keywords] OR "drug treatments"[Title/Abstract/Keywords] OR "pharmacological agents"[Title/Abstract/Keywords] OR "pharmacological agent"[Title/Abstract/Keywords] OR pharmacotherapy[Title/Abstract/Keywords] OR pharmacotherapies[Title/Abstract/Keywords] OR "Drug Therapy"[Mesh:NoExp] OR "insulin sensitizing drugs"[Title/Abstract/Keywords] OR "insulin sensitizing drug"[Title/Abstract/Keywords] OR "insulin sensitizing agent"[Title/Abstract/Keywords] OR "insulin sensitizing agents"[Title/Abstract/Keywords] OR "insulin sensitising drugs"[Title/Abstract/Keywords] OR "insulin sensitising drug"[Title/Abstract/Keywords] OR "insulin sensitising agent"[Title/Abstract/Keywords] OR "insulin sensitising agents"[Title/Abstract/Keywords] OR biguanide[Title/Abstract/Keywords] OR metformin	**985** (23 Reviews 962 trials)	All search terms are searched in the search fields: “title”, “abstract”, “keywords” and in “MeSH” when available. Subject Heading variations compared to PubMed’s MeSH are applied as per availability and recommendations in Cochrane. No filter for English language available.

	[Title/Abstract/Keywords] OR "sustained release metformin "[Title/Abstract/Keywords] OR "Buformin"[Mesh] OR pioglitazone [Title/Abstract/Keywords] OR thiazolidinedione[Title/Abstract/Keywords] OR thiazolidinediones[Title/Abstract/Keywords] OR "Thiazolidinediones"[Mesh] OR rosiglitazone[Title/Abstract/Keywords] OR glitazones[Title/Abstract/Keywords] OR "GLP-1 agonist"[Title/Abstract/Keywords] OR "GLP-1 agonists"[Title/Abstract/Keywords] OR "GLP-1RA"[Title/Abstract/Keywords] OR "GLP-1 R"[Title/Abstract/Keywords] OR "GLP-1 agonist"[Title/Abstract/Keywords] OR "dual GLP-1/GIP receptor agonist"[Title/Abstract/Keywords] OR "incretin mimetics"[Title/Abstract/Keywords] OR incretins[Title/Abstract/Keywords] OR incretin[Title/Abstract/Keywords] OR "Incretins"[Mesh] OR liraglutide[Title/Abstract/Keywords] OR "Liraglutide"[Mesh] OR semaglutide[Title/Abstract/Keywords] OR exenatide[Title/Abstract/Keywords] OR "Exenatide"[Mesh] OR "glucagon like peptide-1"[Title/Abstract/Keywords] OR "Contraceptives, Oral"[Mesh] OR contraceptives[Title/Abstract/Keywords] OR "OCPs"[Title/Abstract/Keywords] OR "oral contraceptive"[Title/Abstract/Keywords] OR "COC"[Title/Abstract/Keywords] OR "COCs"[Title/Abstract/Keywords] OR ethinylestradiol[Title/Abstract/Keywords] OR "Ethinyl Estradiol"[Mesh] OR “ethinyl estradiol"[Title/Abstract/Keywords] OR "ethynyl estradiol"[Title/Abstract/Keywords] OR drospirenone[Title/Abstract/Keywords] OR "cyclical progesterone"[Title/Abstract/Keywords] OR "medroxyprogesterone"[Title/Abstract/Keywords] OR "Medroxyprogesterone"[Mesh] OR "levonorgestrel-releasing intrauterine system"[Title/Abstract/Keywords] OR "LNG-IUS"[Title/Abstract/Keywords] OR "IUCD"[Title/Abstract/Keywords] OR "IUS"[Title/Abstract/Keywords] OR "IUD"[Title/Abstract/Keywords] OR "Clomiphene"[Mesh] OR clomiphene[Title/Abstract/Keywords] OR letrozole[Title/Abstract/Keywords] OR "Letrozole"[Mesh] OR "aromatase inhibitors"[Title/Abstract/Keywords] OR "aromatase inhibitor"[Title/Abstract/Keywords] OR "ART"[Title/Abstract/Keywords] OR clomid[Title/Abstract/Keywords] OR "Aromatase Inhibitors"[Mesh] OR "aromatase inhibitors"[Title/Abstract/Keywords] OR "aromatase inhibitor"[Title/Abstract/Keywords] OR "mineralocorticoid receptor antagonist"[Title/Abstract/Keywords] OR "mineralocorticoid receptor antagonists"[Title/Abstract/Keywords] OR “mineralocorticoid antagonists” [Title/Abstract/Keywords] OR “mineralocorticoid antagonists” [Title/Abstract/Keywords] OR "Mineralocorticoid Receptor Antagonists"[Mesh] OR “receptor antagonists” [Title/Abstract/Keywords] OR “receptor antagonist” [Title/Abstract/Keywords] OR spironolactone[Title/Abstract/Keywords] OR "Spironolactone"[Mesh] OR "aldosterone antagonists"[Title/Abstract/Keywords] OR "aldosterone antagonist"[Title/Abstract/Keywords] OR antiandrogens[Title/Abstract/Keywords] OR antiandrogens[Title/Abstract/Keywords] OR ”Androgen Antagonists"[Mesh] OR finasteride[Title/Abstract/Keywords] OR "Finasteride"[Mesh] OR flutamide[Title/Abstract/Keywords] OR "Flutamide"[Mesh] OR eplerenone[Title/Abstract/Keywords] OR "Eplerenone"[Mesh] OR eflornithine[Title/Abstract/Keywords] OR "Eflornithine"[Mesh] OR vaniqa[Title/Abstract/Keywords] OR saroglitazar[Title/Abstract/Keywords] OR "hydroxymethylglutaryl-CoA reductase inhibitors"[Title/Abstract/Keywords] OR “hydroxymethylglutaryl coenzyme a reductase		

	inhibitor”[Title/Abstract/Keywords] OR "Hydroxymethylglutaryl-CoA Reductase Inhibitors"[Mesh] OR statins[Title/Abstract/Keywords] OR atorvastatin[Title/Abstract/Keywords] OR "Atorvastatin"[Mesh] OR simvastatin[Title/Abstract/Keywords] OR "Simvastatin"[Mesh] OR pravastatin[Title/Abstract/Keywords] OR "Pravastatin"[Mesh] OR fluvastatin[Title/Abstract/Keywords] OR fluindostatin[Title/Abstract/Keywords] OR "Fluvastatin"[Mesh] OR rosuvastatin[Title/Abstract/Keywords] OR "Rosuvastatin Calcium"[Mesh] OR orlistat[Title/Abstract/Keywords] OR "Orlistat"[Mesh] OR tetrahydrolipstatin[Title/Abstract/Keywords] OR gliptins[Title/Abstract/Keywords] OR "DDP-4 inhibitors"[Title/Abstract/Keywords] OR "DDP-4 inhibitor"[Title/Abstract/Keywords] OR "dipeptidyl peptidase - 4 inhibitors"[Title/Abstract/Keywords] OR "dipeptidyl peptidase - 4 inhibitor"[Title/Abstract/Keywords] OR "dipeptidyl-peptidase IV inhibitor"[Title/Abstract/Keywords] OR "dipeptidyl-peptidase IV inhibitors"[Title/Abstract/Keywords] OR "Dipeptidyl-Peptidase IV Inhibitors"[Mesh] OR "Sitagliptin Phosphate"[Mesh] OR sitagliptin[Title/Abstract/Keywords] OR vildagliptin[Title/Abstract/Keywords] OR "Vildagliptin"[Mesh] OR saxagliptin[Title/Abstract/Keywords] OR linagliptin[Title/Abstract/Keywords] OR "Linagliptin"[Mesh] OR alogliptin[Title/Abstract/Keywords] OR dapagliflozin[Title/Abstract/Keywords] OR "SGLT2 receptor antagonists"[Title/Abstract/Keywords] OR "SGLT-2 receptor antagonist"[Title/Abstract/Keywords] OR "SGLT2 receptor inhibitor"[Title/Abstract/Keywords] OR "SGLT2 inhibitors"[Title/Abstract/Keywords] OR "gliflozins"[Title/Abstract/Keywords] OR "gliflozin"[Title/Abstract/Keywords] OR "sodium glucose co-transporter-2 inhibitors"[Title/Abstract/Keywords] OR "sodium glucose co-transporter-2 inhibitor"[Title/Abstract/Keywords] OR "Sodium-Glucose Transporter 2 Inhibitors"[Mesh] OR empagliflozin[Title/Abstract/Keywords] OR inositol[Title/Abstract/Keywords] OR "Inositol"[Mesh] OR "myo-inositol"[Title/Abstract/Keywords] OR "MYO"[Title/Abstract/Keywords] OR "myo-inositol"[Title/Abstract/Keywords] OR rimonabant[Title/Abstract/Keywords] OR "Rimonabant"[Mesh] OR "endocannabinoid receptor blocker"[Title/Abstract/Keywords] OR "weight loss agents"[Title/Abstract/Keywords] OR "weight loss drugs"[Title/Abstract/Keywords] OR "weight loss medications"[Title/Abstract/Keywords] OR "weight loss agent"[Title/Abstract/Keywords] OR "weight loss drug"[Title/Abstract/Keywords] OR "weight loss medication"[Title/Abstract/Keywords] OR sibutramine[Title/Abstract/Keywords] OR "triptorelin"[Title/Abstract/Keywords] OR decapeptyl[Title/Abstract/Keywords] OR "Triptorelin Pamoate"[Mesh] OR "GnRH antagonist"[Title/Abstract/Keywords] OR triptorelin[Title/Abstract/Keywords] OR gonadotropin*[Title/Abstract/Keywords] OR "Gonadotropins"[Mesh] OR "follitropin alpha"[Title/Abstract/Keywords] OR "follicle stimulating hormone"[Title/Abstract/Keywords] OR "follicle stimulating hormone"[Title/Abstract/Keywords] OR "GnRH antagonists"[Title/Abstract/Keywords] OR "GnRH-a"[Title/Abstract/Keywords] OR "GnRH receptor agonist"[Title/Abstract/Keywords] OR triptorelin OR elagolix[Title/Abstract/Keywords] OR "corifollitropin alpha"[Title/Abstract/Keywords] OR acarbose[Title/Abstract/Keywords] OR "Acarbose"[Mesh] OR nimodipine[Title/Abstract/Keywords] OR "Nimodipine"[Mesh] OR amlodipine[Title/Abstract/Keywords] OR "Amlodipine"[Mesh] OR		

	cabergoline[Title/Abstract/Keywords] OR "Cabergoline"[Mesh] OR bromocriptine[Title/Abstract/Keywords] OR "Bromocriptine"[Mesh] OR **"**dopamine receptor agonist"[Title/Abstract/Keywords] OR "dopamine receptor agonists" [Title/Abstract/Keywords] OR "Dopamine Agonists"[Mesh] OR "phosphodiesterase-4 inhibitors"[Title/Abstract/Keywords] OR "phosphodiesterase-4 inhibitor"[Title/Abstract/Keywords] OR "PDE-4 Inhibitor" [Title/Abstract/Keywords] OR "PDE-4 Inhibitors"[Title/Abstract/Keywords] OR "Phosphodiesterase 4 Inhibitors"[Mesh] OR roflumilast[Title/Abstract/Keywords] OR "growth hormone"[Title/Abstract/Keywords] OR "growth hormones"[Title/Abstract/Keywords] OR "GH"[Title/Abstract/Keywords] OR "hGH"[Title/Abstract/Keywords] OR "Human Growth Hormone"[Mesh] OR "phentermine"[Title/Abstract/Keywords] OR topiramate[Title/Abstract/Keywords] OR myoinosital[Title/Abstract/Keywords] OR "Myo-Inositol-1-Phosphate Synthase"[Mesh])) AND (("Randomized Controlled Trial"[Mesh] OR "RCT"[Title/Abstract/Keywords] OR "double blind"[Title/Abstract/Keywords] OR "double blinded"[Title/Abstract/Keywords] OR "single blind"[Title/Abstract/Keywords] OR "single blinded"[Title/Abstract/Keywords] OR "Double-Blind Method"[Mesh] OR "Single-Blind Method"[Mesh] OR "controlled clinical trial"[Title/Abstract/Keywords] OR randomize*[Title/Abstract/Keywords] OR randomis*[Title/Abstract/Keywords] OR "cluster controlled trial" [Title/Abstract/Keywords] OR "cluster controlled trials"[Title/Abstract/Keywords] OR "crossover controlled trial"[Title/Abstract/Keywords] OR "crossover controlled trials"[Title/Abstract/Keywords] OR placebo*[Title/Abstract/Keywords] OR "Placebos"[Mesh] OR "open labeled"[Title/Abstract/Keywords] OR "open labelled"[Title/Abstract/Keywords] OR "open label"[Title/Abstract/Keywords]))		
Total no. of records identified		**6,143**	
Total no. of unique records after de-duplication		**3,008**	

Grey sources

Source and search coverage	Search string	Results	Notes
Source: European Union Drug Regulating Authorities Clinical Trials	("polycystic ovarian syndrome" OR PCOS) AND (drug* OR pharma* OR medication*)	**17**	Only limited search functions available. A basic search string has been used.

Database (EudraCT) **Coverage:** from inception to 2021-03-29			No filters or limitations available.
Source: Open Grey (Grey literature in Europe) **Coverage/search date:** from inception to 2021-03-29	("polycystic ovarian syndrome" OR PCOS) AND (drug* OR pharma* OR medication*)	**3**	Only limited search functions available. A basic search string has been used. A filter for English language is applied.
Source: ClinicalTrials.gov **Coverage/search date:** from inception to 2021-03-29	("polycystic ovarian syndrome" AND "Drug Therapy")	**56**	Only limited search functions available. A basic search string has been used. Filters applied: "Completed" trials only. No filter for English language available
Total no. of records identified:		**76**	
Total no. of unique records after de-duplication within the grey sources and results from the database search:		**71**	

Updated search in PubMed 2021-03-20

Source and search coverage	Search string	Results	Notes
Source: PubMed (NLM) **Coverage:** 2020/4/14 - 2021/12/31	((("PCOS"[Title/Abstract] OR "polycystic ovarian syndrome"[Title/Abstract] OR "polycystic ovary syndrome"[Title/Abstract] OR "polycystic ovary disease"[Title/Abstract] OR "Stein-Leventhal syndrome"[Title/Abstract] OR "Stein Leventhal syndrome"[Title/Abstract] OR "sclerocystic ovarian degeneration"[Title/Abstract] OR "sclerocystic ovary syndrome"[Title/Abstract] OR "sclerocystic ovaries"[Title/Abstract] OR "sclerocystic ovary"[Title/Abstract] OR "Polycystic Ovary Syndrome"[Mesh])) AND (medicine*[Title/Abstract] OR medication*[Title/Abstract] OR "Pharmaceutical Preparations"[Mesh] OR "pharmaceutical preparations"[Title/Abstract] OR "pharmaceutical preparation"[Title/Abstract] OR "pharmacological intervention"[Title/Abstract] OR "pharmacological interventions"[Title/Abstract] OR "drug interventions"[Title/Abstract] OR "drug interventions"[Title/Abstract] OR "drug therapy"[Title/Abstract] OR "drug therapies"[Title/Abstract] OR "therapeutic agents"[Title/Abstract] OR "therapeutic agent"[Title/Abstract] OR "drug treatment"[Title/Abstract] OR "drug treatments"[Title/Abstract] OR "pharmacological agents"[Title/Abstract] OR "pharmacological agent"[Title/Abstract] OR pharmacotherapy[Title/Abstract] OR pharmacotherapies[Title/Abstract] OR "Drug Therapy"[Mesh:NoExp] OR "insulin sensitizing drugs"[Title/Abstract] OR "insulin sensitizing drug"[Title/Abstract] OR "insulin sensitizing agent"[Title/Abstract] OR "insulin sensitizing agents"[Title/Abstract] OR "insulin sensitising drugs"[Title/Abstract] OR "insulin sensitising drug"[Title/Abstract] OR "insulin sensitising agent"[Title/Abstract] OR "insulin sensitising agents"[Title/Abstract] OR biguanide[Title/Abstract] OR metformin [Title/Abstract] OR "sustained release metformin "[Title/Abstract] OR "Buformin"[Mesh] OR pioglitazone [Title/Abstract] OR thiazolidinedione[Title/Abstract] OR thiazolidinediones[Title/Abstract] OR "Thiazolidinediones"[Mesh] OR rosiglitazone[Title/Abstract] OR glitazones[Title/Abstract] OR "GLP-1 agonist"[Title/Abstract] OR "GLP-1 agonists"[Title/Abstract] OR "GLP-1RA"[Title/Abstract] OR "GLP-1 R"[Title/Abstract] OR "GLP-1 agonist"[Title/Abstract] OR "dual GLP-1/GIP receptor agonist"[Title/Abstract] OR "incretin mimetics"[Title/Abstract] OR incretins[Title/Abstract] OR incretin[Title/Abstract] OR "Incretins"[Mesh] OR liraglutide[Title/Abstract] OR "Liraglutide"[Mesh] OR semaglutide[Title/Abstract] OR exenatide[Title/Abstract] OR "Exenatide"[Mesh] OR "glucagon like peptide-1"[Title/Abstract] OR "Contraceptives, Oral"[Mesh] OR contraceptives[Title/Abstract] OR "OCPs"[Title/Abstract] OR "oral contraceptive"[Title/Abstract] OR "COC"[Title/Abstract] OR "COCs"[Title/Abstract] OR ethinylestradiol[Title/Abstract] OR "Ethinyl Estradiol"[Mesh] OR "ethinyl estradiol"[Title/Abstract] OR "ethynyl estradiol"[Title/Abstract] OR drospirenone[Title/Abstract] OR "cyclical progesterone"[Title/Abstract] OR "medroxyprogesterone"[Title/Abstract] OR "Medroxyprogesterone"[Mesh] OR "levonorgestrel-releasing intrauterine system"[Title/Abstract] OR "LNG-IUS"[Title/Abstract] OR "IUCD"[Title/Abstract] OR "IUS"[Title/Abstract] OR "IUD"[Title/Abstract] OR	**107**	All search terms are searched in the search fields: "title" and "abstract" (here marked with TI/AB) and in MeSH (when available). Filters for English language and publication year 2020/4/14 - 2021/12/31 are applied.

	"Clomiphene"[Mesh] OR clomiphene[Title/Abstract] OR letrozole[Title/Abstract] OR "Letrozole"[Mesh] OR "aromatase inhibitors"[Title/Abstract] OR "aromatase inhibitor"[Title/Abstract] OR "ART"[Title/Abstract] OR clomid[Title/Abstract] OR "Aromatase Inhibitors"[Mesh] OR "aromatase inhibitors"[Title/Abstract] OR "aromatase inhibitor"[Title/Abstract] OR "mineralocorticoid receptor antagonist"[Title/Abstract] OR "mineralocorticoid receptor antagonists"[Title/Abstract] OR "mineralocorticoid antagonists"[Title/Abstract] OR "mineralocorticoid antagonists"[Title/Abstract] OR "Mineralocorticoid Receptor Antagonists"[Mesh] OR "receptor antagonists" [Title/Abstract] OR "receptor antagonist" [Title/Abstract] OR spironolactone[Title/Abstract] OR "Spironolactone"[Mesh] OR "aldosterone antagonists"[Title/Abstract] OR "aldosterone antagonist"[Title/Abstract] OR antiandrogens[Title/Abstract] OR antiandrogens[Title/Abstract] OR "Androgen Antagonists"[Mesh] OR finasteride[Title/Abstract] OR "Finasteride"[Mesh] OR flutamide[Title/Abstract] OR "Flutamide"[Mesh] OR eplerenone[Title/Abstract] OR "Eplerenone"[Mesh] OR eflornithine[Title/Abstract] OR "Eflornithine"[Mesh] OR vaniqa[Title/Abstract] OR saroglitazar[Title/Abstract] OR "hydroxymethylglutaryl-CoA reductase inhibitors"[Title/Abstract] OR "Hydroxymethylglutaryl-CoA Reductase Inhibitors"[Mesh] OR statins[Title/Abstract] OR atorvastatin[Title/Abstract] OR "Atorvastatin"[Mesh] OR simvastatin[Title/Abstract] OR "Simvastatin"[Mesh] OR pravastatin[Title/Abstract] OR "Pravastatin"[Mesh] OR fluvastatin[Title/Abstract] OR "Fluvastatin"[Mesh] OR rosuvastatin[Title/Abstract] OR "Rosuvastatin Calcium"[Mesh] OR orlistat[Title/Abstract] OR "Orlistat"[Mesh] OR gliptins[Title/Abstract] OR "DDP-4 inhibitors"[Title/Abstract] OR "DDP-4 inhibitor"[Title/Abstract] OR "dipeptidyl peptidase - 4 inhibitors"[Title/Abstract] OR "dipeptidyl peptidase - 4 inhibitor"[Title/Abstract] OR "dipeptidyl-peptidase IV inhibitor"[Title/Abstract] OR "dipeptidyl-peptidase IV inhibitors"[Title/Abstract] OR "Dipeptidyl-Peptidase IV Inhibitors"[Mesh] OR Sitagliptin[Title/Abstract] OR "Sitagliptin Phosphate"[Mesh] OR sitagliptin[Title/Abstract] OR vildagliptin[Title/Abstract] OR "Vildagliptin"[Mesh] OR saxagliptin[Title/Abstract] OR linagliptin[Title/Abstract] OR "Linagliptin"[Mesh] OR alogliptin[Title/Abstract] OR dapagliflozin[Title/Abstract] OR "SGLT2 receptor antagonists"[Title/Abstract] OR "SGLT-2 receptor antagonist"[Title/Abstract] OR "SGLT2 receptor inhibitor"[Title/Abstract] OR "SGLT2 inhibitors"[Title/Abstract] OR "gliflozins"[Title/Abstract] OR "gliflozin"[Title/Abstract] OR "sodium glucose co-transporter-2 inhibitors"[Title/Abstract] OR "sodium glucose co-transporter-2 inhibitor"[Title/Abstract] OR "Sodium-Glucose Transporter 2 Inhibitors"[Mesh] OR empagliflozin[Title/Abstract] OR inositol[Title/Abstract] OR "Inositol"[Mesh] OR "myo-inositol"[Title/Abstract] OR "MYO"[Title/Abstract] OR "myo-inositol"[Title/Abstract] OR rimonabant[Title/Abstract] OR "Rimonabant"[Mesh] OR "endocannabinoid receptor blocker"[Title/Abstract] OR "weight loss agents"[Title/Abstract] OR "weight loss drugs"[Title/Abstract] OR "weight loss medications"[Title/Abstract] OR "weight loss agent"[Title/Abstract] OR "weight loss drug"[Title/Abstract] OR "weight loss medication"[Title/Abstract] OR sibutramine[Title/Abstract] OR "triptorelin"[Title/Abstract] OR decapeptyl[Title/Abstract] OR "Triptorelin Pamoate"[Mesh] OR "GnRH antagonist"[Title/Abstract] OR triptorelin[Title/Abstract] OR gonadotropin[Title/Abstract] OR "Gonadotropins"[Mesh] OR "follitropin alpha"[Title/Abstract] OR "follicle		

	stimulating hormone"[Title/Abstract] OR "follicle stimulating hormone"[Title/Abstract] OR "GnRH antagonists"[Title/Abstract] OR "GnRH-a"[Title/Abstract] OR "gonadotropin releasing hormone antagonist"[Title/Abstract] OR "gonadotropin releasing hormone antagonists"[Title/Abstract] OR "GnRH receptor agonist"[Title/Abstract] OR triptorelin[Title/Abstract] OR elagolix[Title/Abstract] OR "corifollitropin alpha"[Title/Abstract] OR acarbose[Title/Abstract] OR "Acarbose"[Mesh] OR nimodipine[Title/Abstract] OR "Nimodipine"[Mesh] OR amlodipine[Title/Abstract] OR "Amlodipine"[Mesh] OR cabergoline[Title/Abstract] OR "Cabergoline"[Mesh] OR bromocriptine[Title/Abstract] OR "Bromocriptine"[Mesh] OR "dopamine receptor agonist"[Title/Abstract] OR "dopamine receptor agonists"[Title/Abstract] OR "Dopamine Agonists"[Mesh] OR "phosphodiesterase-4 inhibitors"[Title/Abstract] OR "phosphodiesterase-4 inhibitor"[Title/Abstract] OR "PDE-4 Inhibitor" [Title/Abstract] OR "PDE-4 Inhibitors" [Title/Abstract] OR "Phosphodiesterase 4 Inhibitors"[Mesh] OR roflumilast[Title/Abstract] OR "growth hormone"[Title/Abstract] OR "growth hormones"[Title/Abstract] OR "GH"[Title/Abstract] OR "hGH"[Title/Abstract] OR "Human Growth Hormone"[Mesh] OR "phentermine"[Title/Abstract] OR topiramate[Title/Abstract] OR myoinositol[Title/Abstract] OR "Myo-Inositol-1-Phosphate Synthase"[Mesh])) AND (("Randomized Controlled Trial" [Publication Type] OR "RCT"[Title/Abstract] OR "double blind"[Title/Abstract] OR "double blinded"[Title/Abstract] OR "single blind"[Title/Abstract] OR "single blinded"[Title/Abstract] OR "controlled clinical trial"[Title/Abstract] OR randomize*[Title/Abstract] OR randomis*[Title/Abstract] OR "cluster controlled trial"[Title/Abstract] OR "cluster controlled trials"[Title/Abstract] OR "crossover controlled trial"[Title/Abstract] OR "crossover controlled trials"[Title/Abstract] OR placebo*[Title/Abstract] OR "Placebos"[Mesh] OR "open labeled"[Title/Abstract] OR "open labelled"[Title/Abstract] OR "open label"[Title/Abstract])) AND (English[lang])		
Total no. of records identified		**107**	
Total no. of unique records after de-duplication		**107**	