1 **Title: Effects of empagliflozin on metabolic parameters in polycystic ovary syndrome:**

2 **a randomised controlled study**

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24   **Abstract**

25   **Background:** Empagliflozin is a sodium-glucose-cotransporter-2 inhibitor that improves

26   cardiovascular risk and promotes weight loss in patients with type 2 diabetes. Polycystic ovary

27   syndrome (PCOS) is associated with obesity and increased cardiovascular risk; therefore,

28   empagliflozin may be of benefit for these women. The aim of this study was to compare the effects of

29   empagliflozin vs. metformin on anthropometric and body composition, hormonal and metabolic

30   parameters in women with PCOS.

31   **Materials and methods:** A randomized open-label study was conducted in women with PCOS who

32   were randomised to either empagliflozin 25mg (n=19) or metformin 1500mg (n=20) daily for 12

33   weeks. The main outcomes assessed were changes in anthropometric and body composition,

34   hormonal and metabolic parameters.

35   **Results:** Univariate analysis showed significant differences in weight (empagliflozin: -1.4±3.2% vs.

36   metformin: 1.2±2.3%; p=0.006), body mass index (empagliflozin: -1.4±3.2% vs. metformin:

37   1.1±2.2%; p=0.006), waist circumference (empagliflozin: -1.6±2.8% vs. metformin: 0.2±2.1%;

38   p=0.029) and hip circumference (empagliflozin: -2.0±3.0% vs. metformin: 1.1±1.9%; p=0.001), basal

39   metabolic rate (empagliflozin: -1.8±2.9% vs. metformin: 0.1±1.9%, p=0.024) and fat mass

40   (empagliflozin: -0.7±4.9% vs. metformin, 3.2±5.0%; p=0.023) between the empagliflozin and the

41   metformin groups. These differences were confirmed in linear regression analysis after adjustment for

42   relevant covariates. There were no significant changes in hormonal or metabolic parameters between

43   both groups.

44   **Conclusion:** There was a significant improvement in anthropometric parameters and body

45   composition, in overweight and obese women with PCOS after 12 weeks of treatment with

46   empagliflozin compared to metformin, although no changes were seen in hormonal or

47   metabolic parameters.

48

49   **Key words:** Polycystic ovary syndrome, SGLT2 inhibitors, empagliflozin, body

50   composition, hormones, metabolic parameters

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder featured by hyperandrogenism, menstrual irregularities and polycystic ovaries that affects women of reproductive age.[1] PCOS is also associated with infertility, obesity, metabolic disturbances and increased cardiovascular risk.[1, 2]

Accordingly, the treatment of PCOS is commonly symptom-based, while the ideal treatment would address both the reproductive and metabolic abnormalities related to PCOS.[1, 3] Hormonal contraceptives alone or combined with anti-androgens have been the cornerstone for managing menstrual disturbances and clinical or biochemical hyperandrogenaemia;[4] however, some hormonal contraceptives may unfavourably affect the lipid profile in PCOS[5] and increase the risk of thrombosis and cardiovascular events in the general population.[6] Glucose lowering agents including metformin and thiazolidinedione have been shown to be effective in managing the metabolic abnormalities (*i.e.,* insulin resistance, hyperinsulinaemia, and diabetes mellitus) and chronic anovulation; however, their use has been inconsistently associated with improvements in weight loss and body composition, menstrual irregularity or clinical symptoms of excess androgens.[7, 8]

Empagliflozin, a sodium-glucose cotransporter 2 (SGLT2) inhibitor, is a new treatment option for adults with type 2 diabetes,[9] however, its effects in PCOS have not been previously explored. Its principal action involves inhibition of glucose reabsorption by the kidney and therefore, glucose excretion via urine. Notably, this action mechanism is insulin-independent; as such it does not increase the risk of hypoglycaemia, making it attractive for use in normoglycaemic individuals.[10, 11] Recent trials have demonstrated that treatment with empagliflozin promotes weight loss,[12] exerts positive effects on arterial stiffness, vascular resistance and blood pressure and decreases the relative risk for cardiovascular and all-cause mortality in patients with type 2 diabetes.[10, 13] Evidence from preclinical studies suggests that these cardio-protective effects may be due to the reduction in oxidative stress and suppressed markers of inflammation and fibrosis.[14, 15] In humans, the cardiovascular benefits may also be mediated by reductions in HbA1c, insulin resistance,

79 plasma volume, weight/fat mass and inflammation.[16, 17] Given that these pathological

80 features are also common in PCOS;[1, 2] empagliflozin may be of potential benefit for this

81 population.

82   Therefore, the aim of this study was to explore and compare the effects of empagliflozin

83 *vs.* metformin on anthropometric, body composition, hormonal and metabolic parameters in

84 women with PCOS.

85

86 **Materials and Methods**

87   An open-label, randomised, comparative study in women with PCOS was performed in the

88 Academic Diabetes, Endocrinology and Metabolism research centre at Hull Royal Infirmary. All

89 participants were women, aged between 18 and 45 years, had a body mass index (BMI) ≥ 25 kg/m^2,

90 were diagnosed with PCOS based on the Rotterdam criteria [biochemical hyperandrogenism, as

91 indicated by a free androgen index (FAI) >4, and self-reported oligomenorrhea (cycle length >35 days

92 and 9 or fewer periods per year) or amenorrhea (absence of menses for a period ≥ 3 months)].[18]

93 Women with differential diagnoses of non-classical 21-hydroxylase deficiency, hyperprolactinaemia,

94 Cushing's disease and androgen-secreting tumours were excluded from participation. Additional

95 exclusion criteria included pregnancy or intention to become pregnant, breastfeeding, documented use

96 of oral hormonal contraceptives and hormone-releasing implants, metformin or other insulin-

97 sensitizing medications, clomiphene citrate or estrogen modulators, gonadotropin-releasing hormone

98 (GnRH) modulators and Minoxidil, diagnosis of diabetes, history or presence of malignant neoplasms

99 within the last 5 years, pancreatitis (acute or chronic), recurrent urinary tract infections or

100 gastrointestinal tract surgery, ongoing, inadequately controlled thyroid disorder and known

101 hypersensitivity to the investigational medicinal products or any of their excipients. All participants

102 provided their written informed consent. This study was approved by the Medicines and Healthcare

103 Products Regulatory Authority (MHRA) (Ref: 21411/0254/001-0001), the Yorkshire & Humber

104 Health Research Authority and Leeds East Research Ethics Committee (REC reference: 17/YH/0118),

105 registered at www.clinicaltrials.gov (NCT03008551) and conducted in accordance with the

106 Declaration of Helsinki and local regulations.

Women with PCOS were randomized on a 1:1 ratio using an online web-based randomisation service (https://www.sealedenvelope.com) to receive either empagliflozin 25mg (Jardiance) or metformin SR (slow release) 1500mg (Bolamyn) daily for 12 weeks. The dosage of empagliflozin (25 mg) was chosen to get the maximum metabolic response with comparable duration to metformin treatment group. Metformin group received Metformin SR 1500mg, which is the standard dose commonly used in patients with PCOS in clinical practice.[19] Metformin SR was preferred over immediate release metformin in view of better gastrointestinal tolerability.[20] All participants were advised to maintain their usual dietary and lifestyle habits during the study.

Participants attended three visits (visits 1-3). During Visit 1, participants were screened against inclusion and exclusion criteria by medical history and clinical examination, routine blood tests (i.e., full blood count, liver function tests, urea and electrolytes, clotting screen and a pregnancy test), urine pregnancy test and anthropometric measurements. During Visit 2 (baseline) and Visit 3 (12-week follow-up), participants underwent anthropometric [weight, BMI, waist circumference (WC) and hip circumference (HC)] and body composition assessments and an endothelial function measurement. Blood samples were collected at these time points and analysed for reproductive hormones and cardio-metabolic parameters [fasting glucose, fasting insulin, HOMA-IR, total cholesterol, LDL-C, HDL-C, triglycerides (TG), and hs-CRP].

Procedures

Height and weight were recorded with participants wearing light clothing and no shoes using a weighing scale with attached stadiometer (MS-4202L, Marsden Weighing Machine Group Limited, Rotherham, UK) and BMI was calculated as weight (kg) divided by the square of height (m^2). Blood pressure readings were recorded using an automated device (NPB-3900; Nellcor Puritan Bennett, Pleasanton, CA). Three readings were taken at least two minutes apart, and then the average of the readings was obtained. Waist circumference and hip circumference were measured using a tape measure by wrapping it around the patient's waist at the midway point between the top of iliac crest and the bottom of the ribs. Basal metabolic rate, total body fat percentage, fat mass, fat free mass, total body impedance and total body water were measured by using a body composition analyser (BC

418 MA, Tanita Corporation Itabashi-ku, Tokyo, Japan). All these measurements were performed at baseline and 12 weeks after the empagliflozin and metformin treatments.

Endothelial function was assessed using a plethysmographic device Endo-PAT 2000 (Itamar Medical Ltd, Caesarea, Israel). Participants relaxed for at least 15 minutes in a quiet, controlled temperature (22 – 24 °C) room. Endo-PAT biosensors were placed on the index fingers of both hands. During the measurement, participants were instructed to relax and refrain from talking or making any sudden movements. The probes were inflated, and the signals were recorded on the computer according to manufacturer's instructions. This measurement consisted of 5 minutes of baseline recording, followed by blood pressure cuff inflation to a supra-systolic level (at least 60 mmHg above systolic pressure and no less than 200 mmHg) sustained for 5 minutes and subsequent blood pressure cuff deflation and recording of Endo-PAT readings over a further 5-minute period. Output variables namely Reactive Hyperaemia Index (RHI), a measure for endothelial function, and Augmentation Index (AI), a measure for arterial stiffness, were assessed using an automated computer software (EndoPAT2000 version 3.3.2, Itamar Medical Ltd). Compliance with the treatments was calculated by counting the returned medications at the end of the 12-week period.

Blood sampling and biochemical analyses

Following an overnight fast, blood samples were collected both at the baseline and final visit (end of the 12-week period). The fasting venous blood was collected into fluoride oxalate and serum gel tubes. Blood samples were separated by centrifugation at 3,500 x G for 15 minutes at 5°C, and the aliquots were stored at -80°C within 1 hour of collection.

Serum insulin was assayed using a competitive chemiluminescent immunoassay performed on the manufacturer's DPC Immulite 2000 analyzer (Euro/DPC, Llanberis, UK), with a coefficient of variation (CV) was 6%, and no stated cross-reactivity with proinsulin. The plasma glucose was measured using a Beckman AU 5800 analyser (Beckman-Coulter, High Wycombe, United Kingdom) and according to the manufacturer's recommended protocol. Total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and high sensitivity C-reactive protein (*hs*-CRP) levels were measured

163 enzymatically using a Beckman AU 5800 analyser (Beckman-Coulter, High Wycombe, UK) with

164 CVs of <4.9, 0.9, 1.6 and 8.4 %. Low-density lipoprotein cholesterol (LDL-C) was calculated using

165 the Friedewald equation. Serum testosterone and androstenedione were quantified using isotope-

166 dilution liquid chromatography tandem mass spectrometry (LC-MS/MS). Sex hormone-binding

167 globulin (SHBG), estrogen and dehydroepiandrosterone (DHEAS) were measured using a

168 chemiluminescent immunoassay on the Beckman-Coulter UniCel DxI 800 analyser, applying the

169 manufacturer's recommended protocol. The free androgen index (FAI) was calculated as: (total

170 testosterone/SHBG) x 100.

171

Statistical analysis

173   There are no previous studies on the effect of empaglifozin or any other SGLT-2

174 inhibitors on women with PCOS. Sample size was based on an independent t-test with an

175 arbitrary level of 5% significance (2-tailed) and power of 80%. Assuming a common

176 standard deviation of 16% for radial augmentation index and a 4% reduction as a significant

177 change,[21] a sample size of 16 patients per group allowed us to detect a between-group mean

178 difference of 4%. To allow for loss-to-follow-up we aimed to recruit 20 patients per group.

179   All data were checked for normality according to the Shapiro-Wilk test. Mean

180 differences for all parameters expressed as % change from baseline between women with

181 PCOS in the empagliflozin group and the metformin group were analysed with independent

182 t test or Mann-Whitney U-test for normally and non-normally distributed data, respectively.

183 Mean differences between baseline and 12-week follow-up within each treatment group

184 were analysed with a paired t-test or a signed-rank test for normally and non-normally

185 distributed data, respectively. Values are presented as mean SD, if the variables were

186 normally distributed, or median and interquartile range, if the variables were skewed. All

187 statistical analyses were performed using IBM-SPSS version 24.0 (Chicago, IL, USA) with

188 p-values ≤0.05 considered to be significant. Linear regression analysis using function *ln* R

189 was used to confirm the findings of univariate analysis after adjustments for confounders.

190 Since this was a randomised controlled trial, we only adjusted for age and age + BMI,

191 where relevant.

192

193 **Results**

194 Forty-two participants with PCOS were screened; two participants were excluded from

195 participation because they did not meet inclusion criteria, as such 40 participants were

196 randomised to the two treatment arms (empagliflozin, n=20 and metformin, n=20) (Figure

197 1). During the intervention phase of the study, one participant in the empagliflozin group

198 was lost to follow-up. Finally, 19 participants in the empagliflozin group [age: 26.0 (8.0)

199 years, BMI: 37.1±6.2 kg/m²] and 20 participants in the metformin group [age: 31.5 (20.0)

200 years, BMI: 38.7±7.8 kg/m²) completed the trial and their data were included in the final

201 analysis (Figure 1). The baseline characteristics of both groups are presented in Table and

202 Supplementary Table 1. Compliance was over 90% in both groups. There were no adverse

203 events or serious adverse events in the metformin group. In the empagliflozin group, two

204 patients reported adverse events (headache and dizziness, n=1; mild rash, n=1), which were,

205 however, unrelated to the study drug.

215

216 **Anthropometric and body composition parameters**

217 In the empagliflozin group, waist circumference (p=0.024), hip circumference

218 (*p*=0.013), BMR (*p*=0.016), fat free mass (FFM) (*p*=0.013) and total body water (p=0.014)

219 at 12 weeks decreased significantly compared to baseline, but no changes were seen in total

220 mass (*p*=0.079) or BMI (*p*=0.069) (Table 1). In the metformin group, body mass (p=0.019),

221 BMI (*p*=0.024) and hip circumference (*p*=0.031), total fat percentage (p=0.015) or fat mass

222 (p=0.005) significantly increased after 12 weeks of treatment (Table 1).

223 When data were expressed as percentage change from baseline in each group, significant

224 differences in weight (empagliflozin: -1.4±3.2% vs. metformin: 1.2±2.3%; *p*=0.006), BMI

225 (empagliflozin: -1.4±3.2% vs. metformin: 1.1±2.2%; *p*=0.007), waist (empagliflozin: -

226 1.6±2.8% vs. metformin: 0.2±2.1%; *p*=0.029) and hip circumference (empagliflozin: -

227 2.0±3.0% vs. metformin: 1.1±1.9%; *p*=0.001) were seen between the treatment groups.

228 Similarly, the percentage changes from baseline in BMR (empagliflozin: -1.8±2.9% vs.

229 metformin: 0.1±1.9%, *p*=0.024) and fat mass (empagliflozin: -0.7±4.9% vs. metformin,

230 3.2±5.0%; *p*=0.023) were significantly different between the empagliflozin and metformin

231 groups (Table 1, Figure 2). The proportion of women with PCOS who experienced (a) a

232 decrease >5%, (|b) an increase >5%, or (c) a change ≤5% from baseline in anthropometric

233 and body composition parameters are presented in Supplementary Table 2.

234

Hormonal and metabolic parameters

236 In the empagliflozin group, significant increases in SHBG (*p*=0.049) and oestradiol levels

237 (*p*=0.032) were seen after 12 weeks of treatment (Table 1). There were no other hormonal changes for

238 either group (Table 1). No differences were seen in percentage change from baseline for any of the

239 hormone parameters between groups (Table 1, Figure 2).

240 There were no changes following 12 weeks of treatment in blood pressure, endothelial function

241 (RHI, AI), insulin sensitivity (insulin, fasting glucose, HOMA-IR), fasting lipid profile or *hs*-CRP in

242 either treatment arm (Table 1). Between groups comparisons did not reveal any differences in

243 percentage changes from baseline for any of these metabolic parameters (Table 1, Figure 2).

244

Regression analysis modelling

246 Table 2 shows the results of the linear regression analysis modelling percentage changes from

247 baseline in anthropometric characteristics, hormonal and metabolic parameters as function of

248 metformin or empagliflozin treatment. The results confirm statistically significant reduction in weight,

249 BMI, WC, HC, fat mass and BMR in those randomized to empagliflozin group as compared to those

250 in metformin group.

251

Discussion

253 In this first study on the comparative effects of the SGLT2 inhibitor, empagliflozin, and metformin

254 in overweight and obese women with PCOS, we demonstrated that treatment with empagliflozin over

255   a 12-week period, resulted in significant reductions in weight, BMI, waist and hip circumference, total

256   body fat mass and BMR, compared to treatment with metformin, but did not differentially affect

257   hormonal or metabolic parameters.

258   Obesity is prevalent among women with PCOS and has been associated directly and/or indirectly

259   with negative metabolic, cardiovascular, endocrine, reproductive and mental health outcomes.[1, 2]

260   Weight reduction exerts positive effects on PCOS-related outcomes; therefore, it is a primary goal of

261   the management of the condition.[3] In the present study, women with PCOS in the empagliflozin

262   group had a mean weight loss of 1.5 kg, which is similar to the weight loss reported in previous

263   short-term trials.[12] Mechanistically, initial weight loss in empagliflozin studies is attributed to the

264   calorie loss (approximately 200-300 kcal/day) associated with glucose excretion, but also to the mild

265   diuretic effects of the drug.[9, 12] Conversely, the steady-state weight loss associated with SGLT2

266   inhibitors treatment may result from fat loss.[22, 23] In animal models, SGTL2 inhibitors have been

267   shown to cause reduction in body weight and fat mass by enhancing lipolysis, fatty acid oxidation

268   and adipose tissue browning.[14, 24] These findings coincide with the alterations seen in substrate

269   utilisation from carbohydrates to lipids and potentially, ketone bodies.[9, 25] Reductions in other

270   measures of adiposity including waist and hip circumference, visceral and subcutaneous fat depots or

271   indices, which may better reflect risk for metabolic disturbances and cardiovascular disease, have

272   also been demonstrated in patients with type 2 diabetes following treatment with SGLT2 inhibitors,

273   [22, 23] with these findings being in agreement with the improvements in waist and hip circumference

274   seen in our women with PCOS assigned to receive empagliflozin.

275   In the present study, the metformin group experienced modest increases in body weight. Studies

276   on the effect of metformin on body weight in women with PCOS have yielded mixed results.[8, 26, 27]

277   While some studies have suggested that metformin therapy may result in weight reduction, some

278   randomized controlled trials have failed to confirm this. For example, a large, randomised, double-

279   blind, placebo-controlled trial evaluated the combined effects of lifestyle modification and

280   metformin (850 mg twice daily), by studying 143 anovulatory women in the UK with a mean BMI

281   of 38 kg/m^2 and showed that it is no different than placebo in terms of weight reduction.[26] However,

282   women in this study were not required to have clinical and biochemical evidence of

283   hyperandrogenemia- an essential component of the diagnosis of PCOS. Conversely, a Finnish

284   multicentre randomized study[27] compared metformin with placebo in 320 women with PCOS and

285   the authors reported significantly higher live birth rates in the metformin group (41.9% versus

286   28.8%; p=0.014) and maximal effect was seen in obese women with PCOS. A recent Cochrane

287   review explored the effect of metformin on PCOS (40 studies, total n= 3848 women) failed to

288   provide any conclusive evidence against or for metformin in women with PCOS.[8] These mixed

289   results in the literature with regards to metformin treatment indicate that there are subtypes of PCOS

290   which might respond beneficially to PCOS. Our study is not powered to assess the phenotypic

291   heterogeneity in PCOS with regards to response to metformin- and highlights the need to study this

292   with large scale studies looking at the effect of metformin on PCOS subtypes.

293   Women with PCOS experience higher prevalence of insulin resistance, type 2 diabetes mellitus,

294   dyslipidaemia, endothelial dysfunction and atherosclerosis compared to age-matched women without

295   PCOS.[2] Such metabolic disturbances are characterised by chronic low-grade inflammation and

296   vascular impairments which increase cardiovascular risk.[2] The effects of SGLT-2 inhibitors on

297   glycaemic control have been evaluated as the primary outcome in the majority of the studies

298   investigating this new class of glucose lowering agents in type 2 diabetes. A meta-analysis of 13

299   randomized trials on the efficacy of SGLT-2 inhibitors compared to placebo demonstrated

300   improvements in glycaemic control in type 2 diabetes patients, as evidenced by reductions in HbA1c

301   (-0.49% and -0.50% after one and two years of treatments) and fasting plasma glucose levels (-0.81

302   mmol/L and -0.76 mmol/L after one and two years of treatment).[28] Similar results were shown in a

303   meta-analysis (10 studies, 6203 participants) on the efficacy and safety of empagliflozin only.[29] In

304   addition to glycaemic control, use of SGLT-2 inhibitors results in a reduction in TG levels and

305   increases in HDL-cholesterol levels, but also LDL-cholesterol levels, possibly due to the shifted

306   metabolism favouring lipid utilisation.[30, 31] In contrast to these beneficial effects on glycaemic

307   control and less pronounced lipids effects of SGLT-2 inhibitors reported in type 2 diabetes, we did

308   not observe significant changes in fasting glucose, insulin fasting lipids or *hs*-CRP at 3 months after

309   empagliflozin treatment compared to baseline or any differences between our treatment groups.

310 These results may be related to the short duration of the study or to the baseline characteristics of our

311 participants with PCOS, who were young and did not have diabetes.

312 Further evidence from studies in patients with type 2 diabetes suggest that empagliflozin, and

313 other SGLT2 inhibitors such as canagliflozin and dapagliflozin cause reductions in blood pressure,

314 as a result of their natriuretic effects or due to the intensification of anti-hypertensive therapy.[32, 33]

315 No such blood pressure changes were demonstrated in our women with PCOS, though these subjects

316 were normotensive and changes may not have been expected. Similarly, measures of endothelial

317 function (RHI) or arterial stiffness (AI) were not altered compared to baseline in either treatment

318 groups. Empagliflozin has been shown to improve endothelial dysfunction in preclinical studies in

319 diabetic rat models,[34, 35] but human data are scarce. A recent 16-week study demonstrated that

320 dapagliflozin add-on therapy to metformin improved endothelial function, as evaluated by flow-

321 mediated dilation, in patients with inadequately controlled early-stage type 2 diabetes mellitus.[36]

322 Although there are no comparative data from studies that have investigated the effects of SGLT-2

323 inhibitors in women with PCOS, the results of our 12-week intervention contrast those of a longer

324 study which demonstrated that metformin treatment for 6 months improved or even normalised

325 abnormal flow-mediated dilatation (FMD) on the brachial artery and improved plasma endothelin-1

326 (ET-1) levels in women with PCOS.[37] The discrepancies in these results may be at least partially

327 explained by differences in study duration and the use of different endothelial function measures.

328 There were significant increases in the SHBG and oestradiol levels in the empagliflozin group,

329 but no significant reductions were seen in FAI and serum total testosterone levels. The % changes

330 from baseline in hormonal levels did not differ to metformin. Metformin use in women with PCOS

331 has been associated with improvements in hormonal levels. A recent meta-analysis demonstrated

332 that metformin treatment resulted in small improvements in serum testosterone, but no changes in

333 free testosterone, FAI, SHBG, DHEAS LH, FSH, LH/FSH ratio, oestradiol or progesterone

334 compared to placebo in women with PCOS.[38] Metformin may also have some beneficial effects on

335 ovulation and menstrual frequency.[8] Given the short follow-up of the present study, we did not

336 assess these parameters, which is a limitation of the present study.

337

Conclusions

Empagliflozin treatment over a 12-week period had beneficial effects on weight, BMI, waist and hip circumference and total body fat in overweight and obese women with PCOS compared to metformin, but no differences were seen in hormonal and metabolic parameters including insulin resistance and androgen levels. Placebo-controlled and comparative treatment RCTs of longer-term duration are needed to confirm these findings and provide further insights into the effects of empagliflozin on PCOS-related outcomes in women with PCOS with different PCOS phenotypes and PCOS-related complications (e.g., with/ without diabetes), before empagliflozin gains a therapeutic place in PCOS. Lifestyle interventions (preferably multicomponent including diet, exercise and behavioural strategies) should still be considered the first line of treatment for overweight/obese women with PCOS for reductions in body weight, central obesity and insulin resistance.[3]

Disclosure Statement

The authors report no conflict of interest in this work.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Contributions

ZJ, ESK, SLA, TS participated in study conception and design. ZJ, TS performed the acquisition of data. ZJ, MP, HD, UQ, JA, AYK, ASR, ESK, SLA and TS participated in analysis and/or

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Table 1: Changes in anthropometric, body composition, hormonal and metabolic parameters following 12 weeks of empagliflozin and metformin treatment.

Parameter	Empagliflozin (n = 19)			Metformin (n=20)		
	Baseline	12 weeks	% baseline change	Baseline	12 weeks	% baseline change
Weight (kg)	102.3±16.6	101.5±16.3	-1.4±3.2**	108.8±25.3	110.1±25.7*	1.2±2.3
BMI (kg/m^2)	37.1±6.2	36.6±6.0	-1.4±3.2**	38.7±7.8	39.2±7.9*	1.1±2.2
Waist circumference (cm)	101.2±9.7	99.6±9.5*	-1.6±2.8**	106.2±15.7	106.3±15.4	0.2±2.1
Hip circumference (cm)	121.6±11.5	119.2±11.4*	-2.0±3.2**	124.1±17.4	125.3±16.7*	1.1±1.9
BMR (kcal)[1]	1761±205	1728±200*	-1.8±2.9**	1783 (304)	1797 (305)	0.1±1.9
Body fat (%)[1]	46.7±3.5	47.1±3.4	0.6 (3.2)	46.8±6.2	47.6±5.9*	1.1 (3.8)
Fat mass (kg)[1]	48.9±11.0	48.6±11.0	-0.7±4.9**	52.3±10.9	53.7±18.3*	3.2±5.0
FFM (kg)[1]	54.8±5.9	53.7±5.8*	-2.0±3.2	56.7±7.9	56.5±7.9	-0.3±2.2
TBW (kg)[1]	40.1±4.3	39.3±4.3*	-2.0±3.2	41.5±5.8	41.4±5.8	-0.3±2.2
FAI	10.3±3.0	9.4±3.6	-7.0±31.4	7.5 (6.4)	8.0 (6.4)	-9.7 ±34.0
Testosterone (nmol/L)	1.6±0.4	1.6±0.6	2.6 (37.0)	1.7 (1.2)	1.5 (1.2)	-14.0 (33.6)
SHBG (nmol/L)	17.3±6.4	19.2±8.5*	9.9±22.6	19.5(13.5)	19.5(14.5)	6.4±25.5
Androstenedione (nmol/L)	5.7±1.4	5.7±1.9	-2.2 (24.4)	4.3 (4.4)	5.0 (2.8)	5.6 (59.8)
DHEAS (μmol/L)	6.1±1.6	6.2 ± 2.1	1.0±20.1	5.5 ± 3.3	5.8 ± 3.0	8.1±15.0
Oestradiol (pmol/L)	200 (80)	280 (340)*	39.1 (121)	240 (140)	210 (190)	-8.7 (113.1)
SBP (mmHg)	118.1±11.7	117.5±14.2	-0.8 (5.9)	124.4±15.5	125.9±15.8	1.1 (6.8)
DBP (mmHg)	74.0 (10.0)	73.0 (8.0)	-3.1±9.0	80.3 ± 10.7	80.7 ±9.8	0.8±7.1
RHI	1.6 (0.5)	1.5 (0.7)	2.6 (48.1)	1.7 (0.5)	1.6 (0.7)	-1.9 (53.7)
AI	-3.3 ± 12.0	-3.4± 13.3	-57.0±170	0.6 ± 8.1	2.3 ± 10.4	-47.5±146
Fasting glucose (mmol/L)[1]	4.5 (0.6)	4.5 (0.6)	-0.8±5.8	4.7 (0.5)	4.4 (0.6)	-2.3±8.0
Fasting insulin (μIU/ml)	12.6 (11.6)	12.7 (14.4)	-21.5(80.1)	16.6 (11.4)	14.0 (22.7)	-14.1 (52.5)
HOMA-IR[1]	2.6 (2.1)	2.4 (2.7)	-20.5 (84.6)	3.7 (2.4)	3.2 (4.9)	-18.9 (53.5)
TC (mmol/L)	4.8 ±1.0	4.7±1.1	-1.6±13.7	4.7 ± 0.9	4.5 ± 0.9	-2.2 ±8.5
LDL-C (mmol/L)	2.8 ± 1.0	2.7 ± 1.1	2.7 (30.2)	2.8 (0.6)	2.8 (0.9)	-3.4 (9.6)
HDL-C (mmol/L)	1.1 ± 0.2	1.1 ± 0.2	-0.6 ±9.2	1.2 ± 0.2	1.1 ± 1.9	-3.4 ±9.6
TG (mmol/L)	1.5 (1.3)	1.4 (0.9)	-6.7 (35.8)	1.1 (0.9)	1.2 (0.7)	-9.0 (49.8)
hs-CRP (mg/L)	5.4 (6.6)	3.3 (5.9)	9.8±59.1	6.1 (9.7)	5.1 (10.9)	8.4±34.0

Data are presented as mean ± SD if normally distributed or as median (interquartile range), if skewed. *, *p*<0.05, significant difference from baseline within treatment group; **, *p*<0.05, significant difference from metformin group; AI, Augmentation index; BMI, body mass index; BMR, Basal metabolic rate; DBP, diastolic blood pressure; DHEAS, Dehydroepiandrosterone sulphate; FAI, Free androgen index; FFM, Fat free mass; HDL, high density lipoproteins; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; hs-CRP, high sensitivity-C-reactive protein; LDL, low density lipoproteins; RHI, Reactive hyperaemic index; SBP, systolic blood pressure in; SHBG, Sex hormone binding globulin; TBW, Total body water; TC, total cholesterol; TG, triglycerides. [1]Data available for 18 participants in the empagliflozin group.

Table 2: Linear regression analysis modelling percentage (%) changes in key anthropometric characteristics, hormonal and metabolic parameters as function of metformin or empagliflozin

	Beta	SE	P-value
% change in BMI*	-2.27	0.95	0.02
% change in weight*	-2.28	0.96	0.02
% change in fat mass*	-3.43	1.74	0.05
% change in WC*	-1.76	0.85	0.04
% change in HC*	-2.87	0.88	0.002
% change in BMR*	-1.73	0.86	0.05
% change in FAI**	5.42	11.97	0.65
% change in SHBG**	5.89	8.69	0.5
% change in TC*	2.78	3.85	0.47
% change in fasting glucose*	1.12	2.48	0.65

BMI, body mass index; BMR, Basal metabolic rate; WC, waist circumference; HC, HIP circumference; FAI, Free androgen index; FFM, Fat free mass; SHBH, sex hormone binding globulin; TC, total cholesterol.

Metformin group used as reference group

*adjusted for age

**adjusted for age and BMI

Legends of figures

Figure 1: Flow diagram of the study.

Figure 2: Percentage changes from baseline in anthropometric and body composition parameters after 12 weeks with empagliflozin and metformin treatment.

*, $p<0.05$; BMI, body mass index; WC, waist circumference; HC, hip circumference; BMR, Basic metabolic rate; FFM, Fat free mass; TBW, Total body water. Body composition data (BMR, body fat %, fat mass, FFM and TBW) are presented for 18 participants in the empagliflozin group with available data.