THE UNIVERSITY OF HULL

THE IMPACT OF THYROID DYSFUNCTION ON CARDIOVASCULAR RISK

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fulfilment of the requirements
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by

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To my parents, Zahra and Mehdi for their endless devotion and to my wife Naghmeh for her love

UNIVERSITY OF HULL

ABSTRACT

THE IMPACT OF
THYROID
DYSFUNCTION ON
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by **Alireza M Manuchehri**

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Thyroid dysfunction syndromes (hypo and hyperthyroidism) have major impacts on the cardiovascular system. Whilst the short term effects of thyroid dysfunction on the cardiovascular system are clinically obvious and well studied, the long term effects and particularly the size of its effects on the cardiovascular system remain controversial.

By combining the findings of the following studies with the review of literature that has been provided in the Introduction (Chapter 1) and the 'discussion' sections of individual studies, the author concludes that the effects of thyroid dysfunction on the cardiovascular system are subtle, these effects do not always translate into adverse cardiovascular outcomes, and the magnitude of the effect is proportional to the severity of thyroid dysfunction.

This thesis has been organised in the following 6 chapters:

Chapter 1: 'Introduction', is an overview of the current literature on the subject, summarising the short term and long term effects of hypo and hyperthyroidism on the cardiovascular system. An overview of other forms of thyroid disease has also been provided in this chapter.

Chapter 2: 'The effect of subclinical hypothyroidism on cardiovascular outcomes in type 2 diabetics', for the first time looks at the relationship between subclinical hypothyroidism and cardiovascular outcomes in this high cardiovascular risk population and is currently under peer review for publication. Data analysis showed that there was no relationship between baseline or follow up serum TSH levels and cardiovascular mortality. The unadjusted odds ratio (OR) was 1.27(95%CI=0.86, 1.87, p =0.26). Adjusting for age and sex did not alter the nature of this relationship: OR=1.17(95%CI=0.88, 1.56, p=0.27), and neither did further adjustment for the other baseline covariates (i.e. smoking, body mass index (BMI), diastolic and systolic blood pressures, HbA1c and lipids): OR=1.15 (95% CI= 0.58, 2.27, p=0.68). The data from this study suggest that subclinical hypothyroidism does not contribute to the excess risk of cardiovascular mortality in patients with type 2 diabetes, at least 5 years after its diagnosis.

Chapter 3: 'The effect of thyroid dysfunction on N-terminal pro-B-type natriuretic peptide concentrations', examines the changes

in NT-pro-BNP in relation to hypo and hyperthyroidism. This study showed that treating hypothyroidism to euthyroid state is associated with a rise in NT-proBNP concentrations ($p<0.001$) and that treating hyperthyroidism leads to a fall; however, this trend towards lower NT-proBNP levels in hyperthyroid group after treatment hardly reached statistical significance ($p=0.05$). These changes seem to be secondary to a metabolic rate mediated effect on the peptide production. However, in the context of the high biological variation of NT-proBNP, the magnitude of these changes is likely to be of limited clinical relevance.

Chapter 4: 'The effect of thyroid dysfunction on peptide YY and Ghrelin', examines the changes in these gut hormones in relation to hypo and hyperthyroidism. PYY and ghrelin have been shown to play important roles in body weight regulation. Changes in body weight are frequently seen with thyroid dysfunction. Obesity is a major cardiovascular risk factor. Data analysis showed that there was no significant change in PYY levels in either group before and after treatment (mean PYY in pmol/L±SD before and after treatment: 24.81±12.10 vs. 23.22±15.27, $p=0.428$ hypothyroid; 19.47±8.09 vs. 20.87±10.26, $p=0.464$ hyperthyroid). The observed changes in ghrelin in both groups did not reach statistical significance either (mean ghrelin in pmol/L±SD before and after treatment: 761.32±435.46 vs. 697.60±376.09, $p=0.227$ hypothyroid; $p=0.058$ for hyperthyroid group). This study showed that thyroid dysfunction does not alter plasma PYY and ghrelin concentrations and changes in weight associated with thyroid

dysfunction are more likely to be due to a combination of altered metabolic rate and the direct effect of T_3 or lack of it on hypothalamus.

Chapter 5: 'The effect of dietary soy phytoestrogens on thyroid function and insulin resistance in patients with subclinical hypothyroidism: a double blind crossover study', investigates the possible effect of soy phytoestrogens on thyroid function and cardiovascular risk markers. High content phytoestrogen soy supplement (16 mg phytoestrogen) is compared to a low content phytoestrogen supplement (2 mg phytoestrogen) – both containing equal amounts of soy protein – in a crossover design. The data from this study showed a significant reduction in systolic blood pressure of (mean ± SD) 2.57 ± 3.9 mmHg and diastolic blood pressure of (mean ± SD) 1.64 ± 3.7 mmHg after 16 mg phytoestrogen phase. There was also a significant reduction of systolic blood pressure of (mean ± SD) 1.1 ± 3.9 mmHg after 2mg phytoestrogen phase but no significant reduction in diastolic blood pressure. Other positive findings of the study were a significant improvement in insulin resistance measured by HOMA-IR (mean ± SD) [5.11 ± 12.2 vs. 4.2 ± 9.9 (p value= 0.02) 95% CI 0.12, 1.63] and plasma glucose (mean ± SD) [5.6 ± 1.1 vs. 5.3 ± 1.2 (p value= 0.006) 95% CI 0.11, 0.59] after 16mg phytoestrogen phase but not after 2mg phytoestrogen phase This study concludes that the cardiovascular protection suggested for phytoestrogens epidemiologically, may be through their positive effect on insulin resistance and blood pressure. However, low and

high levels of dietary phytoestrogens have no clinical effect on thyroid function even in patients compromised with subclinical hypothyroidism.

Chapter 6: 'General discussion', this final chapter provides a summary discussion, conclusion, the relevance of the work in this thesis in relation to the latest publications on the subject and scope for future work.

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PUBLICATIONS & PRESENTATIONS

The following papers have been published in peer-reviewed journals as the result of this thesis (Chapters 3 and 4):

1: Manuchehri AM, Jayagopal V, Kilpatrick ES, Atkin SL.
The effect of thyroid dysfunction on N-terminal pro-B-type natriuretic peptide concentrations.
Ann Clin Biochem. 2006 May;43(Pt 3):184-8.

2: Manuchehri AM, Sathyapalan T, Jayagopal V, Kilpatrick ES, Ghatei MA, Bloom SR, Atkin SL.
Alterations in thyroid status do not affect plasma peptide YY (PYY) and ghrelin concentrations.
Clin Endocrinol (Oxf). 2008 May;68(5):836-8. Epub 2007 Oct 30.

Chapter 2 of this thesis, *'the Effect of Subclinical Hypothyroidism on Cardiovascular Mortality in Patients with Type 2 Diabetes'*, has been presented at the Diabetes UK Annual Professional Conference, 5-7 March 2008 – SECC, Glasgow (oral presentation), and is currently under peer review for publication.

Chapter 5 of this thesis, *'the Effect of Dietary Soy Phytoestrogens on Thyroid Function and Insulin Resistance in Patients with Subclinical Hypothyroidism: a Double Blind Crossover Study'*, has been presented at the Society for Endocrinology BES 2008 – Harrogate, 7-10 April 2008 as an abstract (poster), and is currently under peer review for publication.

Chapter 1

INTRODUCTION

The thyroid gland plays a pivotal role in tissue metabolism and development, and in doing so affects various organ systems. Whilst the effect of thyroid hormones on the cardiovascular system is of particular interest to this thesis, this chapter looks into the underlying causes and overall consequences of hyperthyroidism hypothyroidism and other forms of thyroid disease as well.

1.1.History: The naming of the thyroid gland (Greek *thyreos*, shield, plus *eidos*, form) has been attributed to Wharton[1] in 1656 (Figure 1.1), but an endocrine function was not proposed until almost 200 years later[2]. Appreciation of the clinical disorders affecting the thyroid followed, the earliest descriptions of which appeared in the following order: Thyroid cancer in 1811; diffuse toxic goitre by Parry in 1825, Graves in 1835, and von Basedow in 1840; cretinism in 1871; myxoedema in 1874; thyroidectomy for the treatment of toxic goitre in 1884; thyroid extract therapy for myxoedema in 1891; Hashimoto disease in 1912; subacute (de Quervain) thyroiditis in 1936; the structure of thyroxine (T_4) in 1926; identification of triiodothyronine (T_3) in 1952, the earliest evidence of the thyroid-stimulating antibodies of Graves' disease in 1956; the presence of thyroid autoantibodies in Hashimoto's disease in 1957; recognition of medullary thyroid carcinoma as a

distinct entity in 1959; and reports of cases of postpartum thyroiditis with hypothyroidism or thyrotoxicosis only since 1976 to 1977[3].

Other subsequent milestones included the first description of resistance to thyroid hormone in 1967; substantiation that circulating T_3 was derived largely from peripheral monodeiodination of T_4 in 1970; identification of T_3-binding receptors in tissues in 1972 and their homology to the viral oncogene *erb*A in 1986; and demonstrations that point mutations in the thyroid-hormone receptor accounted for hormone resistance in 1989 and 1990. The thyrotropin (TSH) receptor was cloned in 1989; studies since have identified both loss of function and gain of function mutations in the TSH receptor that account for specific types of hypothyroidism and hyperthyroidism, respectively. The gene for the β subunit of TSH was then cloned, facilitating the development of human recombinant TSH (rhTSH)[3]; this agent has great clinical utility for the diagnosis and treatment of thyroid cancer and is used as an adjuvant treatment during cancer diagnostic protocols to detect recurring or remaining thyroid cancer cells in patients with a history of thyroid cancer as an alternative to thyroid hormone withdrawal[4].

EFFECTS OF FEEDING THYROID SUBSTANCE

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PHYSIOLOGICAL ACTIVITY AND CHEMISTRY OF THE THYROID GLAND

ACCORDING to Galen (translation by Daremberg, 1854) all the writers up to his time, including Hippocrates and Plato, described the thyroid body together with the salivary and thymus glands as lubricators of the pharynx, larynx, and trachea. The spongy constitution of these bodies, together with the fact that the upper end of the larynx is entirely closed by the epiglottis while swallowing (thus keeping out all but a very small quantity of liquid) seemed to Galen to be contradictory to the time-honored lubricating function of these glands. The spongy nature of the glands seemed better suited for absorbing purposes than for producing lubricating substances. Then, too, it was puzzling to Galen that if these glands had been created for lubricating the larynx, their product was prevented from entering the very organ it was intended to lubricate.

The thyroid gland was carefully observed by Vesalius (in Ball, 1910), a fact which is attested by its presence in his excellent anatomical drawings. Like many other organs, however, (e.g. the vermiform appendix) it apparently did not seem important enough to receive any discussion as to its function.

So the thyroid was an object of both speculation and controversy, as well as of negative interest, until 1656 when it received its first reasonably accurate description by Thomas Wharton in his *Adenographia*. Wharton's reputation as an anatomist rests principally on his *Adenographia*, the first treatise devoted to a comparative discussion of the glands of the body. Born in 1614, the son of a land owner, Wharton took the degree of doctor of medicine at Oxford, and then practised his profession in London. He was appointed physician to St. Thomas's Hospital in 1659, and was for many years an active and highly esteemed member of the College of Physicians. He died in 1673. In his book, Wharton attempts to explain the term 'gland' and takes the position that secretion is a gland's primary purpose. He gives very detailed descriptions of the anatomy of the glands of the body, and assigns to each of them a group of general functions in the physiology of the organism. The thyroid is described in detail, particular note being made of its great vascularity. The functions of the thyroid, according to Wharton, were (1) to heat the hyoid cartilage which would normally be cold; (2) to lubricate the larynx; and (3) to give rotundity and beauty to the neck. It seems strange that even though Galen, fifteen centuries before, had pointed out the difficulties encountered in explaining the lubricating function of the thyroid, Wharton still held to that view. Today these functions seem fantastic, but with minor variations they were regarded as the essential ones

Figure 1.1

1.2.Anatomy and development: The location of the thyroid makes it readily accessible to both inspection and palpation. The normal adult thyroid gland consists of two lobes connected by an isthmus. Thyroid tissue is light brown, and the cut surface often glistens. The normal gland is surrounded by a delicate fibrous capsule and weighs 15 to 25 g. The normal thyroid is attached loosely to neighbouring structures, and the fascial planes are distinct (Figure 1.2). Four parathyroid glands, which produce parathyroid hormone, are located in the posterior region of each pole of the thyroid. The recurrent laryngeal nerves traverse the lateral borders of the thyroid gland and must be identified during thyroid surgery to avoid vocal cord paralysis.

The thyroid gland develops from the floor of the primitive pharynx during the third week of gestation. The gland migrates from the foramen caecum, at the base of the tongue, along the thyroglossal duct to reach its final location in the neck. This feature accounts for the rare ectopic location of thyroid tissue at the base of the tongue (lingual thyroid), as well as for the presence of thyroglossal duct cysts along this developmental tract. Thyroid hormone synthesis normally begins at about 11^{th} week of gestation.

The mature thyroid gland contains numerous follicles composed of thyroid follicular cells that surround secreted colloid, a proteinaceous fluid that contains large amounts of thyroglobulin, the protein precursor of thyroid hormones[5].

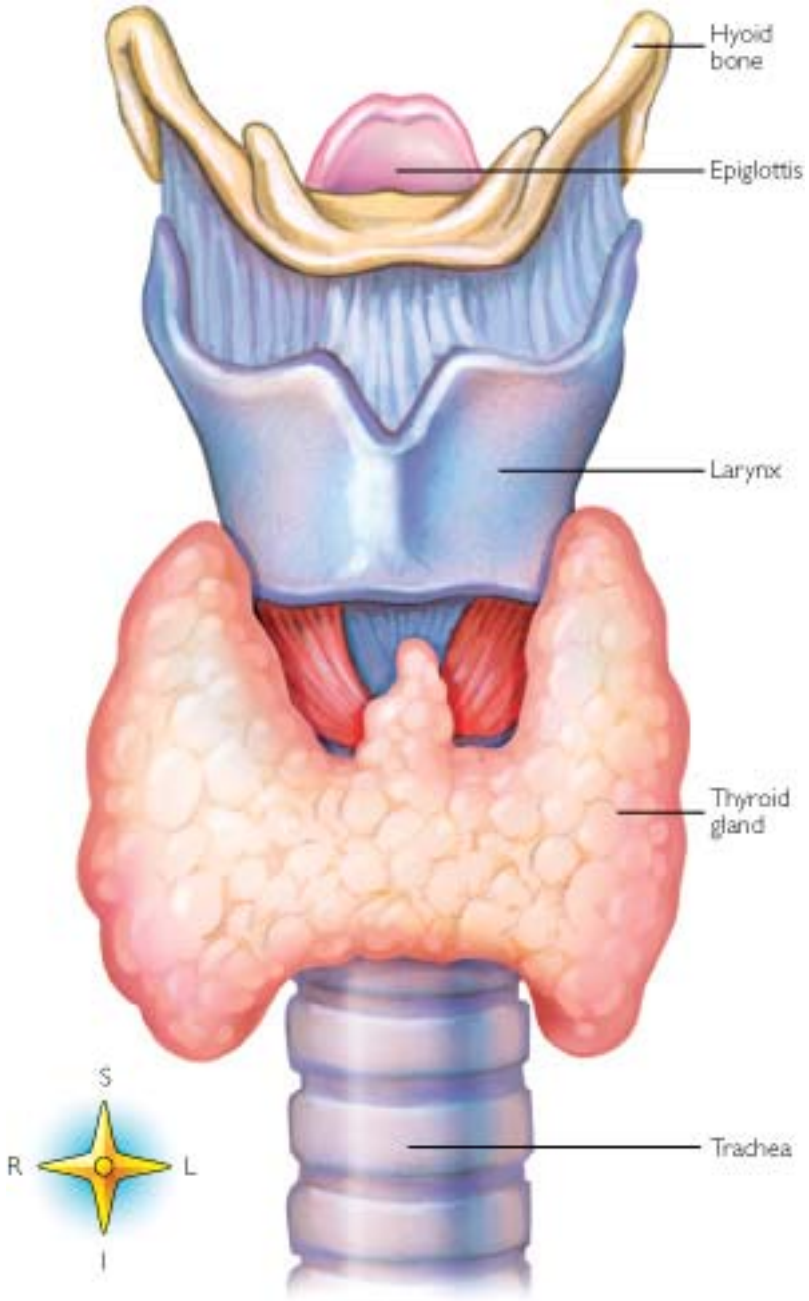

Figure 1.2 The thyroid gland (adapted from Thibodeau / Patton's: The Human Body in Health and Disease, 3rd edition, 2002)

1.3.Regulation of the thyroid axis: Thyroid stimulating hormone (TSH) secreted by the thyrotrope cells of the anterior

pituitary, plays a pivotal role in the control of the thyroid axis and serves as the most useful physiologic marker of thyroid hormone action. TSH is a 31-kDa hormone composed of α and β subunits; the α subunit is common to the other glycoprotein hormones (luteinizing hormone, follicle-stimulating hormone, human chorionic gonadotropin (hCG)), whereas the TSH β subunit is unique to TSH. The extent and nature of carbohydrate modification are modulated by thyrotropin-releasing hormone (TRH) stimulation and influence the biologic activity of the hormone. The thyroid axis is a classic example of an endocrine feedback loop. Hypothalamic TRH stimulates pituitary production of TSH, which, in turn, stimulates thyroid hormone synthesis and secretion. Thyroid hormones feed back negatively to inhibit TRH and TSH production (Figure 1.3). The "set-point" in this axis is established by TSH, the level of which is a sensitive and specific marker of thyroid function. TRH is the major positive regulator of TSH synthesis and secretion. TRH acts through a seven-transmembrane G protein-coupled receptor (GPCR) that activates phospholipase C to generate phosphatidylinositol turnover and the release of intracellular calcium. Peak TSH secretion occurs ~15 min after administration of exogenous TRH. Dopamine, glucocorticoids, and somatostatin suppress TSH but are not of major physiologic importance except when these agents are administered in pharmacologic doses. Like other pituitary hormones, TSH is released in a pulsatile manner and exhibits a diurnal rhythm; its highest levels occur at night. However, these

TSH excursions are modest in comparison to those of other pituitary hormones, in part because TSH has a relatively long plasma half-life (50 min). Consequently, single measurements of TSH are adequate for assessing its circulating level[5].

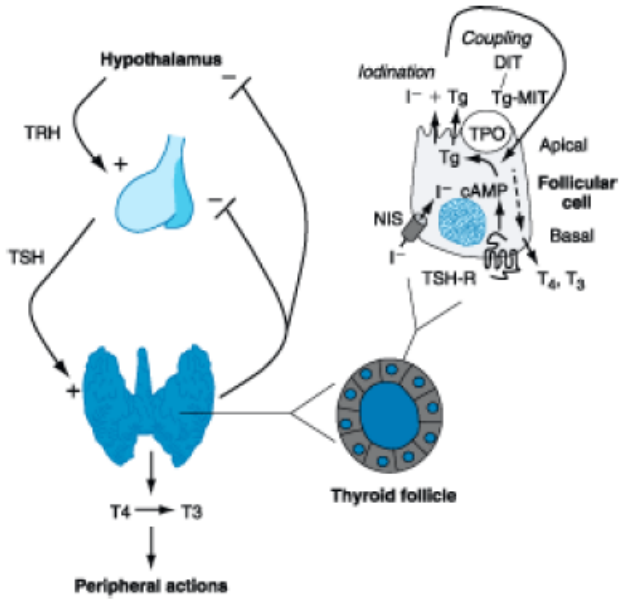

Figure 1.3: Regulation of thyroid hormone synthesis (adapted from Harrison's Principles of Internal Medicine, 15th edition, 2001)

1.4.Thyroid hormone synthesis: The major synthetic steps regulated by TSH are transport of iodide, organification, coupling, thyroglobulin (Tg) synthesis, and endocytotic secretion. Under the stimulation of TSH, the thyroid cells remove iodide from the capillary network at the base of the cell and move the iodide to the apex of the cell, where it is joined with molecules of tyrosine to make mostly T_4 and some T_3. T_4 and T_3 are stored in colloid within the follicles of the gland. They are then released together,

or some T_4 is further deiodinated to T_3 before release. These two steps are also under the influence of TSH or other proteins that bind to the TSH receptor. Mutations of this TSH receptor are continuously activated in some toxic adenomas and multinodular goitres[6]. A small but measurable amount of non-TSH-dependent T_4 secretion also occurs normally[3]. The tyrosine residues to which the iodine is joined in the organification process are part of the Tg molecule, a 660-kDa glycoprotein. Defects in Tg synthesis have been proposed to cause some cases of goitre, although such cases are rare. Serum values of Tg are extremely useful in the management of well-differentiated thyroid cancer as an indicator of biologic activity or recurrence[7]. Figure 1.4 summarises the stages in thyroid hormone synthesis.

1.5.Thyroid hormone transport and metabolism: T_4 is secreted from the thyroid gland in at least 20-fold excess over T_3. Both hormones circulate bound to plasma proteins, including thyroxine-binding globulin (TBG), transthyretin (TTR, formerly known as thyroxine-binding prealbumin, or TBPA), and albumin. The functions of serum-binding proteins are to increase the pool of circulating hormone, delay hormone clearance, and perhaps to modulate hormone delivery to selected tissue sites. The concentration of TBG is relatively low (1 to 2 mg/dL), but because of its high affinity for thyroid hormones (T_4 > T_3), it carries about 80% of the bound hormones. Albumin has relatively low affinity for thyroid hormones but has a high plasma concentration and it binds up to 10% of T_4 and 30% of T_3. TTR

also carries about 10% of T_4 but little T_3[5]. Only the free hormone is biologically available to tissues. Therefore,

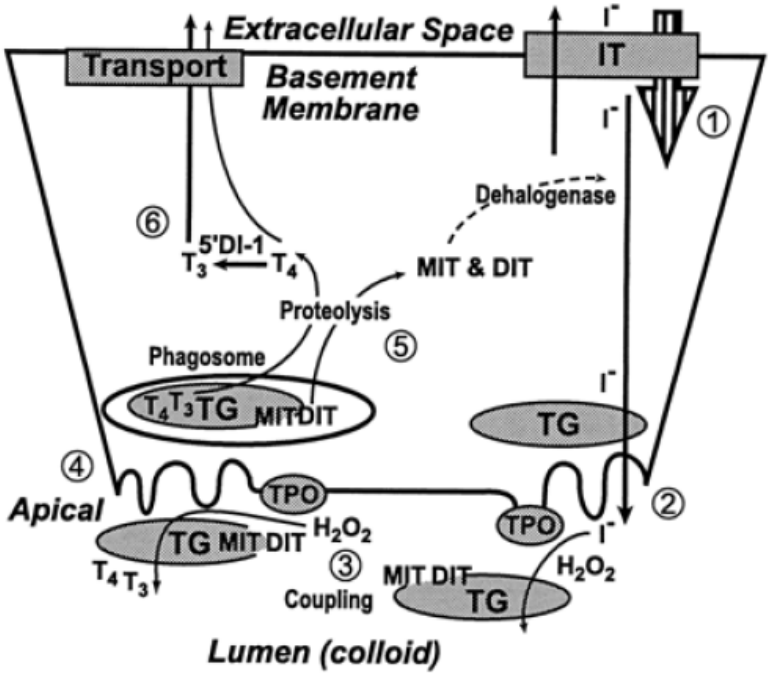

Figure1.4: Thyroid hormone synthesis.
Iodide (I-) is transported by the iodide transporter (IT) from the plasma into the thyroid cell and then I– moves to the apical membrane, where it is organified and coupled under the influence of thyroid peroxidase (TPO) to thyroglobulin (Tg), which is synthesized within the cell. Hormone stored as colloid reenters the cell through endocytosis and moves back toward the basal membrane, where T4 and T3 are secreted. Nonhormonal iodide is recycled. Hormone synthesis may be inhibited at: (1) iodine transport by thiocyanate, perchlorate, interleukin-1, and tumour necrosis factor-α; (2) organification and (3) coupling by propylthiouracil (PTU) and carbiimazole and through decreasing TPO by interferon-γ and interleukin-1; (4) endocytosis by lithium and iodide; (5) thyroglobulin proteolysis by iodide; and (6) intrathyroidal deiodination by PTU and tumour necrosis factor-α (adapted from Becker's Principles and Practice of Endocrinology and Metabolism, 3rd edition, 2002).

homeostatic mechanisms that regulate the thyroid axis are directed towards maintenance of normal concentrations of free hormones. About 80% of T_4 is metabolised by deiodination, 35% to T_3 and

45% to reverse T_3 (rT_3). The remainder is inactivated mostly by glucuronidation in the liver and secretion into bile, or to a lesser extent by sulfonation and deiodination in the liver or kidney. Other metabolic reactions include deamination of the alanine side chain, forming thyroacetic acid derivatives of low biologic activity; or decarboxilation or cleavage of the ether bridge, forming inactive compounds[8].

1.6.Thyroid hormone action: Thyroid hormones act by binding to nuclear receptors, called thyroid hormone receptors (TRs) α and β. Both TRα and TRβ are expressed in most tissues, but their relative levels of expression vary among organs; TRα is particularly abundant in brain, kidney, gonads, muscle, and heart, whereas TRβ expression is relatively high in the pituitary and liver. Both receptors are variably spliced to form unique isoforms. The TRβ2 isoform, which has a unique amino terminus, is selectively expressed in the hypothalamus and pituitary, where it appears to play a role in feedback control of the thyroid axis. The TRα2 isoform contains a unique carboxy terminus that prevents thyroid hormone binding; it may function to block the action of other TR isoforms.

The TRs contain a central DNA-binding domain and a C-terminal ligand-binding domain. They bind to specific DNA sequences, termed thyroid response elements (TREs), in the promoter regions of target genes (Figure 1.5). The activated receptor can either stimulate gene transcription (e.g., myosin heavy chain α) or

inhibit transcription (e.g., TSH β-subunit gene), depending on the nature of the regulatory elements in the target gene.

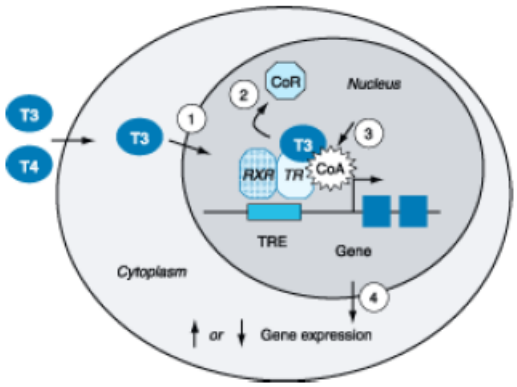

Figure 1.5: Mechanism of thyroid hormone receptor action (adapted from Harrison's Principles of Internal Medicine, 15th edition, 2001)

Thyroid hormones bind with similar affinities to TRα and TRβ. However, T_3 is bound to its receptors with about 10 to 15 times greater affinity than T_4, which explains its increased hormonal potency. Though T_4 is produced in excess of T_3, receptors are occupied mainly by T_3, reflecting T_4 to T_3 conversion by peripheral tissues, greater T_3 bioavailability in the plasma, and receptors' greater affinity for T_3. After binding to TRs, thyroid hormone induces conformational changes in the receptors that modify its interactions with accessory transcription factors. In the absence of thyroid hormone binding, the aporeceptors bind to corepressor proteins that inhibit gene transcription. Hormone binding dissociates the corepressors and allows the recruitment of coactivators that enhance transcription. The discovery of TR interactions with corepressors explains the fact that TR silences gene expression in the absence of hormone binding.

Consequently, hormone deficiency has a profound effect on gene expression because it causes active gene repression as well as loss of hormone-induced stimulation. This concept has been corroborated by the finding that targeted deletion of the TR genes in mice has a less pronounced phenotypic effect than hormone deficiency[5].

1.7.Thyroid autoimmunity and its genetics: Autoimmune thyroid disease (AITD) comprises a series of interrelated conditions including Graves' disease (GD), Hashimoto's thyroiditis (HT), atrophic autoimmune hypothyroidism, postpartum thyroiditis (PPT), and thyroid eye disease. These different manifestations of AITD may coincide, most frequently as the combination of GD and thyroid eye disease. Together, AITDs are the commonest autoimmune disorders in the population, affecting between 2% and 4% of women and up to 1% of men[9, 10]. Furthermore, AITD prevalence increases with advancing age, with more than 10% of subjects over 75 yr of age having biochemical evidence of mild (subclinical) hypothyroidism, the majority of which is due to autoimmune disease[10, 11].

A multifactorial model has widely been accepted to explain the aetiological basis of AITD, indicating a background inherited predisposition to autoimmunity, with additional environmental and hormonal factors that trigger or contribute to the development of disease. This model has been substantiated by familial clustering and twin studies clearly demonstrating that

AITD does not occur because of a single gene defect and does not follow a simple pattern of Mendelian inheritance[12-14]. There is also good evidence that both cigarette smoking and psychosocial stress are associated with the development of GD[15-17]. Similarly, the female preponderance of human AITDs[9], the modulation of animal models of AITD with gonadal steroids[18], the amelioration of GD during pregnancy, and the occurrence of PPT all support the important role of sex steroids in these disorders[19]. Further supporting the role of environmental factors in AITD, a recently published study comparing two neighbouring populations of Karelian Russians and Finish schoolchildren even suggested that inferior prosperity and standard of hygiene may provide protection against thyroid autoimmunity[20]. In an attempt to quantify the contribution of genetic versus environmental factors in the pathogenesis of GD, Brix et al demonstrated that the concordance rate for AITD among monozygotic twins was in the range of 35–55% compared with 3% in dizygotic twins. The same group showed that 79% of the likelihood of developing GD was attributable to genetic factors; and that individual specific environmental factors not shared by the twins accounted for the remaining 21%[21]. Therefore it seems that clarifying the genetic basis of AITD is pivotal to its understanding.

The genetic predisposition to AITD is determined by a series of interacting susceptibility alleles of several different genes. These various genetic loci may also have differing influences on the

predisposition to AITD in different populations (locus heterogeneity), which makes the identification of disease susceptibility genes more difficult. Of the candidate genes studied so far, four loci deserve specific attention. The first two, the human leukocyte antigen (HLA) gene region and cytotoxic T lymphocyte antigen 4 (CTLA4) have stood the test of time and most researchers have found convincing evidence of their association with AITD. The other two candidate genes, i.e. the thyroglobulin gene and CD40 have attracted significant interest in recent years following family-based linkage data. Whilst some replication has been reported, on balance, these regions have generated conflicting data with true confirmation still awaited[22].

The major histocompatibility complex (MHC), which contains the HLA genes, is located on chromosome 6p21. It is subdivided into three regions: 1) the class I region, which encodes HLA antigens A, B, and C; 2) the class II region, which encodes HLA antigens DR, DQ, and DP, each with one or more α and ß chains; and 3) the class III region, encoding several immunoregulatory molecules including complement components, heat shock protein 70 (HSP70), and TNF. The class II region also contains the peptide transporters associated with antigen processing (TAP) and large multifunctional protease (LMP) genes. Tight linkage disequilibrium (i.e. conserved haplotypes) exists between the alleles of the MHC region. The MHC class II molecules play a critical part in the initiation of adaptive immune responses. Peptide antigens can only be recognized by T cell receptors when they are

attached to the binding groove of an MHC molecule on the surface of an antigen-presenting cell. This, together with the presence of several other immunoregulatory genes in the region, makes the MHC a strong candidate locus for AITD and other autoimmune diseases[19].

The association between GD and the alleles of MHC class I, with a higher frequency of HLA-B8 in GD patients was first shown by Grumet et al in 1974[23]; and was later confirmed by several other studies[24-26]. However, a stronger association of GD was found with the MHC class II allele, HLA-DR3, which is in strong linkage disequilibrium with HLA-B8[27]. Many case-control studies in white populations have since consistently shown the association of GD with HLA-DR3, with relative risks between 2.5 and 5[19]. Heward et al showed that linkage disequilibrium between GD and the HLA class II region is due to the extended haplotype DRB1*0304-DQB1*02-DQA1*0501[28]. Although there is now little doubt about the association of GD with the HLA DR3-carrying haplotype in whites, the primary disease susceptibility allele in the region remains unknown. Yanagawa et al have reported an association of GD, particularly in males, with the HLA-DQA1*0501 allele, which was stronger than, and independent of, the HLA DR3 status[29, 30]. This independent association of HLA-DQA1*0501 with GD has been supported by some studies, but not by others[19]. The phenomenon of linkage disequilibrium is, however, extremely strong across the whole HLA region making it difficult to determine the exact location(s)

of aetiological DNA variants detected. This has lead to the suggestion that the HLA region could harbour additional susceptibility loci within either the HLA class II region or the surrounding class I or class III regions. Work within the HLA region is ongoing to determine the exact location(s) of additional aetiological variants within[22].

Data on HLA haplotypes in Hashimoto's thyroiditis (HT) have been less definitive than in GD. A general methodological problem has been disease definition. Although the diagnosis of GD may be relatively straightforward, the definition of HT has been more controversial. HT encompasses a spectrum of manifestations, ranging from the simple presence of thyroid autoantibodies with focal lymphocytic infiltration, which may be of no functional consequence (asymptomatic autoimmune thyroiditis), to the presence of goitrous or atrophic thyroiditis, characterized by gross thyroid failure[31]. Initial studies failed to demonstrate an association between goitrous HT and HLA A-, B-, or C-antigens. Later studies showed an association of goitrous HT with HLA DR5 (RR, 3.1) and of atrophic HT with DR3 (RR, 5.1)[32].

CTLA4 is an immunoregulatory molecule that is expressed on the surface of activated T lymphocytes. The suppressive effects of CTLA-4 on T cell activation have raised the possibility that mutations altering CTLA-4 expression and/or function could result in an exaggerated T cell activation and lead to the

development of autoimmunity[32]. In the human, the CTLA4 gene is located on chromosome 2q33. CTLA4 polymorphisms have been extensively studied for linkage and association in various autoimmune disorders. It has been suggested that the CTLA4(AT)n polymorphism, for instance, may be functionally important because larger-sized alleles of this polymorphism may adversely affect the stability of the mRNA transcript[33]. Following a large re-sequencing effort and fine mapping of all common variants within the gene, disease susceptibility was finally mapped to four single nucleotide polymorphisms (SNP) (CT60, J030, JO31 and JO27–1) within a noncoding 6·1 kb region, with the common allelic variation of the CT60 SNP associated with lower messenger RNA levels of the soluble alternative splice form of CTLA[34]. Association of the CT60 SNP has been confirmed by several independent studies and most recently was shown to be highly associated with GD in a meta-analysis using data collected from 7246 GD patients[35] and similar associations have been reported for HT[34]. The mechanism by which polymorphism of CTLA4 acts to inhibit autoimmunity and specifically the role of soluble, as opposed to full length CTLA4, currently remains unknown, but is the subject of ongoing research. Whilst the noncoding 6·1 kb region described above almost certainly harbours an aetiological DNA variant, it is still possible that there are other polymorphisms within the region attributing to AITD[22].

Looking back at the evolution of AITD genetics over the past 4 decades shows that it has been a slow but steady and progressive process. The most recent step in the evolution of genetic studies of common autoimmune diseases - including AITD - sees association analysis performed at a genome-wide level to produce genome-wide association studies. These studies bring together the latest advances in our genetic maps, large scale automated genotyping technologies and large national DNA collections[22]. In the UK the Wellcome Trust Case Control Consortium has conducted a large association study involving four disease states including AITD in which over 15000 nonsynonymous coding SNPs are being typed in 1000 cases and 1000 controls[36]. Whilst this study has produced data directly related to AITD, it is also important to examine data from another larger study conducted by Wellcome Trust[37] which includes, for example, type 1 diabetes and rheumatoid arthritis to determine whether any novel loci identified in these two diseases are acting as common autoimmunity loci and predisposing to AITD. New discoveries are also influencing the next phase in AITD genetics. The recent discovery of copy number variation (CNV) within the genome which influences gene expression, phenotypic variation and gene dosage are also believed to play a key role in disease susceptibility and await further investigation[38]. The study of epigenetics, concerned with the 'switching off ' of genes, is also starting to a greater extent in GD with most of the work to date mainly focused on X chromosome inactivation and the effect that this

may have in explaining the AITD high female prevalence[22]. The study of AITD genetic susceptibility is an intriguing area with major potentials for producing novel therapeutic targets and treatments.

1.8.Hyperthyroidism:

Hyperthyroidism is the result of excess synthesis and release of thyroid hormone. Thyrotoxicosis is the hypermetabolic state associated with elevated levels of free thyroxine (fT4), free triiodothyronine (fT_3), or both.

1.8.1.Causes of hyperthyroidism: The most common forms of hyperthyroidism include diffuse toxic goitre (Graves' disease), toxic multinodular goitre (Plummer's disease), and toxic adenoma. Together with subacute thyroiditis, these conditions constitute 85-90% of all causes of thyrotoxicosis.

The most common cause of thyrotoxicosis is Graves' disease (50-60%). Graves' disease is an organ-specific autoimmune disorder characterized by a variety of circulating antibodies, including common autoimmune antibodies, antithyroperoxidase (anti-TPO), and antithyroglobulin (anti-Tg) antibodies. The most specific autoantibody is thyroid-stimulating immunoglobulin (TSI). TSI is directed toward epitopes of the thyroid-stimulating hormone (TSH) receptor and acts as a TSH-receptor agonist.

Table 1.8.1 Common, less common and uncommon forms of thyrotoxicosis and hyperthyroidism

Common Forms (85-90% of cases)	**Radioactive iodine uptake over neck**
Diffuse toxic goitre (Graves' disease)	Increased
Toxic multinodular goitre (Plummer's disease)	Increased
Thyrotoxic phase of subacute thyroiditis	Decreased
Toxic adenoma	Increased
Less Common Forms	
Iodide-induced thyrotoxicosis	Variable
Thyrotoxicosis factitia	Decreased
Uncommon Forms	
Pituitary tumours producing thyroid-stimulating hormone	Increased
Excess human chorionic gonadotropin (molar pregnancy/choriocarcinoma)	Decreased
Pituitary resistance to thyroid hormone	Increased
Metastatic thyroid carcinoma	Decreased
Struma ovarii with thyrotoxicosis	Decreased

Similar to TSH, TSI binds to the TSH receptor on the thyroid follicular cells to activate thyroid hormone synthesis and release and thyroid hypertrophy. This results in the characteristic picture of Graves' thyrotoxicosis, with a diffusely enlarged thyroid, very high radioactive iodine uptake, and excessive thyroid hormone levels compared to a healthy thyroid. Thyroid hormone levels can be extremely elevated in this condition. Clinical findings specific to Graves' disease include thyroid ophthalmopathy (periorbital oedema, chemosis [conjunctival oedema], injection, and proptosis) and, rarely, dermopathy over the lower extremities. This autoimmune condition may be associated with other autoimmune diseases such as pernicious anaemia, myasthenia gravis, vitiligo, adrenal insufficiency, and type 1 diabetes mellitus.

The next most common cause of thyrotoxicosis, subacute thyroiditis, comprising approximately 15-20% of cases of hyperthyroidism, is discussed under 1.11.

Toxic multinodular goitre (Plummer's disease) occurs in 15-20% of patients with thyrotoxicosis. It occurs more commonly in elderly individuals, especially in patients with a long-standing goitre. Thyroid hormone excess develops very slowly over time and often is only mildly elevated at the time of diagnosis. As discussed below, very high thyroid hormone levels may occur in this condition after high iodine intake, i.e., with contrast or amiodarone exposure. Symptoms of thyrotoxicosis are mild, often because only a slight elevation of thyroid hormone levels is present, and the signs and symptoms of thyrotoxicosis often are blunted (apathetic hyperthyroidism) in older patients. A typical nuclear scintigraphy scan of a toxic multinodular goitre demonstrates an enlarged thyroid gland with areas of increased and decreased activity.

Toxic adenoma is caused by a single hyperfunctioning follicular thyroid adenoma. Patients with a toxic thyroid adenoma comprise approximately 3-5% of patients who are thyrotoxic. The excess secretion of thyroid hormone occurs from a benign monoclonal tumour that usually is larger than 2.5 cm in diameter. The excess thyroid hormone suppresses TSH levels. Radioactive iodine uptake is usually normal, and the radioactive iodine scan

shows only the hot nodule, with the remainder of the normal thyroid gland suppressed because the TSH level is low.

Several rare causes of thyrotoxicosis exist that deserve mention. Iodide-induced thyrotoxicosis (Jod-Basedow syndrome) occurs in patients with excessive iodine intake, such as after an iodinated radiocontrast study. It occurs in patients with areas of thyroid autonomy such as a multinodular goitre or autonomous nodule. The thyrotoxicosis appears to be a result of loss of the normal adaptation of the thyroid to iodide excess. It is treated by cessation of the excess iodine intake and administration of antithyroid medication. Usually, after depletion of the excess iodine, thyroid functions return to preexposure levels. Euthyroid patients previously treated with antithyroid drugs for Graves' disease are also prone to develop iodine-induced hyperthyroidism[39]. Evidence that iodine can act as an immune stimulator exists, precipitating autoimmune thyroid disease and acting as a substrate for additional thyroid hormone synthesis[40].

Struma ovarii is ectopic thyroid tissue associated with dermoid tumours or ovarian teratomas that can secrete excessive amounts of thyroid hormone and produce thyrotoxicosis.

Patients with a molar hydatidiform pregnancy or choriocarcinoma have extremely high levels of beta human chorionic gonadotropin (βHCG) that can weakly activate the TSH receptor. At very high levels of βHCG, activation of the TSH receptor occurs that is sufficient to cause thyrotoxicosis.

Physiological maximum elevation of βHCG at the end of the first trimester of pregnancy is associated with a mirror-image temporary reduction in TSH. Despite the reduction in TSH, the free T_4 (fT4) levels usually remain normal or only slightly above the reference range. As the pregnancy progresses and the βHCG plateaus at a lower level, TSH levels decrease back to normal levels.

1.8.2.Clinical manifestations of hyperthyroidism: Nonspecific changes due to excessive thyroid hormone include weight loss, nervousness, fatigue, heat intolerance, and rapid heartbeat or palpitations sometimes associated with atrial fibrillation and high-output congestive heart failure (CHF). An increase in the rate of bone resorption occurs and it has been shown that overt symptomatic hyperthyroidism is associated with decreased BMD during the first 3 years after diagnosis and treatment of the disease. This effect is reversible with appropriate treatment after this period of time[41]. Thyroid hormone excess causes left ventricular thickening, which is associated with an increased risk of CHF. Thyrotoxicosis has been associated with dilated cardiomyopathy, right heart failure with pulmonary hypertension, and diastolic dysfunction (discussed in more detail under 1.14.1). Ophthalmopathy and dermopathy specifically associated with Graves' disease include periorbital oedema, chemosis, and proptosis with extraocular muscle dysfunction and diplopia.

The presentation of thyrotoxicosis is variable among patients. Younger patients tend to exhibit symptoms of more sympathetic activation, such as anxiety, hyperactivity, and tremor, while older patients have more cardiovascular symptoms, including dyspnoea and atrial fibrillation with weight loss. The clinical manifestations of thyrotoxicosis do not always correlate with the extent of the biochemical abnormality.

1.8.3.Laboratory measurements: The most reliable screening measure of thyroid function is a TSH level. TSH levels usually are suppressed to immeasurable levels (<0.05 mU/L) in thyrotoxicosis. To estimate the degree of thyrotoxicosis, TSH measurement should be combined with serum levels of free T_4 (and T_3 if T_4 levels are normal). Of patients with thyrotoxicosis, 5% have only elevated T_3 levels.

The most specific autoantibody for autoimmune thyroiditis is an enzyme-linked immunosorbent assay (ELISA) for anti-TPO antibody (thyroperoxidase). The titres usually are significantly elevated in the most common type of hyperthyroidism, Graves' thyrotoxicosis, and usually are low or absent in toxic multinodular goitre and toxic adenoma. A significant number of healthy people without active thyroid disease have mildly positive TPO antibodies, thus the test should not be performed for screening purposes. TSI, if elevated, helps establish the diagnosis of Graves' disease. A positive antithyroglobulin antibody test

does not predict the development of thyroid dysfunction and should not be measured.

1.8.4.Imaging studies: If the aetiology is not clear after physical examination and other laboratory tests, the aetiology of thyrotoxicosis can be confirmed by nuclear thyroid scintigraphy iodine 123 (^{123}I) uptake and scan. Values are elevated in patients with Graves' disease and toxic multinodular goitres. Both ^{123}I and technetium-99m can be used for thyroid scanning, which provides anatomic information on the type of goitre (e.g., diffuse vs. nodular). Scans essentially are pictures of the thyroid and do not necessarily confirm or refute the presence of hyperthyroidism per se; only ^{123}I uptake provides information in this area. If a dominant nodule is found upon examination of a patient with thyrotoxicosis, an ^{123}I thyroid scan is recommended to assure that the dominant nodule is functioning. If the nodule is cold, a biopsy on the nodule by fine-needle aspiration to exclude concomitant malignancy is warranted.

1.8.5.Treatment: With the exception of low ^{123}I uptake hyperthyroidism (e.g., subacute thyroiditis), the treatment of hyperthyroidism includes symptomatic relief and treatment with antithyroid medications, radioactive iodine (^{131}I), or thyroidectomy.

Symptomatic relief from the neurological and cardiovascular symptoms of thyrotoxicosis is normally achieved by beta-blocker therapy. Calcium channel blockers can be used for the same

purposes when beta-blockers are contraindicated or poorly tolerated.

Antithyroid drugs (e.g., carbimazole, propylthiouracil) have been used for hyperthyroidism since their introduction in the 1940s. These drugs inhibit multiple steps in the synthesis of T_4 and T_3, leading to a gradual reduction in thyroid hormone levels over 2-8 weeks or longer. Dose titration should be made every 4 weeks until thyroid functions normalize. Some patients with Graves' disease go into a remission after treatment for 12-18 months, and the drug can be discontinued. Notably, half the patients who go into remission have another recurrence of hyperthyroidism within the following year. Nodular forms of hyperthyroidism (toxic multinodular goitre and toxic adenoma) are permanent conditions and will not go into remission. Antithyroid medications inhibit formation and coupling of iodotyrosines in thyroglobulin, which are necessary for thyroid hormone synthesis. A second therapeutic action of propylthiouracil, but not carbimazole, is the inhibition of conversion of T_4 to T_3. A quick reduction in T_3 is theoretically associated with a clinically significant improvement in thyrotoxic symptoms. The choice between propylthiouracil and carbimazole is somewhat arbitrary. Carbimazole is a more potent and longer-acting drug. Often, patient compliance is better with carbimazole taken once or twice daily than with propylthiouracil given 3 or 4 times daily. Propylthiouracil often is the drug of choice in severe thyrotoxicosis because of the additional benefit of inhibition of peripheral T_4 to T_3 conversion.

The most common side-effects of antithyroids are allergic reactions of fever, rash, urticaria, and arthralgia, which occur in 1-5% of patients usually within the first few weeks of treatment. Serious adverse effects include agranulocytosis, aplastic anaemia, hepatitis, polyarthritis, and a lupuslike vasculitis. All of these adverse effects, except agranulocytosis, occur more frequently with propylthiouracil. Agranulocytosis occurs in 0.2-0.5% of patients, with an equal frequency for both drugs. Patients with agranulocytosis usually present with fever and pharyngitis. After the drug is stopped, granulocyte counts usually start to rise within several days but may not normalize for 10-14 days. Granulocyte colony-stimulating factor (G-CSF) appears to accelerate recovery in patients with mild to moderate but not severe agranulocytosis [42]. Interestingly, significantly elevated serum G-CSF levels were observed in patients with Graves' hyperthyroidism and deficiency of G-CSF as a result of antithyroid therapy (methimazole) was not identified as a cause of agranulocytosis in one study[43].

In severe thyrotoxicosis from Graves' disease or subacute thyroiditis, iodine or iodinated contrast agents have been administered to block T_4 conversion to T_3 and the release of thyroid hormone from the gland. This therapy is reserved for severe thyrotoxicosis because its use prevents definitive therapy of Graves' thyrotoxicosis with radioactive iodine for many weeks. Either a saturated solution of potassium iodide or iopanoic acid/ipodate or Lugol's solution can be administered with rapid reduction in T_3 levels. Care should be taken not to administer

these drugs to patients with toxic multinodular goitre or toxic adenomas. The autonomous nature of these conditions can lead to worsening of the thyrotoxicosis in the presence of pharmacological levels of iodide, a substrate for thyroid hormone synthesis.

Radioactive iodine (^{131}I) has been used to treat Graves' disease since the 1940s, and its use has enjoyed increasing popularity because of its efficacy, few side effects and cost effectiveness[44]. Although the effect is less rapid than antithyroid medication or thyroidectomy, it is effective, safe, and does not require hospitalisation. It is administered orally as a single dose, in capsule or liquid form. The radioactive iodine is quickly absorbed and taken up by the thyroid. No other tissue or organ in the body is capable of retaining the radioactive iodine and, therefore, very few adverse effects are associated with this therapy. The treatment results in a thyroid-specific inflammatory response, causing fibrosis and destruction of the thyroid over weeks to many months. Hypothyroidism is an expected and even desirable result of ^{131}I treatment. After radiotherapy, patients are counselled not to have close contact with family members for ~7 days and, because of salivary radioiodine, should not share eating or drinking utensils. Particular care should be exercised if there is a pregnant woman or a child in the household. An increase in cardiovascular and cerebrovascular mortality has been reported in a number of studies[45-47]. However, the findings suggest that it is hyperthyroidism itself which was the major factor determining

adverse outcome. This is probably mediated through influences of cardiac rate, rhythm and function, as well as exacerbation of any underlying valvular, hypertensive or ischaemic heart disease[48]. Although several studies of cancer risk in patients treated with ^{131}I for hyperthyroidism have been reported, results have been conflicting. The evidence does suggest an increased relative risk of thyroid cancer in hyperthyroid patients treated with radioiodine, although the absolute risk remains small, and indeed is likely to reflect, at least in part, association with underlying thyroid disease rather than ^{131}I exposure per se[48]. Follow-up studies have not associated ^{131}I therapy with a higher risk of carcinoma in general, and leukaemia or lymphoma in particular[49].

Thyroidectomy: Total thyroidectomy, subtotal thyroidectomy and combinations of hemithyroidectomies with contralateral subtotal thyroidectomies have been used for the treatment of hyperthyroidism. Of these, total thyroidectomy appears to be the preferred mode of treatment as it guarantees cure, and, although it also guarantees hypothyroidism, thyroxine replacement treatment is far more predictable as the operation is clearly defined, as opposed to subtotal thyroidectomy where the amount of thyroid tissue left behind varies from centre to centre. Also, subtotal thyroidectomy is associated with a high rate of hypothyroidism and remnants of the thyroid gland have potential for recurrence. Total thyroidectomy obviates these disadvantages and can be performed without increased complication rates[50, 51]. Thyroidectomy may be of particular benefit to those who require

normalisation of thyroid functions quickly, such as pregnant women, women who desire pregnancy in the next 6 months, or patients with unstable cardiac conditions; patients with very large goitres or severe ophthalmopathy; patients who refuse radioactive iodine therapy; those with refractory amiodarone-induced hyperthyroidism and children with severe hyperthyroidism. Preoperative preparation includes antithyroid medication, stable (cold) iodine treatment (to decrease gland vascularity), and beta-blocker therapy. Adverse effects of therapy include recurrent laryngeal nerve damage and hypoparathyroidism due to damage of local structures during surgery. No significant difference in the complication rate exists between subtotal and total thyroidectomy[52].

1.8.6.Complications:

Graves' Ophthalmopathy: Graves' ophthalmopathy is more common in women than in men. Although 50% of patients with Graves' disease have clinical evidence of thyroid eye disease, only 5% develop severe ophthalmopathy, e.g., diplopia, visual-field deficits, blurred vision, tearing, and photophobia. Management of Graves' ophthalmopathy is preferably done in a multidisciplinary setting. Smoking is associated with worse disease outcome. Arguably, ^{131}I therapy for hyperthyroidism can also worsen ophthalmopathy, especially if administered during active disease or to patients who smoke or have severe hyperthyroidism, or those with high levels of TSH-receptor-binding inhibitory immunoglobulins.

Coadministration of steroids and ^{131}I therapy is recommended for such high-risk patients. ^{131}I therapy is safe for patients with inactive Graves' ophthalmopathy. Subtotal thyroidectomy and antithyroid drugs show no benefit or harm to eye changes. There is no good evidence that total thyroid ablation has additional benefit. Artificial teardrops, dark glasses and prisms are very helpful. Dysthyroid optic neuropathy is best treated with intravenous pulsed methylprednisolone; if visual functions do not recover, urgent surgical decompression is indicated. A wait-and-see policy is recommended in mild Graves' ophthalmopathy because the natural history of this condition reveals a tendency to resolve spontaneously. Active, moderately severe Graves' ophthalmopathy qualifies for immunosuppression; intravenous pulsed methylprednisolone is more efficacious and has fewer side effects than oral steroids. Once the disease is inactive, rehabilitative surgery has much to offer. Quality of life is seriously limited in patients with Graves' ophthalmopathy, and remains restricted even after all treatments. Consequently, there is an urgent need for improved treatment modalities, and antibody therapy has shown promise in this respect[53].

Graves' Dermopathy: This is an infiltrative dermopathy, usually over the lower extremities, that is characterized by an accumulation of glycosaminoglycans and inflammatory cells in the dermis. The skin changes usually include a nonpitting erythematous oedema of the anterior shins. Dermopathy and acropachy appear to be markers of severe ophthalmopathy. Occasionally, however, Graves'

dermopathy occurs without clinical ophthalmopathy[54]. No effective treatment exists. Nightly occlusive wraps of the affected site are recommended with plastic wrap after application of a high-potency topical steroid cream.

Osteoporosis: Osteoporosis is characterised by low bone mass, deterioration of bone microarchitecture, increased bone fragility, and susceptibility to fracture. Thyrotoxicosis is an important risk factor for osteoporosis. Thyroid hormones accelerate the rate of bone remodelling, leading to a negative calcium balance and a net bone loss that accelerates the development of osteoporosis, and hence increases bone vulnerability to trauma[47, 55, 56]. This action may be mediated by a nuclear T_3 receptor which has been found in rat and human osteoblast cell lines and in osteoclasts derived from an osteoclastoma[57]. Experimental studies in mice lacking either the thyroid hormone receptor α or β (TRα or TRβ), suggest bone loss is mediated by TRα [58]. Thus, thyroid hormone may affect bone calcium metabolism either by a direct action on osteoclasts, or by acting on osteoblasts which in turn mediate osteoclastic bone resorption[59]. TSH may also have a direct effect on bone formation and bone resorption, mediated via the TSH receptor on osteoblast and osteoclast precursors[60], however bone loss appeared independent of TSH levels in the experiments with mice lacking specific TR isoforms[58]. Post-menopausal oestrogen-deficient women are the ones at particular risk of potential adverse effects of hyperthyroidism upon bone metabolism[61]. Even with effective antithyroid therapy a

complete restoration of bone mineral density (BMD) to pre-morbid levels does not always occur[62].

1.9.Hypothyroidism:

Hypothyroidism is a common endocrine disorder resulting from deficiency of thyroid hormone. It is often the primary process in which the thyroid gland produces insufficient amounts of thyroid hormone. It can also be secondary, i.e., lack of thyroid hormone secretion due to the failure of either adequate thyrotropin (TSH) secretion from the pituitary gland or thyrotropin-releasing hormone (TRH) from the hypothalamus (secondary or tertiary hypothyroidism). The patient's presentation may vary from asymptomatic to, rarely, coma with multisystem organ failure (myxoedema coma). The Whickham survey specifically sought to determine the incidence of thyroid disease in the general population[63]. Hypothyroidism, diagnosed by history and blood analysis, was found in more than 2% of 2800 persons tested. The mean age of diagnosis was 57 years, and the disease was ten-fold more common in women than in men. The disease is particularly prevalent in women older than 40 years of age. Hypothyroidism is prevalent in debilitated geriatric patients of both sexes[64].

1.9.1.Causes of hypothyroidism: Worldwide, iodine deficiency remains the foremost cause of hypothyroidism. In the areas of

adequate iodine intake, autoimmune thyroid disease (Hashimoto's thyroiditis) seems to be the most common cause.

Hashimoto's thyroiditis is part of the spectrum of autoimmune thyroid diseases (cf. 1.12). By strict criteria, it is a histological diagnosis first described by Hakaru Hashimoto, a Japanese surgeon working in Berlin, Germany. In this disease, there is continual replacement of normal thyroid tissue with lymphocytic and fibrous tissue – hence the term chronic lymphocytic thyroiditis – until insufficient thyroid tissue remains to maintain normal hormone production.

Excess iodine, as in radiocontrast dyes, amiodarone, health tonics, and seaweed, can also cause hypothyroidism through inhibition of iodide organification and thyroid hormone synthesis. Most healthy individuals have a physiologic escape from this effect; however those with abnormal thyroid glands may not. These include patients with autoimmune thyroiditis, surgically treated Graves' hyperthyroidism (subtotal thyroidectomy) and prior radioiodine therapy[65].

Medications such as amiodarone, interferon alpha, thalidomide[66], lithium, and stavudine have also been associated with primary hypothyroidism.

Causes of hypothyroidism are summarised in table 1.9.1.

Table 1.9.1 Causes of hypothyroidism

DESTRUCTIVE
Postoperative
Radioactive iodine
External radiation to neck
Infiltrative disease (e.g., sarcoidosis, amyloidosis, lymphoma, metastatic carcinoma)
AUTOIMMUNE
Hashimoto's disease
After Graves' disease
THYROIDITIS
Subacute (e.g., viral)
Silent
Postpartum
DRUG-INDUCED
Iodides
Lithium
Thionamides
HEREDITARY OR CONGENITAL
Enzyme deficiency affecting thyroid hormone biosynthesis
Agenesis
Hormone resistance
Endemic cretinism
HYPOTHALAMIC-PITUITARY DISORDERS
Thyrotropin-releasing hormone deficiency
Thyroid-simulating hormone deficiency
IDIOPATHIC
No cause determined

1.9.2.Clinical manifestations of hypothyroidism: The clinical spectrum of thyroid hormone deficiency ranges from the asymptomatic person without abnormal physical findings to the classic myxedoematous patient in whom the diagnosis can readily be made on physical examination. Physical findings are most striking in young, otherwise healthy patients. Old age or

nonthyroidal disease can commonly produce symptoms or physical findings that suggest thyroid disease and require thyroid function tests to aid in a diagnosis.

Thyroid hormone deficiency generally causes decreased oxygen consumption and defects specific to individual organs. The common symptoms of thyroid hormone deficiency include lethargy and decreased physical ability. The decrease in tissue calorigenesis results in cold intolerance. Clinical findings in hypothyroidism are summarised in table 1.9.2.

Table 1.9.2 Clinical findings in hypothyroidism

Affected Areas	Symptoms	Signs
GENERAL	Cold intolerance Fatigue Mild weight gain	Hypothermia
NERVOUS STSTEM	Lethargy Memory defects Poor attention span Personality change	Somnolence Slow speech Myxoedema wit Psychopathology: myxoedema madness Diminished hearing and taste Cerebellar ataxia
NEUROMUSCULAR SYSTEM	Weakness Muscle cramps Joint pain	Delayed relaxation of deep tendon reflexes
GASTROINTESTINAL SYSTEM	Nausea Constipation	Large tongue Ascites
CARDIORESPIRATORY SYSTEM	Decreased exercise tolerance	Hoarse voice Bradycardia Mild hypertension Pericardial effusion Pleural effusion
PREPRODUCTIVE SYSTEM	Decreased libido Decreased fertility Menstrual disorders	
SKIN AND APPENDAGES	Dry, rough skin Puffy facies Hair loss Brittle nails	Nonpitting oedema of hands, face, and ankles Periorbital swelling Pallor Yellowish skin (i.e., carotenemia) Coarse hair Dry axillae

1.9.3.Laboratory measurements: As with hyperthyroidism, measurement of the serum thyrotropin (TSH) concentration is the most sensitive single test for the diagnosis of hypothyroidism. A number of confounding factors exist when it comes to

determining the normal range of TSH, especially the upper normal limit. In practice the TSH distribution has been found to be right skewed. This deviation from Gaussian distribution is thought to be due to inclusion of individuals with underlying factors that confound the results. Principal among these is occult autoimmune thyroid disease (e.g. Hashimoto's thyroiditis), and the population of individuals at the upper end of the reference range that has a high prevalence of antithyroperoxidase antibodies[67]. Therefore, reference limits have been determined by log transforming the TSH values, calculating the mean ± 2 SD values, and then exponentiating to obtain the reference limits on the original scale[68]. When individuals with thyroid autoantibodies, goitre, or a strong family history of thyroid disease are excluded, as proposed by The National Academy of Clinical Biochemistry (NACB), the upper bound of the 95% TSH concentration reference range decreases to between 2.5 and 3.0 mU/L (from the traditional 4.5-5 mU/L)[11, 68]. It has been argued that such a 'refined normal range' is a better reflection of thyroid health than a standard population-based reference range and that elevated values may predict future hypothyroidism[11, 63, 68-70]. A recent study aimed at establishing new reference intervals for TSH and thyroid hormones based on NACB criteria and regular thyroid ultrasonography concluded that the TSH reference range should be narrowed to a value of approximately 4.0 mU/L as the upper-reference limit[71]. This decreased upper bound would increase the sensitivity of the diagnosis of subtle hypothyroidism while

decreasing its specificity. Fatourechi et al showed that more than 4 times as many patients (20.0% vs. 4.64%) would be classified as having elevated TSH concentrations if 3.0 mU/L rather than 5.0 mU/L were used as the upper limit of normal[72]. The percentages were slightly higher in female patients than in male patients, and in both sexes the percentages increased with age. When 3.0 mU/L was used as the upper limit of normal for evaluating patients without clinically evident thyroid disease, approximately 15% of patients younger than 50 years, 17% to 23% of patients aged 50 to 70 years, and 25% of men and 29% of women older than 70 years were classified as having abnormal TSH concentrations. These percentages were 3.8 to 5.0 times higher than the percentages of patients classified as having abnormal TSH concentrations when the traditional limit of 5.0 mU/L is used[72].

It is essential that physicians consider many other patient-specific factors, including individual patient symptoms, repeat measurement of TSH to confirm it is elevated and does not represent transient elevation or recovery from subacute thyroiditis, and consideration of whether the person has seronegative autoimmune thyroiditis. An additional clinical factor is recognition of the well-established individual reference range for TSH – due to its biovariability – which is typically narrower than the population reference range[73]. Accordingly, the time of phlebotomy is also important, because the TSH level varies throughout the day, with early morning values greater than later ones, and is accentuated by

sleep deprivation, strenuous exercise, or working during the night or evening shifts[74]. The mean TSH for a given patient may be in either the lower or upper TSH reference range. For example, a patient with a confirmed TSH of 3.6 mU/L may have early autoimmune thyroiditis with subclinical hypothyroidism and may benefit from T_4 therapy. However, if the history, physical examination, thyroid ultrasound, and thyroid antibody testing are all normal, it is more likely that this TSH value is normal for that individual, and treatment with T_4 would be inappropriate.

The sensitivity over setting the upper normal limit of TSH is most relevant when it comes to treating mild thyroid failure or the so-called subclinical hypothyroidism (SCH). SCH and its management have been discussed in more detail under 1.14.2.4. The results of the recently published HUNT study are particularly relevant to the debate over the upper normal limit of TSH[75]. In this Norwegian population-based cohort study, 17311 women and 8002 men without known thyroid or cardiovascular disease or diabetes mellitus were prospectively studied over a median follow-up of 8.3 years for the association between thyrotropin levels and fatal CHD. The results showed that compared with women in the lower part of the reference range (thyrotropin level, 0.50-1.4 mU/L), the hazard ratios for coronary death were 1.41 and 1.69 for women in the intermediate (thyrotropin level, 1.5-2.4 mU/L) and higher (thyrotropin level, 2.5-3.5 mU/L) categories respectively; the trend was not statistically significant in men[75].

If TSH levels are above the reference range, the next step would be to measure T_4. As thyroxine is highly protein bound (>99%), and the levels of these binding proteins could vary by hormonal status, inheritance, and in various disease states, free T_4 assays are becoming popular. As the TSH level increases early in the disease, an increased conversion of T_4 to T_3 occurs; this maintains T_3 levels. In early hypothyroidism, TSH levels are increased, T_4 levels are normal to low, and T_3 levels are normal.

In patients with hypothalamic or pituitary dysfunction, TSH levels do not increase in appropriate relation to the low free T_4 levels. The absolute levels may be in the normal or even slightly elevated range but inappropriately low for the severity of the hypothyroid state. Hence, when secondary or tertiary hypothyroidism is suspected, a serum TSH measurement alone is inadequate; a free T_4 should be measured.

1.9.4.Imaging studies: Ultrasound of the neck and thyroid can be used to detect nodules and infiltrative disease. It has little use in hypothyroidism per se unless a secondary anatomic lesion in the gland is of clinical concern. Hashimoto's thyroiditis is usually associated with a heterogeneous image by ultrasound. It can be rarely associated with lymphoma of the thyroid. Serial images with fine-needle aspiration of suspicious nodules may be useful.

Radioactive iodine uptake (RAIU) and thyroid scanning are not useful in hypothyroidism because these tests require some level of

endogenous function in the hypofunctioning gland to provide information. Patients with Hashimoto's thyroiditis may have relatively high early uptake (after 4 h) but do not have the usual doubling of uptake at 24 hours consistent with an organification defect.

1.9.5.Treatment: The treatment goals for hypothyroidism are the reversal of clinical progression and the corrections of metabolic derangements. Thyroid hormone is administered to supplement or replace endogenous production. In general, hypothyroidism can be adequately treated with a constant daily dose of levothyroxine. Clinical benefits begin in 3-5 days and level off after 4-6 weeks. Anticipated full replacement doses may be initiated in individuals who are otherwise young and healthy. In elderly patients or those with known ischaemic heart disease, treatment should begin with one fourth to one half the expected dose, and the dose should be adjusted in small increments no sooner than 4-6 weeks[76, 77].

The correct dose is that which restores the euthyroid state and relieves symptoms. In most patients these will be achieved by a dose of thyroxine resulting in a normal or slightly raised serum thyroxine concentration, a normal serum triiodothyronine concentration, and a normal or below normal serum thyroid stimulating hormone concentration[76]. Controversy exists concerning whether doses of thyroxine which suppress serum thyroid stimulating hormone concentration to below normal, i.e. iatrogenic subclinical hyperthyroidism, are associated with an

increased risk of osteoporosis. However, more recent studies have not consistently confirmed this hypothesis except in postmenopausal women[61]. Achieving a TSH level within the reference range may be slowed because of delay of hypothalamic-pituitary axis readaptation and may take several months. After dose stabilisation, patients can be monitored with annual clinical evaluations and TSH monitoring. Patients should be monitored for symptoms and signs of over treatment, which include tachycardia, palpitations, nervousness, tiredness, headache, increased excitability, sleeplessness, tremors, and possible angina.

A recent metaanalysis of randomized controlled trials of thyroxine-triiodothyronine combination therapy (T_4 + T_3) versus thyroxine monotherapy (T_4) for treatment of clinical hypothyroidism found no difference in the effectiveness of the combination vs. monotherapy in bodily pain, depression, fatigue, body weight, anxiety, quality of life, total cholesterol, LDL-C, HDL-C and triglyceride levels. Hence, T_4 monotherapy remains the treatment of choice[78].

Hypothyroidism in pregnancy is associated with preeclampsia, anaemia, postpartum haemorrhage, cardiac ventricular dysfunction, spontaneous abortion, low birth weight, impaired cognitive development, and foetal mortality. Even mild disease may be associated with adverse affects for offspring. Increased dosage requirements should be anticipated during pregnancy, especially in the first and second trimesters. Recent studies have

suggested that patients with hypothyroidism should augment the thyroxine dose by 30% at the confirmation of pregnancy, followed by adjustments according to TSH levels. For previously diagnosed women serum TSH should be measured every 3-4 weeks during the first half of pregnancy and every 6 weeks thereafter. thyroxine dose should be adjusted to maintain a serum TSH less than 2.5 mU/L. TSH and free T_4 levels should be measured every 3-4 weeks after every dose adjustment[79].

Surgery is indicated for large goitres that compromise tracheoesophageal function; surgery is rarely needed in patients with hypothyroidism.

1.9.6.Complications:

Coma, as an end stage of myxoedema, was described by Ord in 1880. An effective approach is to use intravenous thyroxine at a dose of 4 mcg/kg of lean body weight, or approximately 200-250 mcg as a bolus in a single or divided dose, depending on the patient's risk of cardiac disease followed by 100 mcg 24 hours later and then 50 mcg daily IV or PO along with stress doses of intravenous glucocorticoids. Adjustment of the dose can then be made based on clinical and laboratory along with stress doses of intravenous glucocorticoids. Use of intravenous triiodothyronine is controversial and based on expert opinion. It has a higher frequency of adverse cardiac events and is generally reserved for patients who are not improving clinically on T_4. T_3 can be given initially as a 10 mcg IV bolus and repeated every 8-12 hours until

the patient can take maintenance oral doses of T_4. Advanced age, high dose T_4 therapy, and cardiac complications have the highest associations with mortality[80].

Thyroid hormone replacement can precipitate adrenal crises in patients with untreated adrenal insufficiency. If suspected, the presence of adrenal insufficiency should be confirmed or ruled out and should be treated prior to treatment of hypothyroidism.

Patients should be advised that vision may temporarily worsen when starting hormone therapy. Rarely, pseudotumour cerebri occurs[81].

1.10. Subclinical thyroid disease:

Subclinical thyroid disease is defined as an abnormal serum TSH level and free thyroxine and triiodothyronine levels within their reference ranges. The symptoms, signs, and laboratory abnormalities associated with overt hyperthyroidism and hypothyroidism as reviewed in previous sections are well known to clinicians. Both conditions are unequivocally associated with morbidity and, occasionally, mortality, and no one would dispute the importance of timely diagnosis and therapy. In contrast, subclinical hyperthyroidism and hypothyroidism have subtle clinical manifestations at most, and the importance of timely diagnosis and treatment continue to be contentious subjects of studies, position papers, and editorials[82, 83]. The central issues are whether subclinical thyroid diseases are of sufficient clinical

importance to warrant screening and whether, once these conditions are detected by an abnormal serum thyrotropin value and confirmed by further testing, therapy is justified. Because reliable TSH assays have been available for more than 20 years, one would think that adequate data would have been amassed by now to answer these questions definitively. However, this is not the case, and the debate continues. Subclinical hyperthyroidism and subclinical hypothyroidism have been discussed under 1.14.1.3 and 1.14.2.4., especially with regard to their effects on the cardiovascular system.

1.11.Thyroiditis:

1.11.1. Subacute thyroiditis: Subacute thyroiditis is a self-limiting thyroid condition associated with a triphasic clinical course of hyperthyroidism, hypothyroidism, and return to normal thyroid function. Subacute thyroiditis may be responsible for 15-20% of patients presenting with thyrotoxicosis and 10% of patients presenting with hypothyroidism. Recognizing this condition is important because it is self-limiting, and no specific therapy, such as antithyroid or thyroid hormone replacement therapy, is necessary in most patients. The high thyroid hormone levels are a result of destruction of the thyroid follicle and release of preformed thyroid hormone into the circulation. Eventually, thyroid hormone is depleted and the patient may become hypothyroid. Often the hypothyroidism is mild, and no thyroid

hormone therapy is required unless the patient has signs or symptoms of hypothyroidism. The hypothyroid phase may last up to 2 months. Ninety to 95% of patients return to normal thyroid function.

The 3 types of subacute thyroiditis are subacute granulomatous, also referred to as painful or de Quervain's thyroiditis; subacute painless thyroiditis, which is silent and also referred to as lymphocytic or silent thyroiditis; and postpartum thyroiditis. The aetiology of each of these conditions is different, but all of the conditions follow the same clinical course, including 6-8 weeks of thyrotoxicosis, 2-4 months of mild hypothyroidism, and, finally, a return to the euthyroid state in 90-95% or more of the patients. A patient may experience one or more of these phases.

The most accepted aetiology of de Quervain's thyroiditis is a viral illness. A genetic predisposition clearly exists and patients with HLA-B35 have a significantly increased risk of developing this condition[84]. A weak association with HLA-DRw8 has also been reported[85].

Silent thyroiditis is associated with an excess of HLA-DR3[86]. This association links silent thyroiditis to autoimmune thyroiditis rather than to de Quervain's thyroiditis[87]. Silent thyroiditis has also been reported in conjunction with amiodarone[88] and lithium, and with the use of interferon-α in the treatment of hepatitis C patients[89, 90].

Like silent thyroiditis, postpartum thyroiditis is associated with HLA-DR3, but an association with DR5 is also observed[86]. This postpartum entity occurs in 5% to 9% of all pregnancies[91]. These observations are of particular interest for the probable autoimmune nature of this and the classic form of silent thyroiditis, as it is well established that many autoimmune diseases flare up during the postpartum period after resolution of the immune suppression normally seen to occur during pregnancy. Higher ratios of helper (CD4+) to suppressor (CD8+) T cells seen in the postpregnancy state may play a critical role in the development of this postpartum dysfunction[91]. Patients with type 1 diabetes mellitus are especially prone to the development of postpartum thyroiditis; as many as 25% experience this disorder[92].

1.11.2. Riedel's thyroiditis: Riedel's thyroiditis is an unusual and rare form of thyroid disease, which is characterized by extensive fibrosis of the thyroid gland. In these patients, the gland is hard, woody in consistency, and often fixed to the adjacent tissues. Occasionally, the gland may be asymmetric, presenting as a unilateral mass. In many patients with Riedel's thyroiditis, local symptoms, including hoarseness (recurrent laryngeal nerve involvement) or dysphagia, are present. The aetiology is unclear, but it may represent a variant of Hashimoto's thyroiditis, as evidenced by the mononuclear infiltrate, the detection of thyroid-specific autoantibodies in many patients, and the response to glucocorticoid treatment[93]. Surgical treatment may be required

if there are significant local symptoms or evidence of tracheal compression. Corticosteroids occasionally have been helpful in reducing the goitre size and alleviating local symptoms[94].

1.12. Amiodarone and thyroid dysfunction: Amiodarone is a potent antiarrhythmic drug that is used to treat ventricular and supraventricular tachyarrhythmias. It is an iodine-rich compound with some structural similarity to thyroxine. Amiodarone contains approximately 37% iodine by weight. Each 200 mg tablet is estimated to contain about 75 mg of organic iodide, 8-17% of which is released as free iodide. Standard maintenance therapy with 200 mg amiodarone can provide more than 100 times the daily iodine requirement. It is highly lipid-soluble and is concentrated in the adipose tissue, muscle, liver, lung, and thyroid gland. Total body iodine stores remain increased for up to 9 months after discontinuation of the drug.

Amiodarone effects on thyroid function are mainly related to the inhibition of 5'-deiodinase activity resulting in a decrease in the generation of T_3 from T_4 with a consequent increase in rT_3 production and a decrease in its clearance[95].

Table 1.12.1 Effects of amiodarone on the thyroid gland

Intrinsic drug effect	Iodine-induced effects
Blockade of thyroid hormone entry into cells	Failure to escape from Wolff-Chaikoff effect
Inhibition of type I and type II 5'-deiodinase	Iodine-induced potentiation of thyroid autoimmunity
Decreased T_3 binding to its receptor	Unregulated hormone synthesis (Jod-Basedow effect)
Thyroid cytotoxicity	

Effects of amiodarone on thyroid function tests in euthyroid subjects are summarised in table 1.12.2.

Table 1.12.2. Effects of amiodarone on thyroid function tests in euthyroid subjects

Thyroid Hormone	**Acute effects (up to 3 months)**	**Chronic effects (> 3 months)**
Total and free T_4 range	↑50%	Remains ↑ 20-40% of baseline
Total and free T_3	↓ 15-20%, remains in low-normal range	Remains ↓ 20%, remains in low-normal
rT_3	↑ > 200%	Remains ↑ > 150%
TSH	↑ 20-50%, transient, generally remains < 20 mU/L	Normal

Although most patients remain clinically euthyroid, a significant minority (up to 15% of patients in the UK and the USA) develop amiodarone induced hypothyroidism or thyrotoxicosis[96].

1.12.1. Amiodarone induced thyrotoxicosis (AIT): Less common than amiodarone induced hypothyroidism in iodine replete areas such as the UK and USA (< 2% v 13%), the pathogenesis of amiodarone induced thyrotoxicosis is more complex and the diagnosis and treatment much more difficult. AIT occurs more frequently in men and in iodine deficient areas, but there is no relation between the daily or cumulative dose of amiodarone and the incidence of AIT. The onset of AIT is often acute and may occur several months after the discontinuation of treatment. Spontaneous remissions are common[96].

There are two main forms of AIT (table 1.12.1) that have different aetiologies. Type 1 AIT occurs in patients with underlying thyroid pathology, such as latent Graves' disease or nodular goitre. This iodide induced thyrotoxicosis, an example of the Jod-Basedow phenomenon, is identical to that seen in patients with endemic iodine deficient goitre who are then given iodide replacement, thus explaining why thyrotoxicosis is more common in iodine depleted areas. In contrast, type 2 AIT occurs in an apparently normal thyroid, and results from a direct toxic effect of amiodarone causing a subacute destructive thyroiditis with consequent leakage of preformed thyroid hormones into the circulation[96].

Table 1.12.1. Pathogenesis and clinical features of amiodarone induced thyrotoxicosis

	Type 1	**Type 2**
Underlying thyroid abnormality	Yes	No
Pathogenetic mechanism	Excessive hormone synthesis due to iodine excess	Excessive release of preformed hormones due to thyroid destruction
Goitre	Multinodular or diffuse goitre normally present	Occasionally small, diffuse, firm, sometimes tender
Thyroidal radioiodine uptake	Normal/raised	Low/absent
Serum IL-6	Normal/slightly raised	Profoundly raised
Thyroid ultrasound	Nodular, hypoechoic, increased volume	Normal

In AIT the drug should be stopped, if possible, though this is often impractical because of the underlying cardiac disorder. Discontinuation of amiodarone will not have an acute effect

because of its storage and prolonged half-life. High doses of antithyroid drugs can be used in type 1 AIT but are often ineffective. Potassium perchlorate, 200 mg every 6 h, has been used to reduce thyroidal iodide content. Perchlorate treatment has been associated with agranulocytosis, though the risk appears relatively low with short-term use. Glucocorticoids, administered as for subacute thyroiditis, are beneficial in type 2 AIT. Lithium blocks thyroid hormone release and can provide modest benefit. Thyroidectomy rapidly decreases thyroid hormone levels and may be the most effective long-term solution, if the patient can undergo the procedure safely[5]. In patients with a history of AIT in whom amiodarone becomes necessary after it has been discontinued, ablation of the thyroid with radioiodine before resuming amiodarone should be strongly considered[97].

1.12.2. Amiodarone induced hypothyroidism (AIH): Amiodarone-induced hypothyroidism (AIH) occurs more frequently than AIT in iodine-replete areas. The risk of developing AIH is independent of the daily or cumulative dose of amiodarone, but is enhanced in elderly and female patients with a M:F ratio of 1:1.551[95, 96]. This is to be expected as autoimmune thyroid disease is the principal risk factor for the development of AIH and is particularly common in these patient groups[96].

The most likely explanation for the development of AIH is an inability of the thyroid to escape from the acute inhibitory effects of iodine on hormone release and synthesis. This may reflect

underlying thyroid disease, as AIH is also a well recognised outcome in patients with subclinical autoimmune thyroiditis given excess iodine and generally occurs relatively early (3 to 12 months) after starting treatment with amiodarone. It is also possible that excess iodine may exacerbate preexisting autoimmune thyroiditis directly in some patients, particularly those from areas of low dietary iodine intake[96].

The diagnosis of hypothyroidism is usually straightforward. The clinical features are not affected by amiodarone, although most patients have very few symptoms, most commonly increased lethargy. The diagnosis is confirmed by finding a raised TSH concentration, usually above 20 mU/L, in combination with low T_4 or fT_4. Low T_3 or FT_3 concentrations are unreliable indicators of AIH as they may occur in euthyroid patients during amiodarone treatment. A goitre is found in about 20% of patients with AIH in iodine replete areas, but most of these goitres predate the start of amiodarone treatment[96].

Many patients without preexisting thyroid disease will become euthyroid within two to four months of stopping amiodarone, but permanent hypothyroidism requiring T_4 replacement is common in patients with thyroid antibodies[98]. By far the safest, quickest, and most reliable treatment for hypothyroidism is to continue amiodarone and to add T_4, increasing the dose at monthly intervals until the TSH concentration is well within the normal

range and symptoms attributable to hypothyroidism have resolved[96].

1.13. Nonthyroidal illness

The frequency with which thyroid disease is encountered in the general population, the ease with which it is excluded in otherwise healthy persons, and the poor specificity of the individual signs and symptoms of thyroid dysfunction all have contributed to the practice of screening for thyroid disease in ill patients, in the search for a readily reversible component to the presenting illness. The result is the recognition of multiple abnormalities in *parameters* of thyroid function in these patients. Based on the conviction that patients with these abnormalities are not hypothyroid despite the low hormone levels in blood, the condition has been called the *euthyroid sick syndrome*[99]. An alternative designation, which does not presume the metabolic status of the patient, is nonthyroidal illness (NTI)[100]. Although early cross-sectional studies in such patients had revealed a baffling collection of low, normal, or high values for total and free thyroid hormones, subsequent work has suggested a continuum of change, within which a given patient's status is determined by the severity and duration of illness as well as the presence of mitigating influences that are associated with the specific underlying disorder. Hence, an early and dramatic reduction in serum total and free triiodothyronine levels occurs, followed, in cases of sufficient severity, by a depression in serum total thyroxine and variable changes in serum free thyroxine levels.

Concurrent with decrements in levels of circulating thyroid hormones, a seemingly inept response is seen at the hypothalamic-pituitary level; this persists until the recovery phase of the underlying illness, at which time a rise in TSH to even supranormal levels may be seen that coincides with a rapid recovery in T_4 and T_3 levels[101]. This decrease in serum thyroid hormone levels is seen in starvation, sepsis, surgery, myocardial infarction, cardiopulmonary bypass, poorly controlled diabetes mellitus, bone marrow transplantation, and probably any severe illness[100].

Measurement of serum TSH, free T_4, and free T_3 levels by direct equilibrium dialysis/RIA methods probably yield most accurate information in the setting of NTI. Patients with low free T_4 by these methods and normal or low TSH have secondary hypothyroidism. This may be due to NTI per se, drugs administered for treatment of NTI, or associated pituitary or hypothalamic disease; the latter consideration may require evaluation of cortisol reserve, prolactin, and/or gonadotropins. A serum TSH level above 20-25 mU/L probably reflects primary hypothyroidism; accompanying findings of goitre, low free T_4, and positive antithyroid antibodies help establish the diagnosis. An elevated serum concentration of reverse T_3 argues against hypothyroidism[102].

The following mechanisms have been proposed to explain the abnormalities in thyroid hormone levels during a NTI[103]:

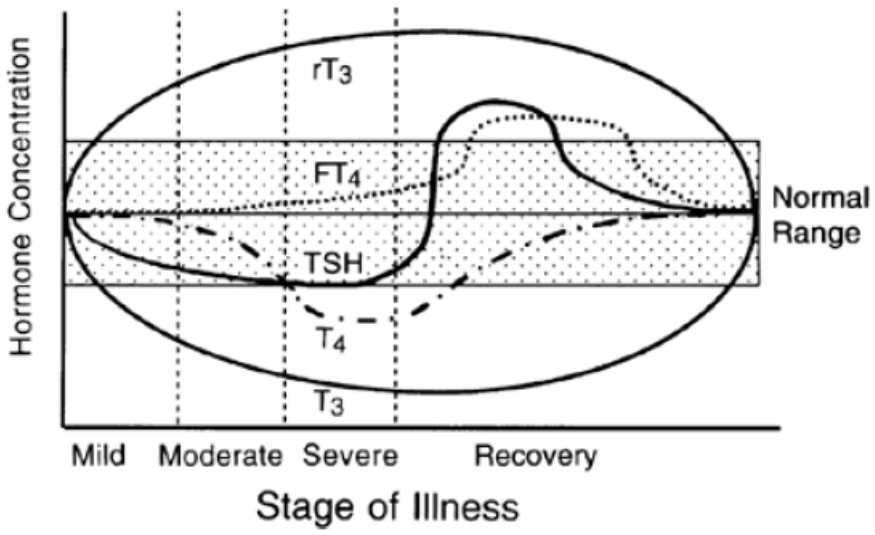

Figure1.6: Influence of the stage and the severity of nonthyroidal illness on thyroid function parameters (adapted from Becker's Principles and Practice of Endocrinology and Metabolism, 3rd edition, 2002).

- Accuracy of test assays in nonthyroidal illness: Abnormalities of thyroid function test results might represent test artifacts or true abnormalities
- Inhibition of thyroid hormone binding to thyroid-binding proteins and tissues, thus preventing tests from appropriately reflecting free hormone levels. This inhibitor is associated with the nonesterified fatty acid (NEFA) fraction in the serum. Contrary to this proposition, substantial evidence indicates that, in an in vivo state, the levels of binding inhibitors do not reach levels sufficient to influence the circulating levels of free T_4, even in patients who are severely ill. Also, some studies have failed to demonstrate an existing binding inhibitor.

- Cytokines are thought to play a role in NTI—particularly IL-1, IL-6, TNFα, and interferon-β. Cytokines are thought to affect the hypothalamus, the pituitary, or other tissues, inhibiting production of TSH, TRH, thyroglobulin, T_3, and thyroid-binding globulins. Cytokines are also thought to decrease the activity of type I deiodinase and to decrease the binding capacity of T_3 nuclear receptors.
- Deiodination: Peripheral deiodination of T_4 to T_3 is impaired, largely secondary to decreased activity of type I deiodinase enzyme, which deiodinates T_4 to T_3.
- Inhibition of TRH and TSH secretion: Cytokines, cortisol, and leptin, as well as changes in brain thyroid hormone metabolism, affect inhibition and secretion of TRH and TSH.
- Serum factors, such as bilirubin, NEFA, furanoic acid, hippuric acid, and indoxyl sulphate, which are present in various NTIs, have been shown to inhibit transport of thyroid hormones.
- Thyroxine-binding globulin decrease and desialation: Diminished T_4 in NTI has been proposed to be due to low TBG caused by protease cleavage at inflammatory sites in acute inflammatory conditions. One other hypothesis for the cause of disproportionately low serum T_4 concentrations in patients with NTI is the presence of abnormal serum binding due to desialation of TBG[103].

Given the clinical context and pattern of laboratory values, the diagnosis of NTI is frequently presumptive. Only resolution of the test results with clinical recovery can clearly establish this disorder.

Treatment of NTI with thyroid hormone (T_4 and/or T_3) is controversial, but most authorities recommend monitoring the patient's thyroid function tests during recovery, without administering thyroid hormone, unless there is historic or clinical evidence suggestive of hypothyroidism. There is no prospective study to date demonstrating benefit or harm of thyroid hormone replacement in NTI. Dr De-Groot has supported the notion that nonthyroidal illness syndrome is a manifestation of hypothalamic-pituitary dysfunction, and in view of current evidence, he proposed that treatment should be considered with appropriate replacement therapies such as pituitary hormones and hypothalamic factors in addition to thyroid hormones[104]. De-Groot also points out that if effective, thyroid hormone replacement will be one of many beneficial treatments given to the patient, rather than a single magic bullet, which would reverse all the metabolic changes going wrong in these severely ill patients[104].

1.14. Thyroid and the cardiovascular system:

Thyroid dysfunction syndromes (hyper and hypothyroidism) are known to affect the cardiovascular system in a number of ways[105].

Whilst the acute effects of thyroid dysfunction on the cardiovascular system are more readily detectable – especially with hyperthyroidism – the evidence on the long term effects of thyroid dysfunction on the heart and on the cardiovascular outcomes is less clear. This is particularly true of the mild or subclinical forms of hypo and hyperthyroidism. For example, a 20-yr follow-up study of the original Whickham Survey[63] found no association between initial hypothyroidism, raised serum TSH levels, or antithyroid antibodies and the development of coronary artery disease. However, the more recent Rotterdam Study[106] concluded that patients with subclinical hypothyroidism have a significantly increased prevalence of aortic atherosclerosis and myocardial infarctions. The common finding between the Whickham Survey and Rotterdam study was that thyroid autoimmunity itself was not associated with myocardial infarction or aortic atherosclerosis.

The following sections of this chapter look at our current knowledge of both short and long term effects of thyroid dysfunction – including the subclinical forms – on the cardiovascular system. Although it has been asserted by various authors that 'subclinical' is a misnomer and 'mild thyroid

dysfunction' is a more appropriate term than 'subclinical' [82, 107, 108], this thesis uses the term 'subclinical' in line with the current trend in the literature. However, it is acknowledged that there is compelling evidence – as detailed later in this chapter – that these mild forms of thyroid dysfunction have significant clinical effects and it may be more appropriate to use 'mild hyperthyroidism' and 'mild hypothyroidism' instead of 'subclinical hyperthyroidism' and 'subclinical hypothyroidism' respectively, in future.

1.14.1. Hyperthyroidism and the cardiovascular system:

1.14.1.1. Overt hyperthyroidism:

Excess thyroid hormone causes tachycardia, palpitations, with some degree of exercise impairment and a widened pulse pressure. These changes occur independently of the cause of the hyperthyroidism[109-111]. The changes in heart rate result from both an increase in sympathetic tone and a decrease in parasympathetic tone[112-114]. Tachycardia, with heart rates greater than 90 beats per minute, is common at rest and during sleep; in addition, the normal increase in heart rate during exercise is exaggerated[114]. In a study of 880 patients of widely varying ages, resting tachycardia was second only to goitre as the most common sign of hyperthyroidism[115].

In patients with hyperthyroidism, cardiac output is 50 to 300 percent higher than in normal subjects. The increase is due to the combined effects of a decrease in systemic vascular resistance, an

increase in resting heart rate, increases in left ventricular contractility and ejection fraction, and an increase in blood volume [105]. The importance of the contribution of decreased systemic vascular resistance to the increase in systemic blood flow in patients with hyperthyroidism is evidenced by the results of studies in which the administration of arterial vasoconstrictors, atropine and phenylephrine, decreased peripheral blood flow and cardiac output by 34 percent in patients with hyperthyroidism but had no effect in normal subjects[116, 117].

Patients with hyperthyroidism have increased left ventricular systolic and diastolic contractile function, a finding consistent with changes in the expression of contractile and calcium-regulatory proteins, as described by Kiss *et al* and Ojamaa *et al*[118, 119]. The rate of increase in intraventricular pressure during systole, the left ventricular ejection fraction, and the rate of blood flow across the aortic valve are all increased[120]. Similarly, the rates of chamber relaxation (isovolumic relaxation) and left ventricular filling, measured as the flow across the mitral valve during diastole, are increased[110]. Administration of a β-adrenergic–receptor antagonist to patients with hyperthyroidism slows the heart rate but does not alter systolic or diastolic contractile performance[110, 111] confirming that thyroid hormone acts directly on cardiac muscle[119, 121, 122].

1.14.1.2. Cardiac arrhythmias associated with hyperthyroidism:

Sinus tachycardia is the most common arrhythmia associated with thyroid hormone excess. Other rhythm disturbances frequently associated with thyrotoxicosis include atrial premature contractions and atrial fibrillation. Less often, patients may present with paroxysmal atrial tachycardia or atrial flutter. Ventricular premature contractions and ventricular tachyarrhythmias are rare[123].

Atrial fibrillation occurs in 5% to 15% of hyperthyroid patients[105]. Higher rates may be found in patients with known or suspected heart disease, or at risk for heart disease such as the elderly or men. TSH should be routinely checked in all patients with AF, as it may be the only manifestation of hyperthyroidism[123].

Whether patients with hyperthyroidism who have atrial fibrillation should receive anticoagulant therapy is controversial[105]. Individual risk-benefit assessment for each patient must weigh the risk of bleeding during anticoagulant treatment against the risk of systemic embolisation[124]. In a retrospective study of 610 patients with hyperthyroidism, age – rather than the presence of atrial fibrillation – was the main risk factor for embolisation[125]. In another study, of 11354 patients with hyperthyroidism, 288 had atrial fibrillation, of whom 6 had systemic embolisation. Of these six patients, five were more than 50 years of age and had atrial fibrillation for more than six months, and four had congestive

heart failure. In younger patients with hyperthyroidism and atrial fibrillation who do not have other heart disease, hypertension, or independent risk factors for embolisation, the risk of anticoagulant therapy probably outweighs the benefits[124]. Conversely, in older patients who are known or suspected to have heart disease, or in those with chronic atrial fibrillation, anticoagulant therapy should be initiated[105].

1.14.1.3. Subclinical hyperthyroidism:

Subclinical hyperthyroidism is characterized by the presence of low or undetectable plasma TSH concentrations and normal circulating free thyroid hormones. Subclinical hyperthyroidism is often present in patients treated with TSH-suppressive doses of thyroxine for thyroid nodular disease or as postoperative treatment for differentiated thyroid carcinoma to prevent local and/or metastatic progression (exogenous or iatrogenic subclinical hyperthyroidism). However, it is also seen in patients with autonomously functioning thyroid nodule or multinodular goitre (endogenous subclinical hyperthyroidism). Its prevalence, in several large community and clinical surveys, has been reported to range from 2–16%[126].

Alterations in cardiac Haemodynamic have been reported in some, but not all, studies of patients with subclinical hyperthyroidism[105]. Although the data concerning the increase of heart rate and the incidence of supraventricular arrhythmias in patients with subclinical hyperthyroidism are not consistent[127-

130], it seems that the elderly population is particularly susceptible to the arrhythmogenic effect of subclinical hyperthyroidism. Sawin et al in their 10 year follow up of 2007 men and women aged 60 or more, demonstrated that a low serum thyrotropin concentration (≤ 0.1 mU/L) was associated with a threefold higher risk of developing atrial fibrillation in the subsequent decade[131]. Their findings concur with those of the more recently analysed data from the Cardiovascular Health Study[132]. Therefore, Serum thyrotropin should be measured in all elderly patients with systolic hypertension, a widened pulse pressure, recent-onset angina, atrial fibrillation, or an exacerbation of underlying ischaemic heart disease[105, 113]. Because of increased morbidity and mortality from thromboembolic events associated with atrial fibrillation, subclinical hyperthyroidism in older patients may be considered a risk factor for thromboembolism[107].

Subclinical hyperthyroidism has also been shown to affect cardiac morphology and function in the young and the middle-aged through increased heart rate and LV mass, enhanced LV function, and impaired LV diastolic filling[107]. The increase in LV mass is mainly the consequence of the long-term increase in cardiac workload, and is responsible for the co-existing diastolic dysfunction and impaired systolic function during exercise [133]. These abnormalities were significantly reverted by ß-blocking drugs[111]. The above effects on the cardiovascular system seem to be consistent with both endogenous and exogenous subclinical hyperthyroidism[107, 134]. Recently, Patanè et al presented a case

of acute myocardial infarction without significant coronary stenoses associated with subclinical hyperthyroidism[135]. Furthermore, an increase in the prevalence of symptoms and signs of thyroid hormone excess and impaired quality of life is seen in this age group[107]. Current recommendations for the treatment of subclinical hyperthyroidism are to observe and monitor patients with partial TSH suppression (0.1– 0.4 mU/L), but to treat patients with complete TSH suppression (<0.1 mU/L)[82].

1.14.2. Hypothyroidism and the cardiovascular system:

1.14.2.1. Hypothyroidism and atherosclerosis

"There was oedema of the skin. . . much serous effusion in the pericardium. . . the heart was large. . . the arteries were everywhere thickened, the larger ones atheromatous." [Dr. William Smith Greenfield, 1878].[136]

This autopsy finding of diffuse atherosclerosis in a 58-yrold woman was published as an appendix to William Ord's classical description of the syndrome of myxoedema. Soon thereafter, the hypothesis of a causal relationship between hypothyroidism and atherosclerosis was first raised in 1883 by E. Theodor Kocher [137], who noted that arteriosclerosis commonly occurred after thyroid extirpation.

In 1967 the first case-control study by Vanhaelst *et al.* [138] compared autopsy findings in 25 patients with myxoedema with

50 age-matched controls and found a greater prevalence and severity of coronary atherosclerosis in the hypothyroid group. In a subsequent case-control study performed by Steinberg in 1968[139], women with myxoedema had more severe coronary artery disease on autopsy than did age matched women without myxoedema. However, this difference was present only between hypertensive cases and controls, with similar degrees of atherosclerosis between normotensive hypothyroid women and normotensive controls. Another autopsy study[140] took the converse approach, examining the thyroid glands of 55 patients who had died of atherosclerotic disease. All of the thyroids were found to have some abnormality, in size or cellular structure, compared with no abnormalities in four controls without atherosclerosis. Although these studies were conducted before the era of TSH testing to confirm the diagnosis of hypothyroidism, they suggested that the relationship between hypothyroidism and atherosclerotic disease exceeded the statistical coincidence of these two common processes[77].

The association between hypothyroidism and atherosclerosis has also been shown in living patients. A study of patients undergoing coronary angiography demonstrated that those who had inadequate therapy for hypothyroidism were more likely to have angiographic progression of coronary artery disease than those with adequate replacement[141]. However, this study should be interpreted with caution because it is based on only 10 individuals and potentially subject to bias from the practices of referring

physicians, who may have been reluctant to increase the dose of T_4 in patients with more symptomatic or severe atherosclerotic disease[142]. In a hospital-based study, men and women with a TSH level of 4.0 mU/L or greater had higher prevalence of coronary artery disease than age matched controls (48% *vs.* 38% for men and 37% *vs.* 20% for women), although this was statistically significant only for women[143].

1.14.2.2. Hypothyroidism and cardiovascular risk markers

Elevated levels of total cholesterol, LDL cholesterol, and apolipoprotein B are well documented features of overt hypothyroidism[144]. Early studies in humans with hypothyroidism, using isotopically labelled LDL, demonstrated a prolonged half-life of LDL cholesterol because of decreased catabolism, an effect that was reversible with T_4 therapy[145]. Additional data in human fibroblasts verified that the T_3-induced increase in LDL degradation was mediated through an increase in LDL receptor number, without any change in the affinity of LDL for its receptor. A specific effect of thyroid hormone on the LDL receptor was suggested by a lack of T_3 effect on LDL concentration in cultured cells without LDL receptors[146]. These findings were supported by an *in vivo* study in a hypothyroid woman whose receptor mediated LDL catabolism was reduced, compared with euthyroid controls, with significant improvement after T_4 replacement therapy[147].

Studies have also shown that hypothyroidism causes qualitative changes in circulating lipoproteins that increase their atherogenicity. Two studies have shown that LDL is more susceptible to oxidation in patients with hypothyroidism, with normalisation after restoration of the euthyroid state[148, 149]. Increased levels of lipoprotein(a) [Lp(a)], a particularly atherogenic LDL variant in which apolipoprotein(a) and apolipoprotein B (Apo B) are covalently bound, have also been reported in hypothyroidism, compared with euthyroid controls. Several studies have shown decreases in the Lp(a) concentration after T_4 treatment of hypothyroid patients[150-154]. However, other reports have not confirmed this relationship[155, 156]. Clinical trials have not demonstrated an effect of T_4 on Lp(a) levels in subclinical hypothyroidism[153-155, 157, 158], with the exception of one trial, which showed a decrease in Lp(a)[159]. Ito *et al* studied the effect of T_4 therapy on lipid profiles of patients with overt and subclinical hypothyroisim, including their non-HDL-C (a measure of total cholesterol minus HDL-C)[154]. They showed that After T_4 replacement, the serum concentrations of all lipoproteins, except Lp(a), were significantly decreased in patients with overt hypothyroidism. In patients with subclinical hypothyroidism, the serum concentrations of total cholesterol, non-HDL-C, remnant-like particle cholesterol, and Apo B were significantly decreased, whereas no significant changes in the serum concentrations of low-density lipoprotein cholesterol, HDL-C, triglycerides, apolipoprotein A-I, and Lp(a) were

observed. In all 39 patients, the reduction in the non-HDL-C levels correlated with the reduction in the low-density lipoprotein cholesterol, remnant-like particle cholesterol, and Apo B levels. However, the reduction in the non-HDL-C levels did not correlate with the reduction in the HDL-C, Lp(a), and apolipoprotein A-I levels. These results suggest that altered serum concentrations of non-HDL-C in hypothyroidism may be related to the disturbed metabolism of low-density lipoprotein, remnant lipoprotein, and Apo B[154].

Additional potentially atherogenic effects of hypothyroidism on lipid metabolism include a reversible reduction in clearance of chylomicron remnants[160]; reduced activity of cholesteryl ester transfer protein, which is involved in reverse cholesterol transport pathway[161, 162]; and decreased activity of hepatic lipase and lipoprotein lipase[163, 164].

Several studies have demonstrated elevated homocysteine levels in hypothyroidism[165-167], with improvement after T_4 replacement[168-170]. This is likely to be caused by impaired renal homocysteine clearance, although an effect of thyroid hormone on enzymes involved in folate metabolism has also been proposed[169, 171]. The magnitude of decline in homocysteine levels after T_4 treatment is sufficient to lower cardiovascular risk, with a decrease of 2–5 μmol/L when hypothyroid patients were treated with T_4 to a level suppressing the serum TSH concentration[168, 169, 171]. One study of patients with

spontaneous hypothyroidism showed a decrease of 4.6 μmol/L on restoring the euthyroid state[170]. In contrast, there are now considerable data showing that subclinical hypothyroidism is not associated with hyperhomocysteinaemia. Three case-control studies[167, 172, 173] have reported no difference in homocysteine levels between individuals with subclinical hypothyroidism and euthyroid controls. Furthermore, Christ-Crain *et al.*[167] found no significant change in homocysteine levels after treatment of subclinical hypothyroidism.

C-reactive protein (CRP), another cardiovascular risk factor, has also been studied in relation to hypothyroidism. Christ-Crain *et al.* [167] measured CRP in 61 overtly hypothyroid and 63 subclinically hypothyroid patients and compared them with 40 euthyroid control subjects. CRP levels were significantly higher in both hypothyroid groups, compared with controls. However, CRP levels did not decrease with T_4 treatment of the subclinically hypothyroid patients.

Davies *et al* showed a reverse statistical relation between serum interleukin 6 (IL-6) concentrations and alterations in circulating thyroid hormone concentrations seen in in-patients with non-thyroidal illness[174]. This negative correlation was also supported by Yamazaki *et al* who suggested that IL-6 in vivo would be capable of inhibiting the synthesis and release of T_4 and, to a greater extent, T_3 from the thyroid gland[175]. The relationship between thyroid status and IL-6 seems to warrant further research,

especially given the results of a recent study showing that among the inflammatory markers, IL-6 was the strongest predictor for CHF[176].

Some studies have shown that insulin resistance or the metabolic syndrome are independent risk factors for cardiovascular disease even in individuals without diabetes[177]. Although hypothyroidism does not appear to cause insulin resistance[178], Bakker *et al.*[179] postulated that relatively lower thyroid hormone levels might amplify the increased cardiovascular risk associated with insulin resistance. Their study did confirm that insulin-resistant subjects with high normal TSH levels had higher LDL cholesterol concentrations, whereas among insulin-sensitive individuals, TSH concentration was not associated with any difference in LDL level.

The impact of hypothyroidism on vascular and haemostatic risk factors for atherosclerosis has also been investigated in a few studies. Alterations in flow-mediated, endothelium dependent vasodilatation, which occurs early in atherogenesis, have been noted in patients with hypothyroidism. It is uncertain whether this is attributable to a direct effect of thyroid hormone deficiency or mediated through the hypercholesterolaemia induced by hypothyroidism[180]. Conflicting data exist regarding the effect of hypothyroidism on coagulation. Both increased[181] and decreased[182] platelet adhesiveness have been reported in hypothyroidism. The degree of hypothyroidism may determine its

ultimate effects on coagulation parameters[183]. In one study comparing moderate (TSH 10 to 50 mU/L) and severe hypothyroidism (>50 mU/L) with the euthyroid state, women with moderate hypothyroidism showed decreased fibrinolytic activity, with lower d-dimer levels, higher α2-antiplasmin activities, and higher levels of tissue plasminogen activator antigen and plasminogen activator inhibitor antigen. In contrast, those with severe hypothyroidism had higher d-dimer levels, lower α2-antiplasmin activities, and lower tissue plasminogen activator antigen and plasminogen activator inhibitor antigen levels[183]. These results suggest a greater risk for thrombosis, which could precipitate myocardial infarction, in moderate hypothyroidism, and a bleeding tendency in severe hypothyroidism[142].

1.14.2.3. Haemodynamic changes associated with hypothyroidism

The haemodynamic changes associated with hypothyroidism are opposite to those of hyperthyroidism, but they are accompanied by fewer symptoms and signs. The most common signs are bradycardia, mild hypertension, a narrowed pulse pressure, and attenuated activity on the precordial examination[105]. The two contributing factors to systemic hypertension in overt hypothyroidism are a marked increase in peripheral vascular resistance – which is the most widely recognised one, and the increase in arterial stiffness, which probably results from myxoedema of the arterial wall[184, 185]. In general, systemic hypertension associated with overt hypothyroidism is poorly controlled by conventional treatments, whereas it promptly

improves with achievement of euthyroidism[184]. These findings should encourage the routine assessment of thyroid function in all patients with pre-existing systemic hypertension that becomes resistant to pharmacological treatment[186]. Other characteristic but nonspecific findings are high serum concentrations of cholesterol and creatine kinase (the skeletal-muscle MM isoform)[105].

The most-consistent cardiac abnormality recognized in patients with overt hypothyroidism is impairment of LV diastolic function, which is characterized by slowed myocardial relaxation and impaired early ventricular filling. LV systolic function usually is only marginally subnormal, as demonstrated by slightly reduced values of ejection fraction and stroke volume. The reduced cardiac preload, in combination with bradycardia and slight reduction in myocardial contractility, accounts for a less than normal cardiac output in overt hypothyroidism[187, 188]. Systemic vascular resistance may increase by as much as 50 percent[105]. The lower cardiac performance and the abnormalities in peripheral and proximal vascular function may contribute to the poor exercise tolerance in overt hypothyroidism [189]. However, because of the lowered demand for peripheral oxygen secondary to an overall lower metabolic rate and the resulting low cardiac output, heart failure is rare[105]. The lack of thyroid hormone effect on myocardial gene expression can be dramatic. Ladenson *et al* reported a substantially lower alpha-myosin heavy-chain mRNA level, a markedly elevated atrial natriuretic factor mRNA level and

decreased phospholamban mRNA level in an individual with profound hypothyroidism and dilated cardiomyopathy. The histological and clinical findings were reversible after 9 months of treatment with thyroxine, suggesting that alterations in gene expression in the dilated myopathic heart may be correctable when a treatable cause is identified[190]. Pericardial effusions and nonpitting oedema (myxoedema) can occur in patients with severe, long-standing hypothyroidism[105, 187, 190].

Positron-emission tomographic studies of oxygen consumption in patients with hypothyroidism have revealed that myocardial work efficiency is lower than in normal subjects[191]. From 10 to 25 percent of patients have diastolic hypertension, which, combined with the increase in vascular resistance, raises cardiac afterload and cardiac work[105, 191]. Although atrial arrhythmias are common and ventricular ectopy is rare in patients with hyperthyroidism, the opposite is true of hypothyroidism[113]. Hypothyroidism prolongs the cardiac action potential and the QT interval[192]. This, in turn, predisposes the patient to ventricular irritability and, in rare cases, acquired torsade de pointes[193]. These changes may arise at least in part from the regulatory effect of triiodothyronine on the expression of various ion channels in the heart[192]. Thyroxine therapy reverses all the cardiovascular changes associated with hypothyroidism[105]. Young patients with no evidence of organic heart disease can be given a replacement dose of thyroxine at the outset. Older patients, or those with known or suspected ischaemic heart disease, should initially be given about 25 percent

of the anticipated replacement dose, and the dose should then be increased in stepwise fashion at six-to-eight-week intervals[187]. In a large study of patients with hypothyroidism who were evaluated for clinical evidence of ischaemic heart disease after the initiation of thyroid hormone therapy, new or worsening angina or acute myocardial infarction was rare, and more patients had improvement in anginal symptoms[194]. These findings reinforce the important and potentially beneficial effects of thyroid hormone in improving the efficiency of myocardial oxygen consumption[191] and simultaneously lowering systemic vascular resistance[195, 196].

1.14.2.4. Subclinical hypothyroidism

Subclinical hypothyroidism (SCH), defined as a raised serum TSH level (>4.5 mU/L) with normal total or free T_4 and T_3 levels, is a commonly encountered condition in primary and secondary care with an overall prevalence of 4-10% in the general population and up to 20% in women above the age of 60[108]. SCH may represent an early stage of thyroid disease with an annual progression rate of 3-18% to overt hypothyroidism. Presence of antithyroid antibodies, serum TSH of more than 20 mU/L, a history of radioiodine ablation for Graves' disease, a history of external radiation therapy for nonthyroid malignancies, a history of autoimmune diseases such as type 1 diabetes, and chronic lithium treatment are the strongest predictors of progression[108, 197].

Patients with SCH are often asymptomatic; however, about 30% of patients may have symptoms suggestive of thyroid hormone deficiency including dry skin, poor memory, slow thinking, muscle weakness, fatigue, muscle cramps, cold intolerance, puffy eyes, constipation, and hoarseness. The Colorado Thyroid Disease Prevalence Study[10] showed that whilst euthyroid subjects experienced a mean 12.1% of all the above mentioned symptoms, patients with overt hypothyroidism had 16.6% and the ones with SCH reported an intermediate 13.7% of the symptoms.

Some, but not all, cross-sectional studies have demonstrated that serum levels of total cholesterol and LDL cholesterol are higher in patients with SCH than in euthyroid controls. Danese *et al*[198] in their meta-analysis of the effect of therapy for subclinical hypothyroidism on serum lipid levels demonstrated a mean reduction in the total cholesterol level of 0.2 mmol/L and in the LDL cholesterol level of 0.26 mmol/L.

Several neurobehavioral abnormalities such as depression, memory loss, cognitive impairment and a number of neuromuscular abnormalities have been shown to be associated with subclinical hypothyroidism; and there is evidence suggesting that SCH in pregnant women may impair the intellectual development of their euthyroid offspring.

Whether SCH is an independent risk factor for cardiovascular disease is controversial. As mentioned earlier in this chapter, the findings of the original Whickham Survey[63] contradict those of

the Rotterdam Study[106] except for the common finding that thyroid autoimmunity was not associated with cardiovascular disease. Cappola *et al* in their analysis of the Cardiovascular Health Study data found no relationship between subclinical hypothyroidism or overt hypothyroidism and prevalence or incidence of atherosclerotic disease, cardiovascular mortality, or all-cause mortality[132].

Controversy exists as to whether the general population should be screened for SCH or whether the condition routinely warrants thyroid hormone replacement. Villar *et al* in their recent systematic review found that based on current randomised clinical trials, thyroxine replacement therapy for subclinical hypothyroidism did not result in improved survival or decreased cardiovascular morbidity. Data on health-related quality of life and symptoms did not demonstrate significant differences between intervention groups. Some evidence indicated that thyroxine replacement improved some parameters of lipid profiles and left ventricular function[199]. Whilst most endocrinologists agree that patients with a TSH of higher than 10 mU/L, asymptomatic patients with positive antithyroid antibody, pregnant women and women who have ovulatory dysfunction and infertility should be considered for a therapeutic trial with thyroxine, routine treatment in patients with a TSH of 4.5-10 mU/L remains a grey area and best clinical practice continues to combine clinical judgment and patients' preferences[82].

1.15. Thyroid and the phytoestrogens:

Phytoestrogens are chemicals of plant origin that have the ability to cause oestrogenic and/or anti-oestrogenic effects. Lignans and isoflavones represent two of the main classes of phytoestrogens of current interest in clinical nutrition. Although ubiquitous in their occurrence in the plant kingdom, these bioactive nonnutrients are found in particularly high concentrations in flaxseeds (mainly a source of lignans such as enterolactone and enterodiol) and soybeans (mainly a source of isoflavons such as daidzein and genistein) and have been found to have a wide range of hormonal and nonhormonal activities[200].

A number of studies have suggested that soybeans can be implicated in diet-induced goitre. Isoflavones have been shown to inactivate TPO *in vitro*. High-performance liquid chromatography (HPLC) fractionation and enzymatic assay of the soybean extract showed that the components responsible for inhibition of TPO-catalyzed reactions coeluted with daidzein and genistein[201]. Similar findings have been observed *in vivo*. Rats exposed to genistein for 20 weeks showed a dose dependent decrease in TPO activity, but their serum thyroid hormone level (T_3, T_4, TSH), thyroid weight and thyroid histopathology remained unchanged, suggesting that the remaining enzymatic activity of TPO was adequate to maintain thyroid homeostasis[202]. Additional factors appear necessary for soy to cause overt thyroid toxicity. These include iodine deficiency but may also include additional soy

components (most importantly, soy protein), other defects of hormone synthesis, or additional goitrogenic dietary factors[203, 204]. Furthermore, if isoflavones do have adverse effects on thyroid function in susceptible individuals, there is no reason to think that isoflavone-containing soy foods would not have similar effects and in fact, a few studies have reported adverse effects of soy consumption on thyroid function in infants [205] and adults [206]. The effect of dietary soy phytoestrogens on thyroid function in patients with SCH is the subject of chapter 5 of this thesis.

Chapter 2

THE EFFECT OF SUBCLINICAL HYPOTHYROIDISM ON CARDIOVASCULAR OUTCOMES IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

2.1. Introduction:

Type 2 diabetes mellitus (T2DM) is a complex metabolic disease characterized by increased peripheral insulin resistance and an insulin-secretory defect that varies in severity leading to raised blood glucose levels. T2DM constitutes about 85% to 95% of all diabetes cases in developed countries and accounts for an even higher percentage in developing countries. In 2007, it was estimated that there were 246 million people with diabetes in the adult population in the seven regions of International Diabetes Federation (IDF). In 2003, the total was 194 million. It is now a common and serious global health problem, which for most countries has evolved in association with rapid cultural and social changes, ageing populations, increasing urbanisation, dietary changes, reduced physical activity and other unhealthy lifestyle and behavioural patterns. The incidence of T2DM is expected to double over the next 20 years. Based on the latest Quality and Outcomes Framework (QoF) data (2006), the estimated national prevalence of diabetes is 3.55% in the United Kingdom.

Circulatory disorders associated with diabetes include coronary heart disease (CHD), stroke, peripheral arterial disease,

cardiomyopathy, and congestive heart failure. Several clinical trials have shown that diabetes generally results in early death from cardiovascular diseases (CVDs). The impact of cardiovascular disease was compared in non-diabetics and diabetics in the Framingham cohort. In the first 20 years of the study about 6% of the women and 8% of the men were diagnosed as diabetics. The incidence of cardiovascular disease among diabetic men was twice that of non-diabetic men. Among diabetic women the incidence of cardiovascular disease was three times that of non-diabetic women[207]. Multiple Risk Factor Intervention Study (MRFIT) also showed that absolute risk of CVD death was three times higher for diabetic than nondiabetic men of every age group, ethnic background, and risk factor level, after adjustment for age, race, income, serum cholesterol level, systolic blood pressure (BP), and reported number of cigarettes per day[208]. The results of the OASIS (Organisation to Assess Strategies for Ischaemic Syndromes) registry later established that diabetic patients with no previous cardiovascular disease have the same long-term morbidity and mortality as nondiabetic patients with established cardiovascular disease after hospitalisation for unstable coronary artery disease[209].

Subclinical hypothyroidism (SCH), defined as a raised serum TSH level with normal total or free T_4 and T_3 levels, has been associated with a greater prevalence of aortic atherosclerosis and myocardial infarction, in some but not in all studies[63, 106, 132] (cf. 1.14.2.4).

SCH is relatively common in patients with type 2 diabetes with a prevalence of 6.8% [210].

We hypothesised that if SCH contributed to an increase in cardiovascular risk then, intuitively, its effect would be exaggerated in patients with type 2 diabetes – a high cardiovascular risk population. Therefore, we conducted a retrospective analysis of cardiovascular outcomes comparing patients with type 2 diabetes with SCH and without SCH, using the Hull and East Yorkshire diabetes database.

2.2. Subjects & Methods:

Patients were identified from an electronic diabetes database of 15760 diabetes patients at Hull Royal Infirmary from 1993 to June 2005. An initial search of 6540 consecutive patients entered on the register up to the end of 2000, a cut off to ensure capture of clinical outcomes at least five years after the initial diagnosis of SCH. A total of 542 patients with a raised TSH (>5 mU/L) and a normal free T_4 were identified. All of the case notes were reviewed and laboratory values/clinical findings were ascertained by the author. Patients with type 1 diabetes, progression to overt hypothyroidism, current thyroxine therapy, those on antithyroid treatment or history of antithyroid treatment, those with a history of or current treatment with lithium and those with a single TSH measurement were excluded. A total of 394 patients with type 2 diabetes and untreated SCH were identified that composed the

case group. To identify the control group, a TSH range of 0.5-3.0 mU/L (normal lab range: 0.5-4.7mU/L) was used as the database search criteria. The reason for setting 3.0 mU/L as the upper cut off TSH value is the controversy over the upper normal limit of TSH, as some authors have suggested that the current upper limit of the population reference range is skewed by the inclusion of persons with occult thyroid dysfunction[70, 71] (cf. 1.9.3). Acknowledging that the concept of a narrower TSH normal range is yet to be widely accepted[74], the researchers felt that 3.0 mU/L would provide a safe margin to ensure exclusion of people with subclinical hypothyroidism in the control group. An initial search of the database identified over 800 patients with TSH measurements in the range of 0.5-3.0 mU/L. After excluding those with type 1 diabetes, previous or current thyroid dysfunction on treatment with thyroxine or antithyroid drugs, those with a history of or current treatment with lithium and those with a single TSH measurement, there were 472 patients in the control group. The 394 case group patients were compared with these 472 type 2 diabetes patients with a TSH of 0.5-3.0 mU/L (normal lab range: 0.5-4.7mU/L) during the same period of time. TSH, free T_4, free T_3, HbA1c, lipid profile, presence of coronary heart disease, peripheral vascular disease, cerebrovascular and cardiovascular events and smoking history were recorded from the review of the notes. Based on one previous study [106] our sample size was powered to detect a difference in odds of 1.90 (80% power, 5% significance).

The relationship between SCH and cardiovascular mortality was assessed by logistic regression from which odds ratios (ORs) and 95% confidence intervals (CIs) were calculated[211, 212]. Three models were constructed: 1. unadjusted odds ratios; 2. adjusted for age and sex; 3. further adjustment for smoking status (yes/no), body mass index (BMI) (weight [Kg]/height2 [m]), diastolic blood pressure (mmHg), systolic blood pressure (mmHg), HbA1c (%) and lipids (mmol/L). An arbitrary level of 5% statistical significance was assumed (two-tailed). P values were calculated by the likelihood ratio method. GLIM4 statistical computer package was used to analyze the data.

2.3. Results:

There were 394 patients with type 2 diabetes having SCH (a prevalence of 6%) and 472 patients with type 2 diabetes without SCH. The baseline demographic parameters of both groups are given in Table 2.3.1. Table 2.3.2 shows the prevalence of CVD in the case and the control groups. The incidences of new cardiovascular events are depicted in Table 2.3.3. Mortality from CVD and other causes in the two groups is summarised in Table 2.3.4.

The mean age of patients with SCH was 73.1 years compared to 71.1 years in patients without SCH. There were more females in patients with SCH (83.7%) compared to patients without SCH (18%). The mean duration from diagnosis of SCH to baseline of the study was 7.9 years. There were 222 new cardiovascular events

(28.1 person years) in patients with Type 2 Diabetes having SCH compared to 246 cardiovascular events (31.1 person years) in patients without SCH. There was no relationship between baseline or follow up serum TSH levels and cardiovascular mortality. The unadjusted odds ratio (OR) was 1.27(95%CI=0.86, 1.87, p =0.26). Adjusting for age and sex did not alter the nature of this relationship: OR=1.17(95%CI=0.88, 1.56, p=0.27), and neither did further adjustment for the other baseline covariates: OR=1.15 (95% CI= 0.58, 2.27, p=0.68). The unadjusted OR for all cause mortality was 1.83 (95%CI=1.36, 2.46, p value <0.001).

Table 2.3.1: The baseline demographic parameters of the case and control groups; values given as mean ± SEM (Range)

Parameters	**Cases (n=394)**	**Controls (n=472)**
Age in years	73.1 ± 0.57 (28 - 98)	71.1 ± 0.57 (34-96)
Sex (m/f)	148/246	387/85
Smokers % (n)	11.93 (47)	16.95 (80)
BMI in kg/m2	30.1 ± 0.40 (18.8-45.2)	29.5 ± 0.33 (17.4-46.4)
TSH in mU/L	8.1 ± 0.01 (5.1 - 9.9)	1.1 ± 0.02 (0.30 - 4.10)
Systolic BP in mmHg	149 ± 1.4 (93 - 217)	148 ± 1.4 (80 - 212)
Diastolic BP in mmHg	80 ± 0.8 (42 - 90)	82 ± 0.79 (40 - 92)
Total Cholesterol in mmol/L	5.7 ± 0.1 (3.4 - 6.9)	5.5 ± 0.1 (3.2 - 6.8)
HbA1c in %	8.2 ± 0.1 (5.8 - 9.3)	8.1 ± 0.1 (5.6 - 9.2)

Table 2.3.2: Prevalence of CVD in the case and control groups

CVD	Cases (n=394)	Controls (n=472)
Coronary artery disease	88 (22.3%)	103 (21.8%)
Cerebrovascular events	12 (3.0%)	12(2.5%)
Peripheral vascular disease	22 (5.6%)	27 (5.7%)
Coronary artery disease and cerebrovascular events	10 (2.5%)	14 (3.0%)
Coronary artery disease and peripheral vascular disease	20 (5.1%)	21 (4.5%)

Table 2.3.3: New cardiovascular events in the two groups

New CVD	Cases (n=394)	Controls (n=472)
Coronary artery disease	95(55.9%)	105(56.8%)
Cerebrovascular events	40(23.5%)	56 (30.3%)
Peripheral vascular disease	34 (20%)	23 (12.4%)
Ischemic colon	1(0.6%)	1(0.5%)
Total	170	185

Table 2.3.4: Mortality from CVD and other causes in the two groups

Mortality	Cases (n=394)	Controls (n=472)
Coronary artery disease	44 (11.2%)	45 (9.5%)
Cerebrovascular events	5 (1.3%)	27 (5.7%)
Other causes	47 (11.9%)	103 (21.8%)
Total	96 (24.4%)	175 (37.1%)

2.4. Discussion:

In this study there was no increase in cardiovascular mortality in patients with type 2 diabetes and SCH compared to patients with type 2 diabetes without SCH. There was no relationship between serum TSH levels and cardiovascular mortality in this cohort of patients with type 2 diabetes. The prevalence of SCH in this population was found to be 6.0% that is not dissimilar to that of 6.8% reported[210]. Sixty percent of patients with SCH were females compared to 18% of patients without SCH. This corresponds to the literature where the highest age-and sex-specific rates are in women older than 60 years . There were more smokers in the control group (17%) than patients with SCH (12%). In spite of adjusting for these variables, there was no increase in cardiovascular disease in patients with SCH and type 2 diabetes.

These data are in accord with the 20-year follow-up of the Whickham cohort, where the rates of death from all causes or from cardiovascular causes were not significantly higher in subjects who had SCH at baseline than in those with euthyroidism at base line[63] and also in line with the results of the Cardiovascular Health Study[132] which did not show an association between SCH and cardiovascular mortality or events. These results are the converse to the Rotterdam Study that found SCH as a strong indicator of risk for atherosclerosis and myocardial infarction[106] in a comparably aged population to this study, though they found a higher SCH prevalence of 11.8%.

Patients with type 2 diabetes have much higher risk of two to fourfold of both coronary heart disease and stroke and increased case-fatality rates[208]. It is reassuring that these data suggest that SCH does not contribute to the excess risk of cardiovascular mortality in patients with type 2 diabetes, at least 5 years after its diagnosis.

In conclusion, the findings of this study do not support the routine use of thyroxine in type 2 diabetic patients with SCH if the aim of the treatment is to minimise their long-term CV risk. However, in the presence of symptoms that can be attributed to hypothyroidism, a trial treatment with thyroxine, based on clinical judgment and patient's preferences, may be warranted.

Chapter 3

THE EFFECT OF THYROID DYSFUNCTION ON N-TERMINAL PRO-B-TYPE NATRIURETIC PEPTIDE CONCENTRATIONS

3.1. Introduction:

Measurement of Brain Natriuretic Peptide (BNP) used in conjunction with other clinical information is useful in establishing or excluding the diagnosis of congestive heart failure in patients with acute dyspnoea[213]. BNP is a prognostically robust tool for risk stratification across the spectrum of acute coronary syndromes and it can reliably predict the presence or absence of left ventricular dysfunction on echocardiogram[214]. In the largest study involving 3177 Framingham probands, BNP showed excellent negative predictive values for excluding severe systolic LV dysfunction, but because positive predictive values were weak, it indicated that BNP was unsuitable for screening in the community or high risk population[215]. The decrease of Natriuretic Peptides (NPs) following treatment of heart failure has been shown to correlate with clinical benefit, improvement of functional parameters such as ejection fraction and surrogate parameters of therapy success such as renin levels and a decrease in heart rate[216, 217].

A change in BNP levels may be associated with other conditions. Pulmonary disease resulting in right ventricular dysfunction leads

to increased BNP values[218]. Kruger *et al* found a good correlation of BNP with right ventricular dysfunction but not with clinical outcome in 50 patients with pulmonary embolism[219]. Other non-cardiac causes associated with elevated NPs are renal failure, ascitic cirrhosis, hyperaldosteronism, hypercortisolism, subarachnoid haemorrhage, COPD, carcinoma of lung, increasing age, gender (higher in women and in the elderly)[220], and lower body mass indices[221].

Although the exact mechanisms of BNP release are still unclear, its release seems to be stimulated by increased left ventricular (LV) wall stress and volume expansion[222]. BNP is synthesized as a pro-hormone and is secreted by proteolytic cleavage. Finally, the biologically active part BNP and the larger aminoterminal part N-Terminal Pro-B-Type Natriuretic Peptide (NT-proBNP) are released in equimolar amounts in circulation[220]. The in-vivo half life of BNP is 20 minutes and shorter than that of NT-proBNP, which is about 2 hours[223], and absolute values of BNP are significantly lower than values of NT-pro-BNP despite equimolar secretion. NT-proBNP was also found to be the only independent predictor of disease for the New York Heart Association (NYHA) class I and NYHA class II and therefore may be a more discerning marker for the detection and evaluation of heart failure than BNP[224].

The actual function of BNP is thought to be cardioprotective through increasing natriuresis and diuresis, vasodilatation and

directly inhibiting the sympathetic nervous system and the renin–angiotensin system[225]. Changes to these parameters have also been found in patients with thyroid dysfunction. Triiodothyronine decreases systemic vascular resistance by dilating the resistance arterioles of the peripheral circulation[195]. As a result of the decrease in systemic vascular resistance, the effective arterial filling volume falls, causing an increase in renin release and activation of the angiotensin-aldosterone axis[226]. This, in turn, stimulates renal sodium reabsorption, leading to an increase in plasma volume. Thyroid hormone also stimulates erythropoietin secretion. The combined action of these two is an increase in blood volume and preload, which further increases cardiac output[105] We have already shown that changes in thyroid function are associated with alteration in the concentration of Cystatin C through a possible metabolic rate mediated mechanism[227]; this study has assessed whether similar changes are reflected with NT-proBNP concentrations following treatment of hypo and hyperthyroidism. Schultz *et al* have shown alterations of NT-proBNP levels across a range of thyroid dysfunctions[228]. Our study assessed whether the previously observed alterations in NT-proBNP levels with thyroid dysfunction are consistent and tries to interpret the results in the light of more recently published data on biological variation of this analyte.

3.2. Subjects and Methods:

To see a significant difference in the NT-proBNP levels between baseline hyperthyroid and hypothyroid groups (80% power and a significance level of 5%) a sample size of 10 per group was calculated using nQuery Advisor (version 4, Statistics Solutions). Seventeen patients (12 female, 5 male, median age 51yrs, range 24-77) with newly diagnosed biochemical hypothyroidism (Thyroid Stimulating Hormone (TSH)>5.0mU/L (reference interval 0.5-4.7mU/L) and free Thyroxine (fT4)<9pmol/L (9-24)) and 21 patients (16 female, 5 male, median 48yrs, range 21-66) with newly diagnosed hyperthyroidism (TSH<0.05mU/L, fT4>24pmol/L and free triiodothyronine (fT3)>5.3pmol/L (0.5-5.3)) had NT-proBNP measured at baseline and when they subsequently became euthyroid (TSH<4.8pmol/L, fT4 9-24pmol/L). Patients were sequentially recruited from the Endocrine out-patient clinic at Michael White Centre, Hull Royal Infirmary over a three month period. The enrolled patients did not have a history of coronary heart disease. Using Boston Criteria[229] heart failure was clinically excluded in all participants at the initial and follow-up visit(s). Hypothyroid patients were treated with thyroxine 100μg for 6 weeks. The 4 patients who were not rendered euthyroid on this regime became so when given 150μg thyroxine for a further 6 weeks. Twelve of the hyperthyroid patients became euthyroid after receiving carbimazole 20mg for 6 weeks. Five became hypothyroid at this stage so had 100μg thyroxine added and were euthyroid by week 12 on the combination. Two patients required an increase in

their carbimazole dose to 40mg after 6 weeks and became euthyroid by week 18. All samples taken, spun immediately and serum frozen at -20ºC, until batch analysed in a single run at the end of the study. The study gained local Ethics Committee approval and patients gave their written informed consent for participation.

All thyroid assays were performed on an Abbott Architect i4000 immunoassay analyser (Abbott Diagnostics Division, Maidenhead, Berkshire, UK). The NT-proBNP measurements were done using Biomedica NT-proBNP assay; this assay has a detection limit of 5 pmol/L at 95% B/Bo and an intra-assay CV of less than 4% at 666 pmol/L. The following values for NT-proBNP were provided by the kit manufacturer: <250 pmol/L: negative result; 250-350 pmol/L: borderline result; >350 pmol/L: positive result. Serum creatinine was measured on a Beckman LX20 instrument (Beckman Coulter UK Ltd, High Wycombe, UK).

3.3. Results:

Kolmogorov-Smirnov test, histograms and normal plots showed that the distribution of data (NT-proBNP values) in both hyper and hypothyroid groups were non-Gaussian; this was also confirmed by Shapiro-Wilk test (using GB-STAT version 9.0, Dynamic Microsystems, Inc). Therefore non-parametric methods were used to analyse the NT-proBNP data. SPSS for Windows (version 12, SPSS Inc) was used for statistical analysis unless

otherwise specified. Mann-Whitney test was employed to compare the NT-proBNP levels in the hypothyroid group versus the hyperthyroid group before and after treatment to euthyroidism; the test did not show any statistically significant difference between NT-proBNP levels in the two groups either before ($p=0.706$) or after treatment ($p=0.179$).Using Wilcoxon Signed-Ranks test, the changes in NT-proBNP were compared before and after treatment in each of the two groups of patients. This showed a statistically significant rise in the NT-proBNP concentrations in the hypothyroid group following treatment ($p<0.001$) (Figure 3.1). The subjects weights in the hypothyroid group did not significantly change after treatment to euthyroidism (mean weight in kg$\pm$SD before and after treatment: 74.84$\pm$9.08 vs 74.14$\pm$9.05; $p=0.417$). There was a marginally significant overall fall in the NT-proBNP levels in the hyperthyroid group of patients after they achieved euthyroidism ($p=0.05$) (Figure 3.2). However, there was a significant weight gain in this group of patients following treatment to euthyroidism (mean weight in kg$\pm$SD before and after treatment 72.27$\pm$18.13 vs 74.78$\pm$17.24; $p<0.05$).

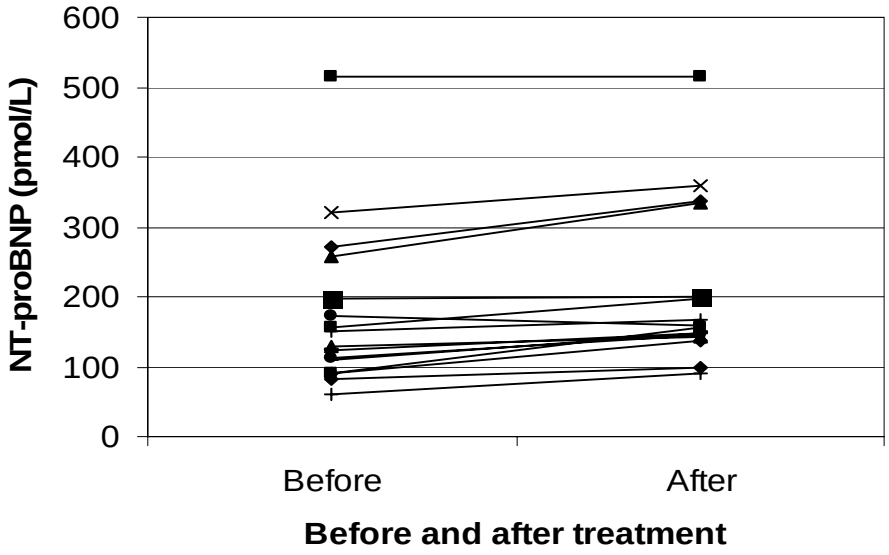

Figure 3.1: Changes in NT-proBNP with hypothyroidism (excluding the patient where NT-proBNP increased from 1211 to 1236 pmol/L from the graph; all values are included in the statistical analysis)

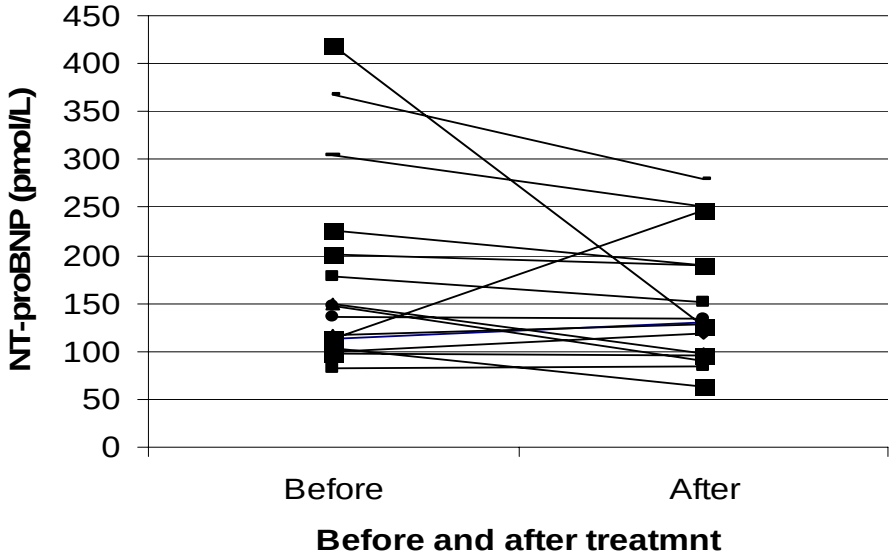

Figure 3.2: Changes in NT-proBNP with hyperthyroidism (excluding the patient where NT-proBNP fell from 584 to 429 pmol/L from the graph; all values are included in the statistical analysis)

3.4. Discussion:

The presented data shows that hypothyroidism reversibly alters NT-proBNP concentrations. Positron-emission tomographic studies of oxygen consumption in patients with hypothyroidism have revealed that myocardial work efficiency is lower than in normal subjects[191]. Also, with an increase in systemic vascular resistance and slowed diastolic relaxation and filling[196], a high incidence of heart failure in patients with hypothyroidism may be expected. However, it seems that other haemodynamic changes associated with hypothyroidism i.e. a reduction of blood volume and a decrease in renin release, can offset these effects and in practice heart failure is rare because the cardiac output is usually sufficient to meet the lowered demand for peripheral oxygen delivery[190]. It therefore seems more likely that the changes in NT-proBNP in this group are as a result of a metabolic rate mediated effect on peptide production, as has been suggested previously with cystatin C[227].

Whilst cardiac failure is certainly recognized in hyperthyroidism, in practice it is uncommon other than in the occasional patient with severe, longstanding hyperthyroidism who develops hyperthyroid cardiomyopathy or the so-called rate-related heart failure[112] This may well help explain the baseline similarities between the hyper- and hypothyroid group. Also, given the known relationship between higher BMI and lower natriuretic concentrations[221, 230], it is surprising that the weight gain found in this group after

treatment did not lead to a larger change in NT-proBNP concentrations than was found in the hypothyroid patients.

In an assessment of NT-proBNP biological variability, Wu *et al* suggest that a change of 90% for NT-proBNP is necessary before results of serially collected data can be considered statistically different[231] and week-to-week intraindividual biological variation of NT-proBNP is estimated to be 30% in patients with stable heart failure[232]. Although this study supports the changes in NT-proBNP with hypothyroidism as demonstrated earlier by Schultz *et al*[228], in the context of the biovariability of this natriuretic peptide the magnitude of the observed changes presented here are unlikely to be as clinically relevant. In contrast to Schultz *et al*, who found 4 fold higher levels of NT-proBNP in hyperthyroid subjects compared to the ones with hypothyroidism, we did not observe a significant difference between the baseline levels of NT-proBNP in the two groups. Apart from using different assays in the two studies (Roche Elecsys NT-proBNP in Schultz *et al* study versus Biomedica NT-proBNP used in this study), a number of factors may account for the different results obtained in the two studies. Schultz *et al* assumed normal distribution of NT-proBNP values based on normal plots and histograms that are useful visual tools to test for normality. However, adding Kolmogorov-Smirnov or Shapiro-Wilk tests to these can give a more accurate assessment of the data distribution. At least two other studies i.e. PRIDE study and Mueller *et al* study that used the same Roche assay with larger number of samples

(204 and 180 respectively) found that NT-proBNP data were non-Gausian[230, 233]. The assumption of normality has significant implications on the statistical power and the results. Other distinguishing factors between the two studies that could be potential sources of bias are:

- Use of carbimazole as the sole antithyroid agent in hyperthyroid group in this study versus use of mercaptoimidazole and/or radio-iodine in Schultz *et al* study.

- Schultz at al study is a dominantly female study with only 1 male subject; the male female ratio in the present study is 1:4, and more reflective of the overall sex distribution of thyroid disease in the general population

- 15 years age difference between the mean age in hyper and hypothyroid groups in Schultz *et al* study compared to 3 years mean age difference in the present study.

In conclusion, this study has shown that treating hypothyroidism to euthyroid state is associated with a rise in NT-proBNP concentrations and the treating hyperthyroidism leads to a fall. These changes seem to be secondary to a metabolic rate mediated effect on peptide production. However, in comparison to the high biological variation of NT-proBNP, the magnitude of these changes is likely to be of limited clinical relevance.

Chapter 4

THE EFFECT OF THYROID DYSFUNCTION ON PLASMA PEPTIDE YY AND GHRELIN

4.1. Introduction:

Hyper and hypothyroidism are typically associated with weight loss and weight gain respectively. Whilst these changes in weight have classically been attributed to changes in energy expenditure and metabolic demand associated with thyroid dysfunction, it is not clear whether thyroid hormones have an additional effect on gut hormones PYY and ghrelin that may further contribute to changes in weight. Obesity is an important and well known independent risk factor for cardiovascular morbidity and mortality[234-236], and the possible intermediary role of PYY and ghrelin in weight changes associated with thyroid dysfunction is the subject of this chapter.

PYY belongs to a peptide family known as the PP-fold peptides – pancreatic polypeptide (PP), and neuropeptide Y (NPY) are other members of this family. Their common tertiary structure, consisting of an α-helix and polyproline helix connected by a β-turn, results in a characteristic U-shaped peptide known as a PP-fold. In addition to a shared tertiary structure, there is significant homology between peptide sequences within the family. They all have 36 amino acids and contain several tyrosine residues. Furthermore, their biological activity is dependent on C-terminal

amidation[237]. PYY is present in two forms, $PYY_{1–36}$ and $PYY_{3–36}$. $PYY_{3–36}$, the major circulating form[238], is a truncated 34-amino acid form created by cleavage of the N terminus Tyr-Pro residues by dipeptidyl peptidase IV (DPPIV) [239]. DPPIV is found in two forms; as a transmembrane protein widely expressed on endothelial, epithelial, and lymphoid tissue and as a circulating form in plasma. It is responsible for dipeptide cleavage from many peptides including hormones, neuropeptides, and chemokines [240]. However, the actions of DPPIV in regulating the relative postprandial concentrations of $PYY_{1–36}$ and $PYY_{3–36}$ are unknown (Cf Chapter 6)[237].

PYY is secreted from the entire gastrointestinal tract but particularly from the distal portion. PYY immunoreactive cells are almost absent in the stomach, sparse in the duodenum and jejunum, common in the ileum and colon, and at very high levels in the rectum. Food intake results in release of PYY from these cells. The PYY level rises to a plateau at 1–2 h post ingestion, with these peak levels influenced by both the number of calories and the composition of the food consumed. Higher plasma concentrations are seen after isocaloric meals of fat, compared with intake of protein or carbohydrate. It has been shown in healthy humans that gastric distension does not affect PYY secretion[241], however, other stimuli, such as gastric acid, cholecystokinin, and luminal bile salts, also stimulate PYY release[242],. The onset of this release occurs even before nutrients have reached the distal parts of the gastrointestinal tract

where PYY is produced. This infers peptide release may occur via a neural reflex, possibly through the vagus nerve. Other factors also alter circulating PYY. Plasma PYY concentrations are increased by IGF-1, bombesin, and calcitonin-gene-related peptide, and they are reduced by glucagon-like peptide 1 (GLP-1)[237].

PYY has long been known to exert numerous effects on the gastrointestinal tract. Administration of PYY increases the absorption of fluids and electrolytes from the ileum after a meal and delays pancreatic and gastric secretions, gallbladder emptying, and gastric emptying. PYY, when administered peripherally, also affects other systems in the body, *e.g.* it results in a reduction in cardiac output, accompanied by vasoconstriction. PYY causes a reduction in glomerular filtration rate, a reduction in plasma renin and aldosterone activity, and a decrease in lipolysis secondary to a direct effect on adipose tissue[237]. The truncated form, $PYY_{3–36}$ has been reported to have potent effects on appetite. In man, $PYY_{3–36}$, given iv at physiological levels, to normal-weight human volunteers, reduces calorie intake by over 30% [243]. Furthermore, the duration of food intake and their subjective feelings of hunger decrease, without an alteration in gastric emptying. This effect persists 2 h after the infusion is terminated, despite the concentration of circulating $PYY_{3–36}$ returning to basal levels. Plasma PYY is suppressed in patients with morbid obesity. However, the anorectic effect is preserved in obese subjects, with calorific intake reduced to the same extent as lean subjects[244].

This suggests that administration of $PYY_{3–36}$ could perhaps be an effective therapy for obesity. Indeed, chronic peripheral administration to rodents results in a depressed food intake throughout the study, and body weight is reduced in comparison with controls [243].

The central actions of PYY, in contrast to peripheral PYY, are orexigenic. PYY injections into the third, lateral, or fourth cerebral ventricles [245], paraventricular nucleus, or hippocampus potently stimulate food intake in rodents [246]. The truncated form, $PYY_{3–36}$, also simulates food intake when administered intracerebroventricularly. This effect is reduced in both $Y1^{-/-}$ mice and $Y5^{-/-}$ mice [247]. Therefore, although $PYY_{3–36}$ does not have a high affinity for these receptors, they may mediate its central feeding effect.

Ghrelin is the first identified peripherally active orexigenic factor. It is a 28-amino acid peptide with an acyl side chain, *n*-octanoic acid, which has been found to be essential for its actions on appetite. Ghrelin is the endogenous agonist of the GH secretagogue receptor (GHS-R)[248].

The oxyntic cells of the stomach are the main site of ghrelin production. About two thirds of circulating ghrelin is thought to be produced by the stomach. However, ghrelin-producing cells have also been found in the duodenum, ileum, caecum, and colon. Both circulating and nutritional factors from within the gut lumen may trigger these cells to release ghrelin. It is, however, thought to

be calorie intake, which is the primary regulator of plasma ghrelin levels [249]. Circulating ghrelin concentrations have been shown to rise during a period of fasting, peak (to double the baseline concentration) just before eating, and fall rapidly after a meal, suggesting a role as a meal initiator [250]. However, recent work has failed to show a relationship between plasma ghrelin concentrations and meal initiation [251]. Furthermore, plasma ghrelin peaks can be conditioned by altering feeding schedule, suggesting a possible role in physiological preparation for a meal, rather than initiation of feeding [252]. Plasma ghrelin concentrations also show a diurnal variation, in phase with leptin, with highest levels in the morning and lowest at night [250].

GHS-Rs are widely expressed. In the central nervous system (CNS), they are found in the pituitary and hypothalamus, whereas peripheral receptor expression has been described in the myocardium, stomach, small intestine, pancreas, colon, adipose tissue, liver, kidney, placenta, and T cells. In addition, there is some evidence of additional receptor subtype(s) that bind the nonoctanoylated form of ghrelin.

Ghrelin has been demonstrated to be a short-term regulator of food intake in both animals and man. Both central and peripheral ghrelin administrations increase calorie intake in animals [253]. Furthermore, infusing antighrelin antibodies into the rat brain inhibits fasting-induced feeding, supporting ghrelin's role as an endogenous regulator of food intake [254]. Ghrelin is also effective

in man, producing a 28% increase in food intake, when given iv to normal weight volunteers [255].

Ghrelin may also be a regulator of long-term energy balance. Plasma ghrelin levels are strongly correlated with body weight. There is a reversible suppression of ghrelin associated with obesity, such that ghrelin levels normalize after diet-induced weight loss. The fall in plasma ghrelin concentration after bariatric surgery for obesity is thought to be partly responsible for the suppression of appetite and weight loss seen after these operations [256]. Seven-day administration of ghrelin to rodents stimulates weight gain and adiposity secondary to increased food intake [249, 253]. This fat deposition is promoted by a change in metabolism from fatty acid oxidation to glycolysis [249]. However, ghrelin null animals do not have significantly altered body weight or food intake when compared with wild-type littermates [257].

In addition to its actions on food intake, ghrelin induces a dose-dependent stimulation of GH release from the pituitary via its actions on the GHS-R in the hypothalamus [248, 249]. However, it is important to note that the effects of ghrelin on food intake are independent of its effects on GH. Whereas ghrelin is known to increase adiposity, GH reduces adiposity. The effect of chronic ghrelin administration on food intake and body weight is still effective in dwarf GH-deficient rats[249].

Ghrelin may be the first of a number of orexigenic factors that have physiological activity in man. However, as yet, no other

circulating hormone derived from the gastrointestinal tract has been shown to stimulate food intake[237].

We have previously demonstrated that changes in thyroid function are associated with alterations in the concentration of low molecular weight peptides cystatin-C and N-Terminal Pro-B Type Natriuretic Peptide through a metabolic rate mediated mechanism[227, 258]. This study has assessed whether changes in thyroid status are associated with changes in plasma PYY and ghrelin concentrations.

4.2. Subjects and Methods:

Seventeen patients (12 female, 5 male, median age 51yrs, range 24-77) with newly diagnosed biochemical hypothyroidism [Thyroid Stimulating Hormone (TSH) > 5.0 mU/L (normal range 0.5-4.7 mU/L)] and free thyroxine (fT4) ≤ 0.70 ng/dl [mean ± SD: 0.46 ± 0.21; normal range 0.70-1.86 ng/dl (9-24 pmol/L)] and 21 patients (16 female, 5 male, median 48yrs, range 21-66) with newly diagnosed hyperthyroidism (TSH < 0.05 mU/L, fT4 > 1.86 ng/dl (mean ± SD: 3.10 ± 1.38) and free triiodothyronine (fT3) > 0.34 ng/dl [mean ± SD: 1.10 ± 0.77; normal range: 0.03-0.34 ng/dl (0.5-5.3 pmol/L)] had fasting plasma PYY and ghrelin measured at baseline and when they subsequently became euthyroid (TSH < 4.8 mU/L, fT4 0.70-1.86 ng/dl). Patients were sequentially recruited from the endocrine out-patient clinic at Michael White Centre, Hull Royal Infirmary over a three month period.

Hypothyroid patients were treated with thyroxine 100μg once daily (od) for 6 weeks. The 4 patients who were not rendered euthyroid on this regime became so when given 150μg thyroxine od for a further 6 weeks. Twelve of the hyperthyroid patients became euthyroid after receiving carbimazole 20mg od for 6 weeks. Five became hypothyroid at this stage so had 100μg thyroxine od added and were euthyroid by week 12 on the combination. Two patients required an increase in their carbimazole dose to 40mg daily after 6 weeks and became euthyroid by week 18. All samples taken, spun immediately and serum frozen at -20ºC, until batch analysed in a single run at the end of the study. The study gained local ethics committee approval and patients gave their written informed consent for participation.

All thyroid assays were performed on an Abbott Architect i4000 immunoassay analyser (Abbott Diagnostics Division, Maidenhead, Berkshire, UK). Plasma ghrelin-like and PYY-like immunoreactivity were measured using specific and sensitive radioimmunoassays as previously described[259, 260]. All samples were assayed simultaneously and in duplicate to eliminate the effect of inter-assay variation. The ghrelin assay cross-reacted fully (100 %) with both acylated and des-acylated ghrelin and did not cross-react with any other known gastrointestinal or pancreatic hormone. The antiserum, SC-10368 (Santa Cruz Biotechnology, California, USA), was used at a final dilution of 1:50,000. 125I

ghrelin was prepared using Bolton & Hunter reagent (Amersham International UK) and purified by high pressure liquid chromatography. The assay detected changes of 25 pmol/L with a 95 % confidence limit. The intra-assay coefficient of variation was 5.5 %.

The PYY assay cross-reacted fully with $PYY_{1–36}$ and $PYY_{3–36}$ and did not cross-react with pancreatic polypeptide, neuropeptide Y, or any other known gastrointestinal hormone. The antiserum (Y21) was raised in a rabbit against synthetic porcine PYY (Bachem, UK) coupled to bovine serum albumin by glutaraldehyde and was used at a final dilution of 1:50 000. 125I PYY was prepared by the iodogen method and purified by high pressure liquid chromatography. The assay detected changes of 2 pmol/L with a 95 % confidence limit. The intra-assay coefficient of variation was 5.8 %.

4.3. Results:

Shapiro-Wilk test, normal plots and histograms (using GB-STAT version 9.0, Dynamic Microsystems, Inc), showed that data distribution for PYY in both groups, and ghrelin in hypothyroid subjects were normal and that data were non-Gaussian for ghrelin in hyperthyroid subjects. Therefore Wilcoxon Signed-Ranks test was used to compare ghrelin levels in hyperthyroid patients before and after treatment and parametric methods were used to analyze the rest of the data. SPSS for Windows (version 12, SPSS Inc) was used for statistical analysis unless otherwise specified.

There was no significant change in PYY levels in either group before and after treatment (mean PYY in pmol/L±SD before and after treatment: 24.81±12.10 vs. 23.22±15.27, p=0.428 hypothyroid; 19.47±8.09 vs. 20.87±10.26, p=0.464 hyperthyroid). The observed changes in ghrelin in both groups did not reach statistical significance either (mean ghrelin in pmol/L±SD before and after treatment: 761.32±435.46 vs. 697.60±376.09, p=0.227 hypothyroid; p=0.058 for hyperthyroid group with 6 negative ranks, 15 positive ranks and a trend towards higher ghrelin levels after treatment). The subjects' weights in the hypothyroid group did not change significantly after treatment to euthyroidism (mean weight in kg ± SD before and after treatment: 74.84 ± 9.08 vs. 74.14 ± 9.05; p=0.417). There was a significant weight gain in the hyperthyroid group of patients following treatment to euthyroidism (mean weight in kg ± SD before and after treatment 72.27 ± 18.13 vs. 74.78 ± 17.24; $p<0.05$).

4.4. Discussion:

The presented data shows that change in thyroid status does not significantly alter gut hormones PYY and ghrelin levels.

Gut hormones have an important physiological role in postprandial satiety. The gut contains a diffuse population of endocrine cells that release several circulating hormones in response to changes in luminal nutriment content. Recent

evidence has shown that gut hormones administered at physiological concentrations can influence appetite in rodent models and humans. Ghrelin is a strong stimulant for food intake and growth hormone secretion. Plasma ghrelin levels are inversely correlated with body weight and rise following weight loss in humans. Though calorie intake appears to be the primary regulator of plasma ghrelin levels, the exact mechanisms mediating ghrelin release are unknown. PYY is an anorectic hormone which is released from the gastrointestinal L-cells post-prandially. Food intake and body weight are reduced in rodents chronically treated with peripheral $PYY_{3–36}$.[261]

The hypothalamus is a center for interpretation and integration of adiposity related long-term humoral signals mediated by insulin and leptin as well as short-term situational and meal related signals from various sources including the gastrointestinal tract, the environment and higher brain centers.[262] Excess food intake, characteristic of hyperthyroidism, is thought to be a compensatory mechanism in response to the higher energy demand associated with this condition. However, Kong *et al* have demonstrated in animal studies that triiodothyronine directly stimulates feeding at the level of hypothalamus via ventromedial nucleous and its numerous projections to other areas of the hypothalamus[263], and this direct effect may account for the observed weight gain in 5-10% of patients with thyrotoxicosis.

In conclusion, thyroid dysfunction does not alter plasma PYY and ghrelin concentrations and changes in weight associated with thyroid dysfunction are more likely to be due to a combination of altered metabolic rate and the direct effect of T_3 or lack of it on hypothalamus.

Chapter 5

THE EFFECT OF DIETARY SOY PHYTOESTROGENS ON THYROID FUNCTION AND INSULIN RESISTANCE IN PATIENTS WITH SUBCLINICAL HYPOTHYROIDISM: A DOUBLE BLIND CROSSOVER STUDY

5.1. Introduction:

As discussed earlier, Subclinical hypothyroidism is a common condition with a prevalence of 4 to 10% in the general population[9, 10, 108]. Studies have suggested that it may lead to an increase in cardiovascular risk through a number of possible mechanisms including a rise in insulin resistance[264].

It has been shown that naturally occurring phytoestrogens can reduce insulin resistance and have beneficial effects on other cardiovascular risk factors in postmenopausal women and in patients with type 2 diabetes[265, 266]. In view of substantial evidence that soy protein intake improves serum lipid profiles, the US Food and Drug Administration and the American Heart Association issued a recommendation of daily consumption of ≥25 g soy protein as a preventive measure to reduce the risk of heart disease[267]. However, the effect and safety of soy phytoestrogens on the thyroid is not well established[201]. Nevertheless, the effect of soy phytoestrogens in patients whose thyroid function is already compromised may be more clinically important, particularly in pregnancy where the prevalence of

subclinical hypothyroidism is around 2 to 5%[10, 268] , and has been associated with a reduced intelligence quotient in the offspring of the affected patient[269].

This double blind, cross over trial was undertaken to determine the effect of soy phytoestrogens on thyroid function and cardiovascular risk markers in patients with subclinical hypothyroidism. We compared the effect of combining 30g of soy protein with either 2mg phytoestrogens or 16mg of phytoestrogens (the latter being the amount expected in a vegetarian diet with high phytoestrogen intake, or in a health food supplementation) with the aim of providing definitive evidence on the activity, safety and/or possible benefits of phytoestrogens in patients with compromised thyroid function.

5.2. Subjects and Methods:

Patients with subclinical hypothyroidism with a TSH value of between 5-15 mU/L (normal range 0.5-4.7 mU/L) with a normal fT_4 were recruited following identification through routine biochemical testing performed at Hull Royal Infirmary between July 2005 and August 2006. The baseline characteristics are given in Table 5.1.

Table 5.1: Baseline characteristics

Characteristics	**2mg followed by 16mg phytoestrogen group**	**16mg followed by 2mg phytoestrogen group**
Number of patients	30	30
TPO antibody positive n (%)	20 (66.7)	18 (60)
Male/Female	4/26	6/24
Age (Mean ± SD)	56.7 ± 18.0	58.7 ± 13.1
Weight kg	76.4 ± 19.1	80.9 ± 20.1
BMI kg/m^2	28.3 ± 6.4	29.0 ± 7.2

Exclusion criteria included patients taking thyroxine or drugs that could interfere with thyroid function such as lithium, carbimazole and propylthiouracil, patients whose dose of antihypertensive or lipid lowering medications had been modified within 3 months of their participation in the trial, and antibiotic use within the last 3 months prior to enrolment in the study, as antibiotics are known to change gut microflora and thus would interfere with phytoestrogen metabolism[270]. At randomisation and during each visit, all subjects were instructed to maintain their diet and level of physical activity throughout the study. In addition, subjects were required to avoid alcohol, herbal, vitamin or mineral supplementation. Compliance checks and dietary reinforcement were performed at each visit. One 24 hour urine collection was made during the study period to measure urine iodine excretion and to exclude iodine deficiency.

A randomized, double-blind, cross over design was employed. In view of the known palatability issue encountered previously with the soy protein powder[271], patients were given a 2 week run in and if they were not able to tolerate the 2mg or 16mg preparation then they were withdrawn from the study. Patients were replaced until the 60 patients needed to give adequate statistical power to the study were recruited. A total of 11 patients in the 2 mg arm and 12 patients in the 16mg arm were unable to tolerate the soy preparation within the initial 2 week period and therefore did not proceed to enter the main study. Sixty patients found the preparation tolerable; a total of 30 patients initiated with the 2mg preparation and 30 patients on the 16mg preparation. After an 8 week wash out period, the participants crossed over to the alternative treatment for 8 weeks (second phase). Each box contained the number of sachets required for eight weeks of treatment plus 6 sachets marked as reserve. A computer generated randomisation list was used. All subjects gave their written informed consent and the protocol was approved by the Hull and East Riding local research ethics committee. The study was registered as 'a double-blind, placebo-controlled, crossover trial of soy phytoestrogens in patients with compensated hypothyroidism' on UK Clinical Trials Gateway website (http://www.controlled-trials.com/ukctg) with International Standard Randomised Controlled Trial Number (ISRCTN) of 55827330.

From a previous study with a significant reduction in T_4 , a sample size of 50 patients in a cross over design was calculated giving

80% power to detect a mean decrease of 0.4 nmol/L of T_4, with a two sided alpha error of 0.05.

At the beginning and end of each phase, following an overnight fast, weight and blood pressure were measured and blood samples were collected. The blood pressure was measured after the patients were seated quietly for at least 5 minutes in a chair and the right arm supported at heart level. A cuff bladder encircling at least 80% of the arm was used to ensure accuracy. Blood pressure measurements were performed using an automated device (NPB-3900; Nellcor Puritan Bennett, Pleasanton, CA) during each study visits. Two readings were obtained at the beginning of each visit at least one minute apart. If there was more than a 5 mmHg difference in systolic BP between the 2 readings, a third reading was obtained. Fasting venous blood samples were collected into serum gel, EDTA, and fluoride oxalated tubes, separated by centrifugation at 2000g for 15 min at 4°C, and the aliquots stored at -80°C within 1 hour of collection. Plasma glucose was measured using a Synchron LX20 analyzer (Beckman-Coulter), and serum insulin was assayed using a competitive chemiluminescent immunoassay performed using the DPC Immulite 2000 analyzer (Euro/DPC, Llanberis, U.K.). The coefficient of variation of this method was 8%, calculated using duplicate study samples. The analytical sensitivity was 2μU/ml and there was no stated cross-reactivity with proinsulin. The insulin resistance was calculated using the Homeostasis Model Assessment method (HOMA-IR = (Insulin x glucose)/22.5)[272] .

Urinary iodine measurements in 24 hour urine collections were undertaken by ICP-MS to monitor for iodine repletion and plasma phytoestrogen measurement was undertaken by LC-MS/MS (Central Science Laboratory, Sand Hutton, York, UK).

The low dose phytoestrogen preparation consisted of 30 g of soy protein concentrate (70% proteins) containing 2 mg of phytoestrogens, whereas high dose phytoestrogen preparation contained 16 mg of phytoestrogens. The phytoestrogen material (Solgen 40) was supplied by Solbar Ltd, Israel, and prepared by Essential Nutrition, Ltd, Brough, UK, who also randomised the sachets.

5.3. Results:

Paired t tests were used to compare thyroid function tests before and after treatment with 2mg and 16mg phytoestrogens, whereas Wilcoxon signed-ranks test was applied to phytoestrogen data that violated the assumptions of normality when tested using the Kolmogorov-Smirnov test. The period and the carryover effect that may have occurred from the cross-over design were also tested using the appropriate Student t test. Independent samples t test was used to compare plasma phytoestrogen levels after wash out and after 2 mg/16 mg phytoestrogens. Statistical analysis was performed using SPSS for Windows NT version 14.0 (SPSS, Chicago, IL). An arbitrary level of 5% statistical significance (two-tailed) was assumed.

Of the 60 patients randomized, 10 patients withdrew in the first arm and 6 patients in the second phase (loose stools: 3, progression to symptomatic hypothyroidism: 6, transient thyroiditis: 4, use of antibiotics: 1 , worsening of diabetes control: 1, moved out of area: 1). Six patients completing both arms of the study were excluded due to plasma phytoestrogen values greater than 3 SD above the mean suggesting consumption of additional phytoestrogens. Intention to treat analysis was done. A flow chart describing the progress of the patients in the study is given in figure 5.1 as per the CONSORT (Consolidated Standards of Reporting Trials) statement[273].

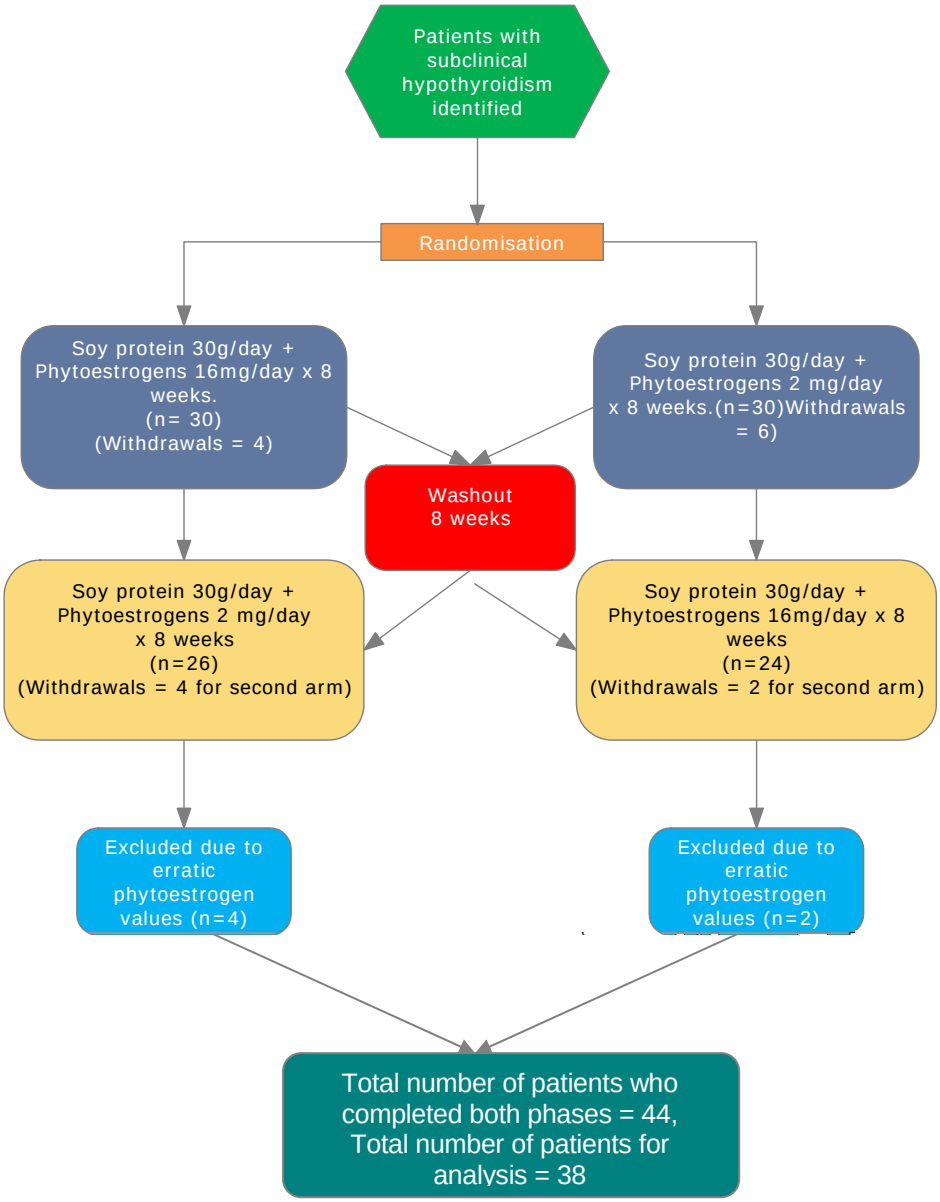
Patients with subclinical hypothyroidism identified
Randomisation
Soy protein 30g/day + Phytoestrogens 16mg/day x 8 weeks. (n= 30) (Withdrawals = 4)
Soy protein 30g/day + Phytoestrogens 2 mg/day x 8 weeks.(n=30)Withdrawals = 6)
Washout 8 weeks
Soy protein 30g/day + Phytoestrogens 2 mg/day x 8 weeks (n=26) (Withdrawals = 4 for second arm)
Soy protein 30g/day + Phytoestrogens 16mg/day x 8 weeks (n=24) (Withdrawals = 2 for second arm)
Excluded due to erratic phytoestrogen values (n=4)
Excluded due to erratic phytoestrogen values (n=2)
Total number of patients who completed both phases = 44, Total number of patients for analysis = 38

Figure 5.1

There was a significant reduction in systolic blood pressure of (mean ± SD) 2.57 ± 3.9 mmHg and diastolic blood pressure of (mean ± SD) 1.64 ± 3.7 mmHg after 16 mg phytoestrogen phase (Table 5.2). There was also a significant reduction of systolic blood pressure of (mean ± SD) 1.1 ± 3.9 mmHg after 2mg phytoestrogen phase but no significant reduction in diastolic blood pressure. There were no changes in the lipid profile and hsCRP pre and post treatment (Table 5.3).

Table 5.2: Weight and blood pressure of patients after 16mg phase and after 2 mg phase by paired t test

	16mg phytoestrogen phase (95% CI)(p value)	**2mg phytoestrogen phase (95% CI)(p value)**
Weight (kg)	79.45 ± 20.45 vs. 79.29 ± 20.50 (0.18 – 0.50) (0.939)	78.35 ± 19.41 vs. 78.36 ± 19.10 (-0.30 – 0.53) (0.585)
Systolic BP (mmHg)	140.71 ± 7.7 vs. 138.14 ± 8.5 (1.51 – 3.64) (<0.001)	140.75 ± 8.2 vs. 139.64 ± 7.8 (0.05 – 2.2) (0.04)
Diastolic BP (mmHg)	76.76 ± 6.7 vs. 76.00 ± 5.6 (1.62 – 3.74) (0.002)	76.77 ± 6.9 vs. 77.38 ± 6.2 (-1.67 – 0.44) (0.245)

There was a significant improvement in insulin resistance measured by HOMA-IR (mean ± SD) [5.11 ± 12.2 vs. 4.2 ± 9.9 (p value= 0.02) 95% CI 0.12, 1.63] and plasma glucose (mean ± SD) [5.6 ± 1.1 vs. 5.3 ± 1.2 (p value= 0.006) 95% CI 0.11, 0.59] after 16mg phytoestrogen phase but not after 2mg phytoestrogen phase (table 5.4).

Table 5.3: Lipid Profile and hsCRP of patients post 16mg phase and post 2 mg phase by paired t test

Tests*	Mean ± SD	Sig. (2 tailed)
Total Cholesterol post 16mg vs. post 2mg phytoestrogen (mmol/L)	5.50 ± 1.15 vs. 5.71 ± 1.34	0.12
HDL Cholesterol post 16mg vs. post 2mg phytoestrogen (mmol/L)	1.37 ± 0.32 vs. 1.40 ± 0.34	0.22
LDL Cholesterol post 16mg vs. post 2mg phytoestrogen (mmol/L)	3.49 ± 1.10 vs. 3.70 ± 1.25	0.17
Total Cholesterol/HDL ratio post 16mg vs. post 2mg phytoestrogen	4.13 ± 0.99 vs. 4.21 ± 1.03	0.47
Triglycerides post 16mg vs. post 2mg phytoestrogen (mmol/L)	1.34 ± 0.67 vs. 1.34 ± 0.64	0.83
Hs-CRP post 16mg vs. post 2mg phytoestrogen (mg/L)	3.85 ± 3.32 vs. 3.72 ± 3.38	0.70

Table 5.4: Insulin resistance of patients before and after phytoestrogens

	Mean ± SEM	Sig.(2tailed)
Insulin pre and post 16mg phytoestrogen phase (µIU/mL)	17.30 ± 5.8 vs. 15.25 ± 4.6	0.09
Insulin pre and post 2mg phytoestrogen phase (µIU/mL)	12.54 ± 1.4 vs. 11.14 ± 1.2	0.20
Glucose pre and post 16mg phytoestrogen phase (mmol/L)	5.64 ± 0.2 vs. 5.30 ± 0.2	0.006
Glucose pre and post 2mg phytoestrogen phase (mmol/L)	5.33 ± 0.2 vs. 5.42 ± 0.2	0.19
HOMA-IR pre and post 16mg phytoestrogen phase	5.11 ± 1.9 vs. 4.28 ± 1.5	0.02
HOMA-IR pre and post 2mg phytoestrogen phase	3.14 ± 0.5 vs. 2.97 ± 0.5	0.48

Thyroid function tests after 2mg and 16mg phytoestrogen treatments are given in table 5.5. The fT_3 values were significantly higher after treatment with 2mg phytoestrogen (mean ± SD) (4.07

± 0.67 vs. 4.39 ± 0.63 pmol/L p <0.01) but there were no changes in other thyroid parameters after treatment. Subgroup analysis revealed that fT_3 values were significantly higher after 2 mg phytoestrogen phase (4.11 ± 0.62 vs. 4.56 ± 0.65 p value <0.01) but not after 16mg phytoestrogen phase (4.22 ± 0.71 vs. 4.25 ± 0.61 p value=0.80) in patients who received 16 mg phytoestrogen in phase 1. There were no significant differences in thyroid function tests in patients entering the 2 mg phytoestrogen in phase 1. Thyroid antibody titres did not alter before or after either treatment.

Table 5.5: Thyroid function tests before and after phytoestrogens

Thyroid Function Tests **fT3 in pmol/L, fT4 in pmol/L and TSH in mU/L**	**Mean ± SD**	**Sig. 2 tailed**	**Mean % change**
fT3 pre and post 16mg phytoestrogen phase	4.22 ± 0.71 vs. 4.25 ± 0.61	0.80	2%
fT3 pre and post 2mg phytoestrogen phase	4.07 ± 0.67 vs. 4.39 ± 0.63	<0.01	11%
fT4 pre and post 16mg phytoestrogen phase	11.74 ± 1.68 vs. 11.9 ± 1.57	0.43	2%
fT4 pre and post 2mg phytoestrogen phase	11.61 ± 1.47 vs. 11.87 ± 1.40	0.20	3%
TSH pre and post 16mg phytoestrogen phase	6.26 ± 2.18 vs. 6.31 ± 2.44	0.84	2%
TSH pre and post 2mg phytoestrogen phase	6.40 ± 1.79 vs. 6.07 ± 2.03	0.36	-1%

The plasma extracts were screened for the presence of sixteen different phytoestrogens. The lignan profile, including enterolactone and enterodiol, was constant but low throughout the study. The levels of non-soy phytoestrogens such as formononetin, biochanin A and coumestrol were also low and static. The baseline values were consistent with the standard United Kingdom low content soy diet. The plasma phytoestrogen levels attained after 2 mg phytoestrogens (mean ± SD) ((daidzein (9.5 ± 10.2 ng/ml vs. 4.6 ± 5.5 ng/ml p = 0.06) (genistein (10.9 ± 16.0 ng/ml vs. 8.2 ± 10.3 ng/ml p = 0.52)) and 16mg phytoestrogens (mean ± SD) ((daidzein (20.2 ± 21.5 ng/ml vs. 24.8 ± 28.5 ng/ml p = 0.56) (genistein(35.6 ± 39.2 ng/ml vs. 51.5 ± 63.6 ng/ml p = 0.33)) were comparable in both groups. The baseline levels of phytoestrogens were comparable to the levels after the washout phase (mean ± SD) (daidzein 2.6 ± 2.9 ng/ml vs. 2.9 ± 2.7 ng/ml p = 0.82) (genistein 2.6 ± 3.7ng/ml vs. 3.5 ± 5.3ng/ml) which confirmed that after outliers were excluded, the remaining patients had both adhered to the protocol and avoided extra dietary sources of phytoestrogens.

The 24 hour urine iodine estimation revealed urinary iodine excretion of 206 ± 105 microgram/day (mean ± SD) (range: 106 - 672 microgram/day).

5.4. Discussion:

This study showed that there was a significant reduction of blood pressure and insulin resistance after 16mg phytoestrogen

preparation and a significant reduction of diastolic blood pressure after 2mg phytoestrogen phase as well as no clinical effect on thyroid function even in patients with subclinical hypothyroidism. Although the magnitude of reduction in blood pressure in our study is small, the public health implications may be important, given that a small reduction in population wide blood pressure can lead to a substantial decrease in cardiovascular risk in the society.

Historically, epidemiologic observations of diet and cardiovascular disease in Japan and China have linked soy-product consumption with a reduced cardiovascular risk[271, 274] . Studies of phytoestrogen intakes in Western countries, indicate average daily intakes around 2 mg phytoestrogens[275, 276] equivalent to the low dose phytoestrogen preparation used here. Higher phytoestrogen levels are found in United Kingdom vegetarians[277] , with levels corresponding to the 16mg phytoestrogen phase of this study, which was consumed in addition to a Western diet avoiding soy products. The hypotensive effect of soy phytoestrogens is reported though the literature is conflicting[278-280]. These studies are difficult to compare as different soy phytoestrogen/placebo/comparator preparations have been used in different study populations. To attempt to see if this was a real effect, we added soy phytoestrogens to soy protein and then compared two different phytoestrogen doses. We intentionally chose patients with subclinical hypothyroidism who are reported to have a higher cardiovascular risk[106] and probably a more sensitive thyroid

gland to external insult, and therefore a greater chance to see if changes were to take place. Whilst the 16mg of phytoestrogen was more effective than 2 mg at reducing blood pressure, it could be a matrix effect with the soy protein. In another study with high dose phytoestrogens alone (132mg daily) without soy protein we found that there was no effect on cardiovascular disease markers in patients with type 2 diabetes[281].

There was unexpectedly no significant improvement in lipid profile with neither high nor low dose phytoestrogens in this study. On the other hand, when 132mg of phytoestrogens with 30 g soy protein was used, cardiovascular parameters were reduced within the same time frame in patients with type 2 diabetes[266]. The lack of improvement of lipid profile in this study might be due to the lower dose of phytoestrogens used. However, supplementation with phytoestrogens alone (40–150 mg/day) have not altered lipid profiles[282, 283]. Conversely, isolated soy protein has been shown to improve lipid profile[284]. Beneficial effects have also been observed with different combinations of soy protein and phytoestrogens concentrations[266, 280, 285, 286].

Overall there was no significant difference in any of the thyroid parameters after 2mg or 16mg soy phytoestrogen supplementation. However, subgroup analysis showed that patients who received the low dose, 2 mg phytoestrogens, after the high dose (16 mg phytoestrogens) phase had an increase in the fT_3

levels although the T_4 or TSH values did not differ. The magnitude of the rise of fT_3 in this group is unlikely to be of clinical significance and this was not seen in the 2mg phytoestrogen phase leading to the 16mg phase. In a similar study that we have performed in patients with type 2 diabetes there was an increase in the T_4 levels by 8.4% but T_3 levels were unchanged by 132mg of phytoestrogens in soy protein[266]. There is evidence to show synergism of soy phytoestrogens with iodine deficiency in producing hypothyroid effects in rats[287]. A urinary iodine concentration of 100 microgram/L corresponds to an intake of about 150 microgram per day, which is the daily requirement of iodine in adults[288]. In our study, all patients had sufficient iodine excretion of more than 100microgram per day. It has been shown that soy phytoestrogens inactivate thyroid peroxidase both in vitro and in vivo[289] , in this study there was no alteration in thyroid peroxidase antibody status in patients with subclinical hypothyroidism. That there was no clinically significant change in thyroid parameters is reassuring, especially in view of the observation that children of women with high serum TSH concentrations during pregnancy performed less well on tests of neuropsychological development[290].

The 16mg phytoestrogen used in this study is the amount expected in a vegetarian diet with high phytoestrogen intake, or health food supplementation. This study shows that with respect to thyroid function, 16 mg of phytoestrogen is safe and there is no need to alter dietary advice to this subgroup of the population

with regard to soy consumption. However, dietary intake of phytoestrogens in Asian diets has been estimated to be in the range of 30-50 mg per day of combined phytoestrogen aglycone equivalents[291, 292] and the effect of this dose on compromised thyroid function remains unclear.

There was a significant improvement in insulin resistance after the 16mg phytoestrogen phase indicating the activity of the preparation. This is in accord with reports of diets containing soy protein rich in phytoestrogens that improve insulin resistance in ovariectomised cynomolgus monkeys[293], and other studies showing reduced insulin levels in patients with type 2 diabetes as well as in postmenopausal women[266, 285]. This is particularly relevant in view of studies suggesting increase in cardiovascular mortality in patients with subclinical hypothyroidism[264]. In a cross-sectional cohort study TSH was significantly associated with insulin resistance in euthyroid range after adjustment for age and sex[294]. However, it is important to note that we found no effect on insulin resistance when using phytoestrogen alone with no soy protein, whereas this study has shown an effect of high dose phytoestrogen with soy protein, but not low dose phytoestrogen with soy protein. This would suggest that soy protein on its own (like phytoestrogen) would not be affective, but rather it is the combination with phytoestrogen that is important. The reduction of insulin resistance in patients with subclinical hypothyroidism should in theory have a protective effect on their cardiovascular risk profile.

In conclusion, the cardiovascular protection suggested for phytoestrogens epidemiologically may be through their positive effect on insulin resistance and blood pressure. However low and high levels of dietary phytoestrogens have no clinical effect on thyroid function even in patients compromised with subclinical hypothyroidism.

Chapter 6

GENERAL DISCUSSION

This thesis examines the relationship between thyroid dysfunction and the cardiovascular risk by combining a retrospective cardiovascular outcome study (chapter 2) with 3 clinical trials that employ surrogate cardiovascular risk markers (chapters 3 to 5). By combining the findings of these studies with the review of literature that has been provided in the Introduction (Chapter 1) and the 'discussion' sections of individual studies, the author concludes that the effects of thyroid dysfunction on the cardiovascular system are subtle, and these effects do not always translate into adverse cardiovascular outcomes.

As noted in the introduction to this thesis, considerable amount of evidence points to various effects of excess thyroid hormone or the lack of it on the cardiovascular system. However, the exact mechanisms through which these effects are exerted are not wholly understood. There also remain unsolved clinical dilemmas. The debate on the treatment of SCH for example, has been ongoing for years, and despite published consensus statements on the subject, there does not seem to be unanimity among the experts as to when to treat SCH [82, 83]. Today, the controversy goes beyond the experts and extends itself to patients, patient advocacy groups and politicians[295].

The first study presented in this thesis, 'Chapter 2: the effect of subclinical hypothyroidism on cardiovascular outcomes in patients with type 2 diabetes mellitus', is an attempt to see whether SCH incurs additional risk of CVD in patients with type 2 diabetes. The study is genuine in that this relationship had never been studied in T2DM population before. This study did not show an increase in cardiovascular events or mortality as a result of SCH in the study population. The main limitation of the study is a marked difference in sex distribution of patients in the case versus the control group (83.7% females in the cases vs. 18% females in the controls). This disparity was imposed on the researchers by the limited number of control patients extracted from the database after limiting the upper cut off point of TSH value to 3.0 mU/L for the reason explained before (cf. 1.9.3), and after implementing the rest of the exclusion criteria as detailed in section 2.2. This limitation is at least partly accounted for by statistically adjusting for the sex distribution difference. Although female predominance in the case group might as well provided a protective effect against CVD, their 2 years higher mean age has no doubt attenuated this protective effect to some extent.

One should also consider the generic limitations of retrospective studies in establishing causal relationships compared to prospective randomised controlled clinical trials. However, given the nature of SCH, prospective studies are likely to be confounded by progression to overt hypothyroidism during the course of the study, and that the magnitude of change and response to

treatment would likely to be small and difficult to distinguish from controls[296].

All the same, the study is novel in terms of its target population and its findings are in line with 2 major studies on the subject, i.e., the Whickham Survey[63] and the Cardiovascular Health Study[132]; as well as recent Cochrane review[199], and a smaller study that did not find an association between SCH and presence or severity of cardiovascular disease[297].

The lack of significant effect from SCH on cardiovascular outcomes (Chapter 2) is in a way reflected in the results of the second study (Chapter 3, 'The effect of thyroid dysfunction on N-terminal pro-B-type natriuretic peptide concentrations'), which shows that NT-proBNP – a marker of heart failure – is not significantly affected by thyroid dysfunction. Compared to a previous publication by Schultz et al[228] on the subject, here we adopted a new approach in interpreting the findings in the context of the wide biological variation of NT-proBNP (cf. 3.4.). Along the way, Schultz et al paper was also critically examined and we explained the reason why our findings differ from those of Schultz et al [228] (cf. 3.4.).

There is a body of evidence pointing to a negative correlation between levels of circulating thyroid hormones and IL-6[174, 175, 298]. Although this relationship has been well studied in non-thyroidal illness, the evidence on such relationship with hypothyroidism is lacking. Given the association of IL-6 with low

levels of thyroid hormones, and the recent findings indicating the importance of IL-6 as an independent predictor of CHF[176], one might assume that hypothyroidism and CHF may have a mutually deteriorating effect on each other. This hypothesis has yet to be tested in clinical trials and may be of interest for future research.

Chapter 4 assesses the plasma levels of gut hormones PYY and ghrelin in relation to hyper and hypothyroidism, exploring the possibility of a role for these gut hormones in the weight changes associated with thyroid dysfunction. Similar to the case of NT-proBNP, we did not found a change in plasma PYY and ghrelin levels.

The role of gut hormones PYY and ghrelin in regulating food intake and therefore weight is well established. Abdominal obesity is a major component of the metabolic syndrome and is incorporated in both the National Cholesterol Education Program's Adult Treatment Panel (ATP) III definition and the International Diabetes Federation (IDF) criteria along with dyslipidaemia, hypertension, and glucose intolerance[299, 300]. It has been shown that disrupting ghrelin signalling by targeting the ghrelin or ghrelin receptor genes blunts weight gain from a high-fat diet in mice[301, 302]. Also, simultaneous deletion of ghrelin and its receptor (double knock-out mice) enhances the metabolic phenotype of single gene-deficient mice compared with wild-type mice, possibly suggesting the existence of additional molecular components of the endogenous ghrelin system[303]. The double

knock-out mice in this study showed decreased body weight, increased energy expenditure, and increased motor activity on a standard chow diet[303]. Therefore, inhibiting ghrelin can serve as a therapeutic means for weight reduction and provides ample food for future research. One recent try along this line is the identification and characterisation of human GOAT (ghrelin *O*-acyl transferase), the lipid transferase that modifies the O-linked octanoyl side group of ghrelin molecule[304]. This modification is crucial for ghrelin's physiological effects including regulation of feeding, adiposity, and insulin secretion. Because octanoylated ghrelin promotes food intake and adiposity and also suppresses insulin secretion and impairs glucose tolerance, GOAT may provide a critical molecular target in developing novel therapeutics for obesity and type 2 diabetes[304].

Another anorexigenic gut hormone, which may at least partly exert its effect on appetite and weight through suppression of circulating ghrelin levels, is oxyntomodulin[305]. Subcutanously self-administered oxyntomodulin in healthy overweight and obese volunteers resulted in weight loss and a change in the levels of adipose hormones consistent with a loss of adipose tissue[306]. The anorectic effect of oxyntomodulin was maintained over the course of the trial (4 weeks)[306] and can potentially be used in the treatment of obesity and the metabolic syndrome.

PYY is a high affinity substrate for dipeptidyl peptidae IV (DPP-IV)[307]. Whereas cleavage of glucagons-like peptide-1 (GLP-1)

by DPP-IV causes inactivation, cleavage of PYY yields the active form, $PYY_{3–36}$. Thus, DPP-IV does not inactivate PYY but, rather, alters its biological activity. Given the role of obesity in type 2 diabetes, prevention of the formation of such an endogenous food intake inhibitor by a DPP-IV inhibitor may be undesirable. Although the results of clinical trials on DPP-IV inhibitors indicating that they are weight neutral[308] argues against this, with rising popularity of DPP-IV inhibitors as promising anti-diabetic agents, the relationship between PYY and DPP-IV warrants further experimentation.

The recurring theme of Chapters 2-4 was also seen in Chapter 5, where we found that a daily supply of phytoestrogens and 30 g of soy protein improved 2 components of the metabolic syndrome, i.e. insulin resistance and blood pressure, without affecting the thyroid function tests. It was interesting to see that these reductions in insulin resistance and blood pressure were independent of body weight. However, longer term prospective trials are needed to see whether the improvement seen in these 2 markers of the metabolic syndrome actually translate into better cardiovascular outcomes.

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