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The interaction between growth hormone and the thyroid axis in hypopituitary patients: in vivo and ex vivo studies

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Candidate Thesis Declaration

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a higher degree, Doctor of Medicine (MD), is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

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Abbreviations

AGHDA	Assessment of growth hormone deficiency in adulthood
AO-GHD	Adult-onset growth hormone deficiency
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BALP	Bone alkaline phosphatase
BMI	Body mass index
BMR	Basal metabolic rate
CH	Central hypothyroidsim
CO-GHD	Childhood-onset growth hormone deficiency
CTX-1	C-terminal telopeptides of type I collagen
Cu	Copper
DIO1	Type 1 deiodinase isoenzyme
DIO2	Type 2 deiodinase isoenzyme
DIO3	Type 3 deiodinase isoenzyme
EF	Ejection fraction
ET	Ejection time
Fe	Iron
GH	Growth hormone
GHD	Growth hormone deficiency
HDL-C	High density lipoprotein cholesterol
HPT	Hypothalamic pituitary thyroid axis
IDL	Intermediate density lipoprotien
ICT	Isovolumetric contraction time
IRT	Isovolumetric relaxation time
LDL-C	Low density lipoprotein cholesterol
Lp(a)	Lipoprotein a
LVET	Left ventricular ejection time
LVM	Left ventricular mass
MPHD	Multiple pituitary hormone deficiencies

Abbreviations (contd)

MPI	Myocardial performance index
NHP	Nottingham Health Profile
OCI	Osteocalcin
P1NP	Procollagen type 1 amino-terminal propeptide
REE	Resting Energy Expenditure
rT3	Reverse 3,5,3’-triiodothyronine(T3)
Se	Selenium
SF36	Short Form 36
T3	3,5,3’-triiodothyronine
T4	Thyroxine
TBI	Traumatic brain injury
TFT	Thyroid function tests
TPO	Thyroid peroxidase
Trig	Triglyceride
TR	Thyroid hormone receptor
TRE	Thyroid hormone responsive element
TRH	Thyrotrophin releasing hormone
TSH	Thyroid stimulating hormone

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Summary of Thesis

Alterations in the hypothalamo-pituitary-thyroid (HPT) axis have been reported following growth hormone (GH) replacement. The aim of this study was to examine potential mechanisms responsible for GH-induced changes in the HPT axis. Furthermore, we aimed to explore the relationship between changes in the serum concentration of thyroid hormones and peripheral biomarkers of thyroid hormone action, before and after GH replacement.

Twenty men with severe GH deficiency participated in a prospective, observational study of physiological GH replacement. Research measurements were conducted immediately prior to commencing, and 3-6 months after, GH replacement.

Following GH replacement, the ratio of freeT3:freeT4 in serum increased. In subcutaneous fat, Type 2 deiodinase enzyme activity declined. Type 1 and Type 3 deiodinase activity remained unchanged following GH substitution. Serum TSH, thyroglobulin and thyroid binding globulin levels were unchanged by GH therapy.

Hepatic-derived serum markers of thyroid hormone action, including ferritin and caeruloplasmin, declined following GH replacement. In contrast, serum markers of bone turnover increased in parallel with changes in serum concentration of thyroid hormones. Resting energy expenditure and cardiac time intervals did not change throughout the study. Health-related quality of life (QOL) improved following GH replacement in the full study group. However, the improvement in QOL was poorer than expected in subjects who experienced a fall in serum free T4.

This study confirms the significant impact of GH replacement on the thyroid axis. In vitro analysis of subcutaneous fat from hypopituitary subjects demonstrates that GH replacement is associated with significant changes in deiodinase

isoenzyme activity. However, the observed variation in enzyme activity does not explain changes in the circulating concentration of thyroid hormones induced by GH replacement. Notwithstanding the underlying mechanism, changes in the HPT axis, induced by GH replacement, have complex clinical implications for patients with hypopituitarism.

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Chapter 1

Introduction

1.1 Hypopituitarism

Hypopituitarism, a biochemical deficiency in one or more of the hormones released from the pituitary gland, can result from disease of the pituitary itself or the hypothalamus (1). It has a reported incidence of 42 cases per million and a prevalence ranging from 300 - 450 cases per million (2). Hypopituitarism is a heterogeneous disease with a variable degree of hormone deficiency among different patients – some will have isolated hormone deficiencies while others may have multiple pituitary hormone deficiencies (MPHD).

Benign adenomas arising within the pituitary gland are the commonest cause of acquired hypopituitarism in adulthood (3, 4). A recent population study in the south of England estimated the prevalence of pituitary adenomas to be approximately 78 cases per 100,000 inhabitants (5). Other tumours, adjacent to the pituitary (parasellar area) and in the hypothalamus (e.g. craniopharyngioma) are another common cause of hypopituitarism. Pituitary hormone deficiency may result from the compressive effect of the tumour itself or from the treatment of the tumour i.e. surgery and/or radiotherapy (1). Less common causes of acquired hypopituitarism in adulthood include cranial radiotherapy, post-partum haemorrhage causing pituitary necrosis (Sheehan’s syndrome), infiltrative conditions such as sarcoidosis, lymphocytic hypophysitis, infections such as tuberculosis and metastatic tumours. Also, traumatic brain injury is an increasingly recognised cause of hypopituitarism (6, 7). Finally, less than 10% of cases are idiopathic and may be accompanied by an empty sella turcica on imaging (empty sella syndrome) (2, 4).

The symptoms of hypopituitarism are highly variable due to interaction of multiple different hormone deficiency syndromes (8). For examples ACTH deficiency can cause weight loss, whereas weight gain can occur due to fluid retention in hypothyroidism (TSH deficiency). Furthermore, partial deficiency of pituitary hormones may not cause any noticeable symptoms but in the case of ACTH deficiency, could be unmasked by stress or intercurrent illness. Specific symptoms of ACTH deficiency include weight loss, fatigue, anorexia and loss of body hair. Severe ACTH deficiency may result in hyponatraemia due to water retention. Gonadotrophin deficiency leads to oligo/amennorhoea in women while men suffer impotence and erectile dysfunction. TSH deficiency results in the classical symptoms of hypothyroidism including cold intolerance, fatigue, muscle stiffness, weight gain, constipation and dry skin.

1.2 Growth Hormone Deficiency

Growth hormone secreting cells (somatotrophs) account for the majority of cells composing the anterior pituitary in humans. Despite this, GH deficiency (GHD) is among the commonest pituitary hormone insufficiency observed in adults with hypopituitarism (9).

Adults with GHD comprise two distinct groups – those with a prior diagnosis of GHD in childhood and those who acquire GHD in adulthood due to hypothalamic and/or pituitary disease (10). Childhood-onset GHD is most commonly isolated and idiopathic. Children usually present with short stature or impaired height velocity (11, 12). GH replacement is long established for treatment of childhood-onset GHD in order to optimise linear growth and height potential before closure of the epiphyses. Interestingly, up to 25% of cases with idiopathic childhood-onset GHD will have normal GH reserves if re-tested in adulthood. The underlying pathophysiology in such cases is poorly understood. Children with severe GHD and additional pituitary hormone deficiencies secondary to organic pituitary disease

such as craniopharyngioma will have persistent GHD in adulthood and do not routinely require re-testing (12, 13).

The symptoms of adult-onset GHD are subtle and common-place, including fatigue, poor exercise capacity, abdominal obesity and impaired psychosocial function. Essentially, there is no pathognomonic feature. This contrasts with childhood-onset GHD where growth failure acts as a useful biological marker of GHD. In addition, the majority of adults with GHD have deficiencies of other pituitary hormones, further complicating the clinical picture. For example, ACTH deficiency can cause weight loss, whereas weight gain can occur due to hypothyroidism and GHD. We cannot, therefore, rely on symptoms alone for case detection in GHD.

Identifying patients at risk of GHD, such as those with hypothalamic pituitary disease, previous cranial radiotherapy and head injury, is crucial (14). Toogood et al have demonstrated that in patients with MPHD, the severity of GHD is related to the degree of hypopituitarism (15). Therefore, patients with other clinically or biochemically detectable pituitary hormone abnormalities are also a high risk group. They should be screened for GHD if GH replacement would be appropriate. GHD is established on both clinical and biochemical criteria but despite significant advances in our understanding of adult GHD, accurate diagnosis remains challenging. Selecting the appropriate patient, performing a reliable diagnostic test and understanding the clinical caveats, as well as the analytical limitations, are the crucial steps (14).

1.2.1 Diagnosis of GHD

Multiple tests are available for the diagnosis of GHD in adulthood and debate still exists about the most appropriate test. The availability of multiple testing modalities emphasises the complexities involved in making an accurate diagnosis and the need to individualise testing for each patient's clinical circumstances. The

‘ideal test’ will provide clear separation between normal and GHD patients even allowing for factors that may attenuate GH secretion such as age and obesity.

International consensus guidelines has converged around the insulin tolerance test and the growth-hormone releasing hormone (GHRH) + Arginine test (combined test) as the best available test of GHD in adults. These tests provide sufficient sensitivity and specificity to establish a reliable diagnosis when appropriate cut-offs are used (16, 17). The glucagon stimulation test is a second line test but is nonetheless well validated for assessing GH secretory capacity when first line tests are unavailable or contra-indicated (18, 19). Other tests, such as clonidine stimulation or arginine alone are available but less well validated.

IGF-1 is a peptide hormone that mediates most of the biological actions of growth hormone. Circulating IGF-1 is principally composed of endocrine IGF-1 produced in the liver under GH stimulation. A small amount of autocrine IGF-1 is also produced in peripheral tissues such as bone and can be controlled by other factors released from surrounding cells. IGF-1 has a very high affinity for binding proteins (IGFBP) and circulates in a ternary complex, bound to IGFBP-3 and the acid-labile subunit. It exerts its effect by activation of the IGF-1 receptor which is widely distributed in many tissues (20).

The value of serum IGF-1 and IGF binding protein-3 (IGFBP-3) in the diagnosis of GH deficiency is a matter of contention among endocrinologists. While serum IGF-1 levels less than 2 standard deviations (SD) below the age-matched mean, in a well- nourished adult with pituitary disease, is highly suggestive of GHD (21), it is clear that serum IGF-1 and/or IGFBP-3 can be normal in patients with undisputed GHD. Various investigators have reported normal IGF-1 values in 37-70% of GHD adults (22-24). Further studies, however, showed that age, the time of onset of GHD, and the degree of hypopituitarism, all had a significant influence on serum IGF-1 levels - sometimes expressed as standard deviation scores (IGF-1 SDS) or Z scores. In the study by *Aimaretti et al*, 70% of GHD adults under the age

of 40 years had a serum IGF-1 level below the age-related 3rd centile, but the corresponding percentage for those over the age of 40 was only 35% (25). In a large retrospective analysis of patients with GHD from the KIMS database, *Lissett et al* found that 86% of patients with childhood-onset GHD compared to 52% with adult-onset GHD, had serum IGF-1 SDS less than -2 (26). The latter study also identified gender, BMI and number of additional pituitary hormone deficiencies as factors which influence serum IGF-1 SDS. While recognising the above-mentioned caveats, it is now generally accepted that in well-nourished patients without liver disease, a low IGF-1 in the presence of 3 or more anterior pituitary hormone deficiencies, provides very strong evidence of GHD. Further testing in this context is optional (21). However, for many patients with suspected GHD, a provocative test of growth hormone reserve is required. In addition, since the presence of other pituitary hormone deficiencies is the strongest predictor of GHD and no provocative test has 100% specificity, it is recommended that adult patients who appear to have isolated GHD undergo two provocative tests to confirm the diagnosis, particularly if the serum IGF-1 is not low.

1.3 Clinical manifestations of the syndrome of adult GHD

Severe GHD in adults can give rise to several clinical abnormalities. Body composition is altered due to increased fat mass and reduced muscle mass. Exercise capacity is reduced and quality of life is impaired. The plasma lipid profile is unfavourable and cardiovascular morbidity may be increased (27). A growing recognition of this clinical syndrome in the last 20 years has led to the therapeutic use of growth hormone (GH) replacement in adults with severe GHD. This treatment has been shown to improve many abnormal parameters.

1.3.1 Body composition

Both adults and children with GHD have increased visceral fat mass and reduced lean body mass. A variety of studies, using different methodologies to estimate body composition, have demonstrated similar results. In a six-moth study of GH replacement in hypopituitary adults (n=68*), Johannson et al* used dual energy x-ray absorptiometry and bioimpedance measurements to evaluate changes in body composition (28). They reported a median decline of 2.6kg in fat mass accompanied by a 2kg increase in lean body mass. A similar study by *Whitehead et al* (n=14), using computed tomography, showed a median reduction of 2.2kg fat mass an 3.6kg increase in lean body mass over six month (29).

In routine clinical practice, anthropometric measurement of waist:hip ratio is a useful surrogate of visceral fat mass in GHD patients. A recent, international trial of 1,034 patients receiving GH replacement, as part of routine clinical care, has confirmed the utility of waist:hip ratio in longitudinal monitoring of body composition (30).

1.3.2 Lipid profile

GHD is associated with an adverse lipid profile; total and LDL cholesterol (LDL-C) is elevated in comparison to healthy controls; HDL cholesterol (HDL-C) is variably reported to be reduced or similar to GH sufficient subjects (31). Sustained reductions in total cholesterol and LDL-C have been reported with the use of physiologic doses of GH replacement in adulthood (32, 33). An effect on HDL cholesterol is less convincing and GH replacement does not appear to impact on serum triglyceride concentration. The impact of GH on the pro-atherogenic lipid particle, Lp(a), is controversial. Variable results have been reported using different assay methodologies. However, the balance of evidence is in favour of GH increasing the serum concentration of Lp(a) (34, 35).

1.3.3 Exercise capacity and cardiac function

Cardiorespiratory function is impaired in adults with GHD. Robust evidence supports an improvement in exercise capacity following GH replacement (36). The relative contributions of cardiac function and muscles strength are debated. Studies examining the effects of GH replacement on muscle strength, using a variety of methodologies have produced mixed results. A recent meta-analysis concluded that there was insufficient evidence supporting a positive effect of GH on muscle strength (37).

Childhood-onset GHD is associated with reduced cardiac output and impaired diastolic function. However, the abnormalities in adult-onset GHD appear to be less marked (38, 39). GH has been associated with a subtle increase in LV wall thickness and stroke volume; diastolic function may also improve. However, there is only limited evidence, from placebo-controlled studies, that GH replacement, improves cardiac function in adults (40). In addition, the therapeutic window for the cardiac effect of GH replacement is likely to be relatively narrow since acromegaly, a condition of excess GH secretion, is clearly associated with a dilated cardiomyopathy and cardiac failure (41).

1.3.4 Bone strength

GH plays an important role in the accrual of adult bone mass following the completion of linear growth in adolescence (42). GHD adults have a reduced bone density in comparison with healthy individuals; the severity of GHD appears to correlate with the degree of bone loss (43, 44). GH activates the bone remodelling cycle. This is reflected in the increased serum and urine concentration of bone turnover markers after systemic exposure to GH (45). Placebo controlled studies have shown that GH replacement leads to an initial decline in bone mineral density (BMD) due expansion of the bone remodelling space. However, the overall effect of

sustained, long-term GH replacement in adulthood is an improvement in BMD (46). The fracture rate in GHD subjects is increased; however there is currently insufficient data to support a decreased fracture rate in adults receiving GH replacement (47).

1.3.5 Quality of life

Improved vitality was one of the main benefits reported in the first use of GH in adulthood (48). Since then, impaired well-being and a deficit in self-reported health status have been clearly defined in GHD adults (49, 50). This may, in part, be related to altered body composition and reduced exercise capacity. However, studies which have employed both generic and disease-specific health status questionnaires have outlined deficits in numerous physical and emotional domains of quality of life (QOL) (29, 51, 52). Placebo-controlled trials of GH replacement in adulthood have clearly demonstrated an improvement in QOL (49, 53). Intriguingly, there does not appear to be a dose-response element to the effect of GH on QOL. Subjects over-treated with pharmacological doses of GH, resulting in a high serum IGF-1 level, achieve a similar increment in QOL scores as those treated with physiological dose of GH whose IGF-1 level was maintained within the normal range (54).

Poor QOL is seen as a core clinical feature of the syndrome of adult GHD and is a key indication for replacement of GH in adulthood. The disease-specific questionnaire –Assessment of Growth Hormone deficiency in Adulthood (AGHDA) is a sensitive tool for the measurement of QOL in adults with GHD and it has demonstrated excellent reproducibility in monitoring the response to GH replacement (55). The reasons underlying the variable response to GH, in respect of QOL, are unclear. However, the interaction of GH with other pituitary axes may be an important consideration in this respect.

1.4 Interaction of GH with other hypothalamic–pituitary axes

GHD in adulthood is typically part of a syndrome of multiple pituitary hormone deficiencies. GH replacement has been shown to have significant interaction with other pituitary axes and their replacement regimens. In vitro human studies have shown that GH inhibits the activity of 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) (56). This enzyme catalyses the conversion of the inactive compound, cortisone, to the hormonally active cortisol. Therefore, GH replacement, at physiological doses, reduces tissue exposure to cortisol. Cortisol promotes adipocyte differentiation, adipogensis and visceral fat deposition (57). It has been postulated that modulation of glucocorticoid metabolism may account, in part, for the reduction in fat mass observed after GH replacement (58).

In addition, female sex steroids have an important influence on the response to GH replacement. Oestrogen confers a degree of hepatic GH resistance (59). Therefore, pre-menopausal women, in comparison to men, require a higher dose of GH to generate an equivalent serum concentration of IGF-1. In contrast, replacement of the adrenal androgen, dihydroepiandosteone (DHEA), which is under the control of ACTH, reduces GH dose requirements in women with hypopituitarism (60). The mechanism underlying the interaction of DHEA with the GH-IGF-1 axis is still unclear.

Previous research has described complex alterations in the hypothalamic-pituitary-thyroid (HPT) axis following GH replacement (61). Studies in adults with hypopituitarism have reported a reduction in circulating T4 levels, with between 36-47% of previously euthyroid subjects requiring thyroxine replacement (62, 63). Also, 15-18% of patients with concurrent central hypothyroidism required an increase in thyroxine dose to maintain serum T4 concentration in the normal range. However, this effect has not been demonstrated universally (64, 65). Serum T3 levels have been reported to rise or remain unchanged (64, 66-69). A variety of changes in serum TSH have been reported. Older studies reported an increase in TSH which

may be explained by contamination of cadaveric GH with TSH (70). Contemporary studies more commonly report no change (62, 63, 66) or a decrease in TSH (68, 69) after replacement of GH. The serum concentration of reverse T3, an inactive hormone by-product of T4, has been reported to decline in previous, small studies of GH replacement (63, 67).

The mechanism underlying the changes observed in the HPT axis is a matter of debate. Some authors have speculated that alterations in serum binding proteins, such as thyroid binding globulin (TBG) may account for the observed fluctuations following GH replacement (71). However, the ratio of freeT4: total T4 is unchanged in most prospective studies which argues against an impact of GH on TBG concentration. The hypothesis that GH affects the peripheral interconversion of thyroid hormones is supported by a number of studies in this field.

The prohormone, T4, accounts for the majority of thyroid hormone produced from the thyroid. T4 is activated in numerous peripheral tissues, by deiodination, to produce T3 (Figure 1.1). Several studies of GH replacement have shown a rise in serum T3 and fall in reverse T3, concurrent with a fall in T4, suggesting that GH directly affects the interconversion of thyroid hormones in peripheral tissue. However, no direct human evidence exists to support this theory. Furthermore, the clinical impact of the changes in the HPT axis, recorded after GH replacement in adults, is unclear.

1.5 Iodothyronine deiodinase enzymes

Iodothyronine deiodinase enzymes are responsible for the local activation and inactivation of thyroid hormones. In healthy humans, they maintain 3,5,3'-triiodothyronine (T3) homeostasis, both in the serum and in peripheral tissue, by controlling the formation and degradation of T3 (72). When replete in iodine, the major hormone produced by the thyroid is thyroxine (T4); this is a prohormone and

must be activated into T3 in peripheral tissues, by removal of an iodine moiety from the outer (phenolic) ring (Figure 1.1).

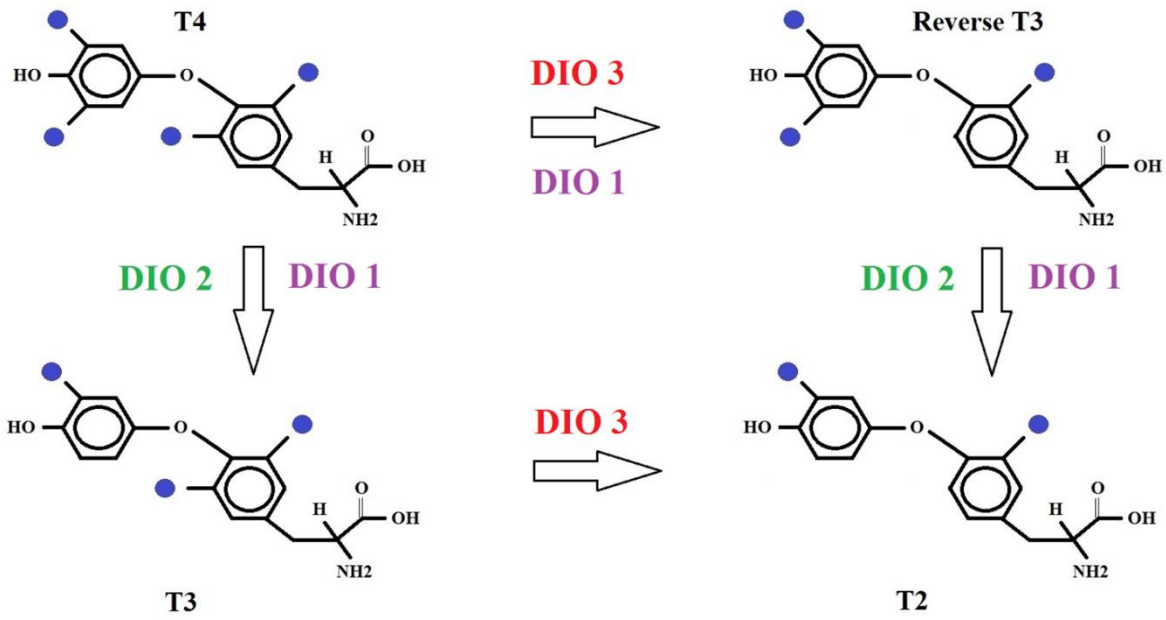

Figure 1.1

Peripheral metabolism of iodothyronines by deiodinase isoenzymes (DIO1, DIO2, DIO3). Removal of an iodine moiety (blue circles) from the outer ring of T4 (deiodination) produces the active hormone T3 which can interact with the thyroid hormone nuclear receptor. Alternatively, removal of an iodine moiety from the inner ring of T4 deactivates the compound into reverse T3 (rT3). T3 can be deactivated, by undergoing further deiodination with DIO3, producing the inactive T2 molecule which is rapidly metabolised.

The activity of deiodinase enzymes in certain tissues, most notably the liver, is also reflected in the serum concentration of thyroid hormones. In euthyroid human subjects, 80% of circulating T3 is formed by deiodination of T4 in peripheral tissue (73). Reverse T3 (rT3), an inactive hormone by-product, can also be produced peripherally, by removal of the inner (tyrosyl) ring.

There are three subtypes of deiodinase enzymes - DIO1, DIO2 and DIO3 - which are differentially expressed depending on the tissue type (Table 1.1). For example, DIO1 is highly expressed in liver and kidney while DIO2 is expressed more avidly in brain, pituitary and brown adipose tissue. T4 is converted into the biologically active hormone T3 by outer ring deiodination – a reaction catalysed by DIO2 and DIO1. DIO3 converts T4 and T3, into the biologically inactive rT3 and T2 respectively. The enzymes work synergistically to control the cytosolic pool of T3 and the saturation of the nuclear thyroid hormone receptor (TR) depending on the needs or function of a particular tissue. The activity and expression of deiodinase isoenzymes can be affected by a variety of factors.

Table 1.1

Properties of iodothyronine deiodinase isoenzymes. Details of their physiological role as well as their known actions in disease states are outlined.

Property	DIO 1	DIO 2	DIO 3
Cellular location	Plasma membrane	Endoplasmic reticulum	Plasma membrane
Half-life	12 hours	20 minutes	Several hours
Tissues with high activity	Liver, kidney, thyroid	Brown adipose tissue, placenta, brain, skeletal muscle	Brain, placenta, skin, haem(74)angiomas
Physiological role	Clearance of rT3	Major source of plasma T3 and intracellular T3, thermogenesis	Embryonic development, clearance of T4 and T3
Role in disease	Generates most of plasma T3 in thyrotoxicosis		Increases clearance of T3 and T4 in sick euthyroid syndrome. Consumptive hypothyroidism in large haemagiomas

The complex, dynamic and adaptive nature of the deiodinase enzyme system is highlighted by the changes that occur in enzyme activity and mRNA expression during certain illness. Critical illness, in particular, is notable for significant alterations circulating concentrations of thyroid hormone (75). A low circulating T3 concentration is the most consistent finding, accompanied by a low or normal serum T4 level or elevated rT3 in the presence of a normal TSH level. The syndrome has been termed “low T3 syndrome” or “non-thyroidal illness”. Alterations in deiodinase activity are, at least in part, responsible for this syndrome. Various lines of evidence point to a reduction in DIO1 activity – with reduced conversion of T4 to T3 – as well as up regulated DIO3 activity leading to increased clearance of T3 (76). The lack of a rise in serum TSH, in response to a lowering of serum T3 suggests that central hypothyroidism is also a component of the syndrome (77). The severity of illness and risk of poor outcomes correlates with the degree of T3 suppression; however, whether this syndrome is adaptive or pathological (and therefore worthy of treatment) is a matter of controversy.

Deiodinase enzymes are also influenced by fluctuations in the circulating concentration of T3 and T4 (78). A rise in serum T3 leads to reciprocal changes in DIO2 and DIO3 activity. DIO3 expression is upregulated, increasing the clearance of T3 while the expression of DIO2 is downregulated, reducing the production of T3. In contrast, if T3 levels decline, the DIO3 expression becomes suppressed (72).

Deiodinase enzymes are membrane associated proteins – DIO1 and DIO3 are found in the plasma membrane while DIO2 is intracellular but bound to the membrane of the sarcoplasmic reticulum. They belong to the family of selenoproteins which are specialised peptides which contain the rare amino acid selenocysteine in their active centre (79). Experimental evidence suggests that selenium (Se) is essential for enhancing the catalytic efficiency of deiodinase enzymes – a mutant enzyme containing cysteine alone has a 100 fold lower activity in driving the deiodination of thyroid hormone moieties (79, 80). Clinically, the

activity of deiodinase enzymes can be affected by severe selenium deficiency or marked alteration in selenium exposure (81, 82).

Drugs can also affect deiodinase enzyme activity. The anti-thyroid drug, propyltiouracil (PTU) inhibits DIO1 activity (83). In hyperthyroidism DIO1 (as opposed to DIO2) is responsible for much of the circulating T3. Therefore, inhibition of DIO1 in liver, kidney and thyroid accounts for the rapid reduction in the circulating concentration of T3 observed when thyrotoxicosis is treated with PTU (84). Amiodarone, an iodine-containing anti-arrhythmic, also inhibits the activity of DIO1 and perhaps DIO2 (85, 86). This leads to an initial fall in serum T3 followed by a compensatory rise in both T4 and TSH (87).

The direct effect of GH on deiodinase enzyme activity or expression in human tissue has, thus far, not been studied. The well described alterations in the HPT axis following GH replacement, in both adults and children, are highly suggestive of changes in peripheral deiodinase enzyme activity. In particular, the rise in the serum T3:T4 ratio implies an increased production, or decreased clearance, of T3 in peripheral tissue.

1.6 Central hypothyroidism

Central hypothyroidism refers to TSH and/or TRH deficiency and is a rare cause of thyroid hormone deficiency. In adults, central hypothyroidism is normally acquired in the context of organic pituitary and/or hypothalamic disease in patients with multiple pituitary hormone deficiencies (MPHD). In children and adolescents congenital causes of central hypothyroidism, due to specific genetic defects, are more common (discussed below).

1.6.1 Epidemiology & Aetiology

The rarity of the disease and difficulties making the diagnosis make estimation of the true prevalence and incidence very challenging. A recent Spanish study estimated the prevalence of central hypothyroidism to be 45.5 cases per 100,000 of the general population; pituitary adenomas were the commonest cause of central hypothyroidism accounting for 60% of cases (2). Further research, from disease-specific studies, provides further insight into the epidemiology of central hypothyroidism. In patients with pituitary adenomas, the commonest cause of hypopituitarism in adults, rates of TSH deficiency between 15 – 60% have been reported (88-91). The wide range is likely due to differences in biochemical diagnostic criteria, tumour size and extent of treatment. Indeed a study of macroproalctinomas in men found a rate of central hypothyroidism of 26% in patients with a tumour diameter greater than 40mm; larger tumour diameter correlated strongly with lower serum freeT4 concentration following treatment (92). Pituitary tumour apoplexy is also associated with substantial rates of TSH deficiency; Lubina et al reported that 54% were diagnosed with central hypothyroidism (93). Less common causes of hypopituitarism in adults may be associated with differing rates of central hypothyroidism. Treatment of craniopharyngioma, for example, has been reported to result in central hypothyroidism in up to 90% of cases (94, 95). This is locally invasive tumour, derived from the embryonic squamous remnants of Rathke's pouch, which often requires multi-modality treatment including radiotherapy. This may account for the high rates of central hypothyroidism. In contrast, Rathke's cleft cyst is associated with a much lower rate of TSH deficiency, between 7-35% in symptomatic patients (96, 97).

Non-classical causes of acquired central hypothyroidism include radiotherapy, lymphocytic hypophysitis, traumatic brain injury and medication. Radiotherapy to the head and neck, for non-pituitary tumours, is associated with significant rates of hypopituitarism. Agha et al reported a 9% rate of central

hypothyroidism in adults treated with radiotherapy for primary non-pituitary brain tumours (98). Furthermore, TSH deficiency has been observed in approximately 5% of patients irradiated for nasopharyngeal or paranasal sinus tumours (99). The risk of hypopituitarism is directly related to the total radiation dose. Lymphocytic hypophysitis is an autoimmune inflammatory condition of the pituitary and infundibulum which is more common in women. ACTH deficiency is particularly common in lymphocytic hypophysitis in comparison with other hypothalamic pituitary disease; however, central hypothyroidism is present in 59% of published cases (100).

Damage to the hypothalamic-pituitary axis in the setting of head trauma has been recognised for several decades, with post-mortem data demonstrating pituitary gland infarction in up to one third of patients after fatal TBI (101). Recently, several clinical studies have demonstrated a high frequency of hypothalamic-pituitary hormone deficiencies among adult TBI survivors. Notwithstanding the methodological differences between various studies, there is a broad agreement that anterior hypopituitarism is a common finding after moderate and severe head injury with an estimated prevalence of approximately 25% among adult long-term survivors (102). GH and gonadotrophin are, by far, the commonest deficiencies; however, central hypothyroidism has been reported in between 1-10% of TBI survivors (103).

Bexarotene is a synthetic retinoid analogue (rexinoid) approved for the treatment of cutaneous T-cell lymphoma. In clinical studies, Bexarotene induced clinical and biochemical evidence of central hypothyroidism in 40% of subjects (104). This side effect appears to be dose dependent and those previously treated with interferon α appear to be particularly susceptible. Under physiological conditions, thyroid hormone activates the thyroid hormone receptor (TR) in the pituitary by forming a heterodimer with the retinoid X receptor and providing negative feedback against TSH secretion (105). It would seem that rexinoid drugs,

such as Bexarotene, can directly suppress TSH secretion via the rexinoid X receptor. This side effect is reversible upon discontinuation of the drug.

Congenital causes of central hypothyroidism are increasingly recognised. Familial isolated TSH deficiency is caused by a single base pair substitution in the gene encoding the TSHβ subunit. This syndrome is autosomal recessive and has now been described in several kindreds (106). Also, TRH receptor mutations have been rarely described (107). Central hypothyroidism due to a genetic mutation is, more commonly, part of a MPHD syndrome. For example, mutation of *POU1F1*, a pituitary-specific transcription factor typically causes severe GHD and central hypothyroidism (108). Similarly, mutation in *PROP1*, a transcription factor involved in embryonic pituitary maturation, leads to combined pituitary hormone deficiencies. The phenotype in *PROP1* mutations is variable but often includes central hypothyroidism (109). Germline mutations in the HESX1 gene have also been reported to cause MPHD and are often accompanied by septo-optic dysplasia. Mutations in LHX3 and LHX4 can also cause congenital hypothyroidism; the former is associated with a rigid cervical spine, while the latter is often accompanied by cerebellar defects and abnormalities of the central skull base.

1.6.2 Diagnosis

The diagnosis of central hypothyroidism is very challenging. Typical symptoms of hypothyroidism may be milder than in primary thyroid failure and goitre is absent (110). In acquired central hypothyroidism there appears to be constitutive activation of the TSH receptor which maintains some thyroxine secretion in the face of falling TSH levels (111, 112). Also, hypothyroid symptoms may be obscured due to co-existing features of other pituitary hormone deficits such as GH or ACTH deficiency.

There is no reliable marker of central hypothyroidism and the diagnosis is largely biochemical, based on the finding of a low free serum thyroid hormone concentration with inappropriately low serum TSH concentration (110). The diagnosis is complicated by the fact that a serum T4 concentration within the population reference range, similar to the situation of primary hypothyroidism, does not exclude central hypothyroidism.

Previous investigators have described a mathematical model to define the relationship between serum TSH and free T4 – the TSH index – in patients with pituitary disease (113). This model may detect a pathologically subnormal serum TSH in patients with pituitary disease or inappropriate suppression of TSH in patients receiving thyroxine. However, this model has not been validated in other cohorts of patients with pituitary disease or using other T4 and TSH assays. Also, this model does not account for alteration in the serum T3/T4 ratio induced by GH replacement.

Furthermore, some patients with genuine central hypothyroidism have an elevated serum TSH concentration despite a low serum freeT4. Abnormal glycosylation of the TSH molecule, largely a function of TRH, leads to impaired TSH bioactivity despite a high circulating concentration. In vivo and ex vivo experiments suggest that increased sialation in the carbohydrate moiety probably accounts for the reduced bioactivity of the TSH molecule (114). Therefore, patients with central hypothyroidism may have immunologically active TSH in the serum, detected in vitro by commercial assay kits; however, this is not biologically active in vivo. Despite the putative role of TRH in the post-translational modification of the TSH molecule, abnormal TSH bioactivity was observed in patients with both pituitary and hypothalamic lesions.

TSH dynamics have been proposed as a mechanism for differentiating patients with TSH deficiency from those who are euthyroid. The normal, nocturnal surge in TSH release is blunted or ablated in central hypothyroidism; in fact this

appears to be one of earliest abnormalities in TSH secretion in patients developing central hypothyroidism (115). Previous investigators have proposed frequent serum sampling for TSH over a 24 hour period as a potential mechanism for diagnosing central hypothyroidism (116). Using this technique, some investigators have claimed to have diagnosed mild (or early) central hypothyroidism which was not apparent on baseline thyroid function tests (117). However, this approach to diagnosis is controversial. There is a lack of correlation between abnormalities in TSH circadian rhythm and circulating thyroid hormone concentration. In addition, the nocturnal surge can be blunted, temporarily, in other circumstances such as following pituitary surgery (115, 118). Also, this test of TSH dynamics is clearly cumbersome and costly, requiring admission to hospital for 24 hours.

Thyrotrophin-releasing-hormone (TRH) stimulation of TSH has also been proposed as a dynamic evaluation which could separate TSH sufficient patients with pituitary disease from those who have insufficient TSH reserves (90, 115, 119). The test involves injection of 10µg/kg of TRH intravenously and measuring the TSH response in the serum with repeated sampling over one hour. Healthy individuals show a consistent and prompt rise in TSH levels. A normal response is defined as a rise in TSH, greater than 4.0 - 5.0mU/L, (typically five-fold above baseline) within 30 minutes and a rapid decline in serum TSH thereafter (120). Early data suggested that central hypothyroidism due primary pituitary disease led to a blunted TSH response to TRH (less than 150% rise from baseline) while hypothalamic disease resulted in a delayed or prolonged response (121). However, defects at the hypothalamic level are common in patients treated for pituitary disease and vice versa. In addition, it is now clear that patients with undoubted central hypothyroidism may occasionally exhibit a normal response to TRH (117, 122). Therefore, this test has limited clinical utility in routine clinical practice.

Finally, in patients treated for hypothalamic-pituitary disease, the pattern of fluctuation of serum thyroid hormone concentration over time may provide an indication about TSH reserve. Regulation of serum freeT4 concentration in healthy

individuals is highly conserved such that variation typically does not exceed 10% over time (123). Therefore, temporal decline in serum free T4 greater than 20%, in comparison with the initial freeT4 (for example before pituitary surgery or radiotherapy) would support a diagnosis of central hypothyroidism. Limited data supports this approach and clearly this diagnostic metric will require long-term follow up of patients with repeated measurement of serum free T4 in the same bioassay (124).

1.6.3 Treatment and monitoring

Thyroxine dosing in central hypothyroidism has largely been extrapolated from data in primary hypothyroidism although limited data in patients with pituitary disease supports a body weight adapted dose of 1.6µg/kg/day (125). Recent studies, in patients with central hypothyroidism, have highlighted the difficulty in optimising thyroxine replacement (126). As a consequence, patients with central hypothyroidism, compared to those with primary thyroid failure, are at risk of under-replacement with thyroxine .

Serum TSH cannot be used as a homeostatic guide in the same manner as in primary hypothyroidism. However, measurement of serum TSH is not pointless. Suppression of TSH, in central hypothyroidism, is considered an appropriate response to thyroxine in the context of hypothalamic pituitary disease (110). A database study of patients with pituitary disease prescribed thyroxine in a tertiary referral centre in USA showed the majority of patients, considered euthyroid by their physician, had a suppressed TSH (127). These results were concordant with a Belgian study of 108 patients with central hypothyroidism who were taking a replacement dose of thyroxine comparable to that in primary hypothyroidism (mean dose 1.6±0.5µg/kg); 75% had a suppressed TSH (124). Ferretti et al also showed that half of the final thyroxine replacement dose was sufficient to suppress TSH secretion in 80% of patients with central hypothyroidism (128). A normal, non-

suppressed TSH, in the context of thyroxine replacement for central hypothyroidism, should raise the suspicion of an insufficient thyroxine dose.

Beyond suppression of TSH, serum freeT4 is, currently, the most clinically useful measure of the adequacy of thyroxine replacement in central hypothyroidism. Limited evidence suggests that freeT4 concentration correlates well with clinical impression of thyroid status (127). Adjusting the dose of thyroxine with the aim of raising the serum T4 into the upper half of the reference range has been recommended by many experts (100). This is based on research data showing a high-normal serum freeT4 is associated with a more favourable metabolic phenotype (lower BMI, lower serum cholesterol) than a low-normal serum freeT4 in patients with central hypothyroidism (124).

Serum freeT3 is less useful for judging thyroxine replacement in central hypothyroidism. In fact, serum freeT3 levels are sometimes low in this condition. This led investigators to assess the role of combined T3 and T4 treatment but this did not confer any metabolic advantages or an improved quality of life in comparison with T4 replacement alone (128).

Therefore, the limited utility of routine thyroid function test in central hypothyroidism, has led some investigators to evaluate the utility of peripheral biomarkers of thyroid hormone action as a means of assessing tissue exposure to thyroid hormone. Thyroid hormone receptors are expressed in virtually every tissue and a variety of biomarkers have been measured in both primary and central hypothyroidism (128). Serum markers derived from muscle, liver and bone, which are sensitive to the circulating concentration of thyroid hormone, have been most commonly evaluated. More recently, metabolic rate measurements and cardiac time intervals on echocardiography have demonstrated potential as biomarkers of thyroid hormone action.

1.7 Peripheral biomarkers of thyroid hormone action

1.7.1 Serum biomarkers

Sex hormone binding globulin

Sex hormone binding globulin (SHBG) is a glycoprotein, produced by the liver, which acts as a transport protein for androgens and oestradiol in the plasma (129). The circulating concentration of SHBG is influenced by many factors including gender and age (130). Pre-menopausal women have a higher concentration in the plasma compared with men. SHBG levels are highest in the pre-pubertal boys and lowest in post-menopausal women (130). In vivo and in vitro studies have demonstrated that a variety of hormones can influence the plasma SHBG concentration. Testosterone suppresses, while 17βoestradiol stimulates, SHBG production (131). Therefore, SHBG levels decline markedly during puberty in boys and following the menopause in women (132). Also, in vitro studies demonstrated SHBG inhibition by cortisol, prolactin, insulin and IGF-I (133-135). However, the plasma concentration of SHBG has also been shown to be particularly sensitive to the circulating thyroid hormone levels (136).

Several human studies have demonstrated thyroxine to be a potent stimulus for SHBG production with higher concentrations observed in thyrotoxic patients and lower levels in patients with hypothyroidism (137, 138). Previous research has confirmed the utility of plasma SHBG as a marker of hepatic exposure to thyroid hormone. In patients with inappropriate TSH secretion, (hyperthyroxinaemia with a non-suppressed TSH) plasma SHBG determination has been promoted as a method of differentiating patients with thyroid hormone resistance (THR) from those with a TSH secreting pituitary adenoma (139). In the latter disease state, the patient is truly thyrotoxic and the liver will manufacture excessive amounts of SHBG. In contrast, in THR the liver is resistant to the action of thyroxine due to a germline

mutation in the gene encoding thyroid hormone receptor β and, therefore, will not produce excess SHBG despite high circulating levels of thyroid hormone (140).

While circulating SHBG levels are considerably lower in patients with primary hypothyroidism, there is a paucity of clinical data in patients with central hypothyroidism. Limited clinical data suggest that the confounding effect GH and oestrogen may limit the utility of SHBG, as a marker of thyroid hormone action in hypopituitarism (110, 128). Isolated TSH deficiency is rare and the impact of other pituitary hormone deficiencies may confound the interpretation of SHBG levels. In particular, GH has been shown to influence SHBG production. GH deficient subjects have higher SHBG levels than age and gender matched controls, while patients with acromegaly a have lower circulating concentration (137, 141). The interaction of GH and SHBG may have clinically important consequences with previous researchers suggesting that the higher SHBG levels in boys with isolated GHD may be responsible for the delay in pubertal development which often accompanies this condition - high SHBG level results in less tissue exposure to free androgens, including testosterone (142).

There is inconsistent data on the effect of GH replacement on SHBG production. In non-hypopituitary, obese men, a continuous infusion of GH results in an acute suppression of circulating SHBG levels (143). In patients with hypopituitarism, various studies report that GH replacement either has no effect or suppresses SHBG levels. Studies in GHD children have shown that SHBG levels decline after GH replacement but this effect may take up to twelve months to be observed (141, 144). The effect of GH on SHBG levels in adult patients is conflicting; some studies show suppression similar to that observed in hypopituitary children and other studies, in hypopituitary women, report no changes in SHBG levels (145, 146). The conflicting results of studies in this field may be attributable to different study populations, with differing aetiology of GHD, as well as the confounding effect of alterations in other hormone levels i.e fluctuations in thyroid and sex hormone levels, both of which can influence SHBG production. Overall, the literature supports a suppressive effect of GH on SHBG production from the liver.

It is unclear if GH directly suppresses SHBG production or whether this is dependent on IGF-1 which mediates most peripheral effects of GH. Previous ex vivo experiments in a hepatoma cell line have shown than IGF-1 can reduce SHBG production (135). However, the expression of IGF-1 receptors in human liver is believed to be considerably less than in hepatoma cells (147). Furthermore, human studies of GH replacement which have demonstrated a suppression of SHBG levels have failed to show a correlation between the rise in serum IGF-1 favouring a direct effect of GH on SHBG inhibition (145).

Ferritin

Serum ferritin is manufactured in the liver and widely distributed in the body. It is a well-established marker of hepatic iron stores. Many factors affect serum ferritin concentration - iron stimulates synthesis of ferritin, delayed hepatic uptake prolongs its clearance from the plasma and inflamed endothelial cells release the peptide into the circulation (148). In addition, serum levels display sexual dimorphism with higher levels observed in men.

Several clinical studies have observed that serum ferritin levels are higher in patients with thyrotoxicosis and decline, in line with serum thyroid hormone levels, after treatment with anti-thyroid drugs (149). Similarly, thyroid hormone replacement, in patients with primary hypothyroidism, results in an increase in serum ferritin (150). Since several factors may influence serum ferritin concentration, the reference range in healthy individuals is quite broad and overlaps with that of the hyper- and hypothyroid range. Epidemiological research has failed to show an association between serum ferritin and thyroid hormone levels at a broad population level (151). However, there is generally a good correlation between longitudinal changes in thyroid hormone and ferritin concentration during treatment of thyroid disorders (150). This suggests that changes in the serum ferritin concentration reflect alterations of the thyroid status in individual subjects. The

effect appears to be is rapid and measureable after seven days of T3 replacement in hypothyroid patients.

Serum ferritin has been used to try and differentiate thyroid hormone resistance from thyrotroph pituitary adenomas, in patients with inappropriate TSH secretion (152). However, there is very little data available for its use in patients with central hypothyroidism, either to detect those with mild TSH deficiency (in which T4 levels are still maintained in the normal range) or to judge the adequacy of thyroxine replacement (128).

The mechanism by which thyroid hormone influences serum ferritin concentration is poorly understood. Serum iron levels are not affected but the metabolism of iron and generation of ferritin may be altered by fluctuations in circulating thyroid hormone levels (30).

Patients with GHD are reported to have low haematopoetic precursor cells and occasionally a normocytic anaemia (153, 154). Human and animal studies have shown that GH replacement has a beneficial effect on haemoglobin levels and red cell mass (155-157). Available data suggests that this is due to direct of GH/IGF-1 on bone marrow (158, 159). The impact of GH replacement on serum ferritin has only been assessed in a small number of human trials with conflicting results. Some investigators found that serum ferritin declined after the introduction of GH replacement in adults (155, 160). They hypothesised that the stimulation of erythropoiesis by GH led to the consumption of iron stores and a consequent decline in serum ferritin. However, they did not report a correlation between the decline in serum ferritin and the rise in serum IGF-1 concentration. In addition, many patients in these studies were anaemic before starting GH replacement. Other authors have reported that serum ferritin levels did not change in children who received GH substitution despite an rise in haemoglobin and red cell number which showed a positive correlation with the rise in IGF-1 (156).

Therefore, serum ferritin has the potential as a biomarkers of the hepatic action of thyroid hormone in patients receiving GH replacement.

Creatinine Kinase

Muscle pain and stiffness is a common sign inpatients with hypothyroidism. Indeed, human studies have confirmed a clinically important myopathy, with impairment of muscle function and structure, in hypothyroidism (161). The muscle enzyme, creatinine kinase (CK), was first reported to be a marker of peripheral thyroid hormone action several decades ago (162, 163). The original studies demonstrated a direct correlation between the degree of hypothyroidism and seum CK levels in overtly hypothyroid patients. Since then, it has been confirmed that CK is elevated in the serum of approximately 60% of overtly hypothyroid patients – usually less than ten times the upper limit of normal (164-166). A direct correlation between serum TSH and CK elevation is evident in both overt and subclinical hypothyroidism; however, statistically significant elevations of CK are usually confined to patients with overt hypothyroidism (167, 168). A direct relationship between circulating T4 levels and CK has been demonstrated in some but not all studies in this field (168-170). This may be due to marked inter-individual variation in serum CK activity in healthy individuals dependent on age, race, muscle mass and physical activity.

Elevations in serum CK appear to occur early in the development of hypothyroidism and can be observed during a brief withdrawal of thyroid hormone for radioiodine treatment following thyroidectomy for thyroid cancer (171, 172). In addition, the release of large amounts of CK into the circulation, in extreme cases of profound hypothyroidism, has been reported to cause rhabdomyolysis and consequent renal failure in rare case reports (173, 174). Treatment of hypothyroidism, both primary and central, with thyroxine therapy has been shown to suppress serum CK (128, 175, 176). The relationship between serum CK and T4

levels in hyperthyroid patients is less well established, although some studies have also shown a correlation between T4 levels and CK in thyrotoxic patients (163).

The mechanism whereby hypothyroidism leads to skeletal muscle damage and elevation of serum CK is not fully understood. Indeed, the mechanism may differ depending on the degree of hypothyroidism. Deregulation of mitochondrial oxidative function in muscle fibres occurs in the presence of subclinical hypothyroidism while overt hypothyroidism leads to more marked abnormalities of muscle metabolism including impairment of glycolysis and accumulation of glycogen (161, 177).

Muscle is also a target for GH/IGF-1 signalling. GHD is associated with reduced lean skeletal muscle mass and reduced muscle strength (178). GH replacement has been shown to improve lean muscle mass while reducing fat mass (179). Prospective controlled trials of recombinant GH replacement in adulthood, using conventional, physiological dosing, have not reported any significant change in serum CK levels. However, serum CK levels can be elevated in active acromegaly and isolated reports of elevated CK have been reported in children receiving GH replacement (180, 181). The latter situation may be attributable to a high dose of GH or unrecognised central hypothyroidism.

Trace elements

It has long been observed that thyroid hormones can affect the homeostasis of trace elements including copper (Cu), selenium (Se), magnesium (Mg) and zinc (Zn) (182-184). Alterations in Se and Cu, in particular, appear to reflect the hepatic actions of thyroid hormone. Cu metabolism has been shown, in human and animal studies, to be regulated by thyroid hormone, principally through a direct hepatic effect on the regulation of the copper transport protein caeruloplasmin. Animal experiments have confirmed that administration of T4 increases hepatic

caeruloplasmin mRNA expression with a parallel increase in serum caeruloplasmin concentration (185). The induction of hypothyroidism in the animals produced the opposite effects on caeurloplasmin activity. Similarly, serum Cu has been shown to correlate with serum thyroid hormone concentration in an animal model (186). In human, clinical studies, serum Cu showed a significant positive correlation with T3 and T4 before and after treatment of hyperthyroid patients with radioiodine ^{131}I ablation (187). Therefore, some investigators have suggested that serum Cu and/or caeruloplasmin could be useful markers of hepatic exposure to thyroid hormone.

There is a complex relationship between serum Se and thyroid hormone levels. Se is an essential component of the iodothyronine deiodinase enzymes which are responsible for the peripheral activation of T4 to T3 and deactivation of into reverse T3. All three deiodinase isoenzymes contain essential selenocysteine residues in their active site. The activity of these enzymes can be affected by severe selenium deficiency or marked alteration in selenium exposure (81, 82). Also, Se has an important, direct role in the thyroid hormone production in the thyroid gland - adequate Se intake increases the efficiency of thyroid hormone synthesis (188). The precise role of Se in thyrocytes is not clear but it may protect the thyroid peroxidase (TPO) enzyme from inactivation by degrading excess H_20_2.

Serum Se concentrations are mainly controlled by hepatically-derived Sepp, the Se transport protein accounting for most of the circulating Se in both humans. Some investigators have suggested that serum Se levels could be a reflection of the hepatic action of thyroid hormone. Mittag et al demonstrated, in a murine model, that thyroid hormone positively affects serum Se levels and regulates the production of several selenoproteins (189). The euthyroid sick syndrome (or non-thyroidal illness), observed in critically ill patients, provides a model for this theory. The syndrome is characterised by a down-regulation of the HPT axis with low serum TSH levels and T3 levels. Interestingly, circulating Se concentrations decline in parallel to the deranged HPT-axis in critical illness (190, 191). However, the trials of Se supplementation in critically ill patient have produced conflicting results. The

potential benefits of this treatment are still debated and the relationship between serum thyroid hormone levels and serum selenium is lost during Se supplementation (192-194).

In addition, serum selenium status is altered in many thyroid conditions; low selenium levels have been reported in Graves's and Hashimoto's thyroiditis where a poor correlation exists between serum selenium and thyroid hormone levels (195). Therefore, given the complex interaction between Se and the HPT axis, Se as opposed to Cu, is less likely to be a reliable biomarker of thyroid hormone action. Indeed, both Se and Cu, along with caeruloplasmin, are acute phase reactants and are their production is responsive to inflammatory cytokines (196). This may limit their utility as thyroid hormone biomarkers in routine clinical practice. However, there are no studies to date which have analysed the utility of trace elements as markers of peripheral thyroid hormone exposure in patients with central hypothyroidism.

There is a paucity of data regarding the impact of GH on Cu or Se metabolism. Preliminary studies in humans suggest that Cu metabolism is not significantly affected by GH replacement in adults (197, 198). A small, single study in patients with acromegaly found that caeruloplasmin activity, but not its plasma concentration, was significantly increased in patients with acromegaly in comparison with healthy controls. The authors concluded that the enhanced activity of caeruloplasmin may lead to greater oxidation of LDL particles in the serum and a consequent higher risk of atherosclerosis (199).

The relationship between Se and GH appears more complex with data showing that plasma Se levels increased both with human GH replacement and following surgical treatment of acromegaly (197). Further evidence for a complex relationship between Se and GH is derived from historical data, which showed that rats fed a Se deficient diet had smaller pituitary glands with less immunopositivity

for GH than control animals fed a normal diet (200). Selenium can become deposited in the pituitary gland at very high serum concentrations where it appears to have a toxic effect on somatotroph function; this effect appears to be partially reversible (201). In humans, however, standard supplemental doses of selenium do not appear to impair GH release or IGF-1 generation in healthy subjects (202).

Lipoproteins

The relationship between lipid metabolism and thyroid status has been recognised for nearly 80 years (203). Thyroid hormone regulates some of the key enzymes involved in lipoprotein transport and metabolism. Thyroid hormone stimulates hepatic synthesis of cholesterol by inducing hydroxymethyl glutaryl co-enzyme A (HMG CoA) reductase, resulting in an increased synthesis of cholesterol in hyperthyroidism and a reduction in hypothyroidism (204). However, thyroid hormone also affects with the degradation of low-density lipoprotein (LDL) cholesterol; thyroid hormone enhances the expression of LDL receptors on hepatic cell surface at an mRNA level and promotes clearance of LDL from the plasma (205, 206). The net effect is a decrease in serum LDL in hyperthyroidism and an increase in hypothyroidism (207). Therefore, hypothyroidism, both overt and subclinical, is an important cause of secondary hyperlipidaemia and may represent a risk factor for ischaemic heart disease.

Thyroid status also regulates hepatic lipase and cholesterol ester transfer protein (CETP) (208). The latter enzyme is responsible for the net transfer of lipids between lipoproteins; specifically CETP transfers cholesterol esters between from high-density lipoprotein 2 (HDL_2) to very low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) and conversely from triglyeride to HDL_2. Hepatic lipase catalyses the conversion of HDL_2 to HDL_3. The differential effect of thyroid hormone on these two enzymes has resulted in conflicting results in respect of the impact of thyroid status on HDL. Serum HDL level has been reported normal

or elevated in hypothyroidism. However, the ratio of total to HDL cholesterol is normally increased (204).

Lipoprotein (a) [Lp(a)] is rich in cholesterol ester and contributes modestly to the circulating pool of cholesterol. It contains a glycoprotein, apo(a) which has a high degree of homology with plasminogen and may compete with plasminogen at its receptor to promote the synthesis of plasminogen activator inhibitor 1 (PAI1) which has prothrombotic effects (209). Therefore, elevated Lp(a) levels have been linked with atherosclerosis. The effect of thyroid hormone on serum Lp(a) is controversial although some studies have shown suppression of Lp(a) levels in patients with over hypothyroidism receiving thyroxine replacement (210).

Interestingly, there is generally a good correlation between serum LDL and the degree of thyroid failure, both in overt and subclinical hypothyroidism (211, 212). Also, changes in the lipid profile in hypothyroidism are reversible with treatment. Thyroxine replacement results in a significant decrease in total and LDL cholesterol; triglycerides and HDL:total cholesterol ratio remains unchanged (207, 213).

The close relationship between thyroid status and certain serum lipoprotein levels, in particular LDL, makes changes in the lipid profile an attractive tool for monitoring the adequacy of thyroid hormone replacement in hypopituitary patients with central hypothyroidism. Higher serum LDL levels have been reported in retrospective studies of patients with secondary hypothyroidism (214, 215). In fact, the lipid profile in secondary hypothyroidism is reported to be more atherogenic than that in primary hypothyroidism, largely owing to lower HDL levels (216). Previous research has shown that withdrawal of thyroid hormone in patients with central hypothyroidism, where serum T4 levels are allowed to fall into the sub-normal range leads to a significant rise in serum LDL levels; this is reversible upon reintroduction of thyroxine replacement. However, the authors did not report whether there was a significant negative correlation between the serum LDL and T4 (or T3) levels (128). Furthermore, a prospective trial in patients with central hypothyroidism, comparing a

body weight-adjusted dose of thyroid hormone with an empiric dose, resulted in a more euthyroid phenotype with the former dosing schedule; in particular, the lipid profile was more favourable with significantly lower total and LDL cholesterol levels (125). Taken together, this data suggests that the cholesterol may be a useful marker of thyroid hormone action in central hypothyroidism. However, the data in this field is not consistent. Research in patients with central hypothyroidism has also shown no significant difference in serum lipid profiles between patients taking a high dose (≥1.58μg/kg/day) versus low dose (≤1.18μg/kg/day) thyroxine replacement where T4 levels were maintained within the normal range (214). Most studies in this field have been retrospective in design and have not measured LDL or HDL subclasses. A more detailed assessment of the lipid abnormalities in patients with central hypothyroidism, with the measurement of lipoprotein subclasses, may better define the lipid phenotype in this cohort and allow refinement the thyroid hormone replacement regimen.

Central hypothyroidism is typically a component of a multiple pituitary hormone deficiency syndrome and other pituitary hormone deficiencies may confound the interpretation of the lipid profile. Notwithstanding the suppressive effects of oestrogen on LDL cholesterol, there is a wealth of data confirming the influence of GH on the serum concentration of lipoprotein particles. An adverse lipid profile has long been recognised in both childhood onset and adult-onset GHD (217-219). Several placebo controlled trials have demonstrated that GH replacement in adulthood results in a reduction in circulating total and LDL cholesterol (220-222). However, the results of one study suggests the effect may be transient (29). Animal studies and ex vivo studies have demonstrated that GH acts to recruit LDL receptors to the hepatic cell surface thus explaining the LDL lowering effects of the hormone.

However, the effect of GH replacement on LDL concentration is likely to be modest. The original studies of GH replacement in adulthood used relatively high doses, based on weight, which would be considered excessive by contemporary

standards. A high replacement doses of 3U/m^2/day only produced a fall of 12% in total serum cholesterol. In contrast, modern, open-label studies using dose titration of GH to maintain serum IGF-1 levels above the median, but within the age-adjusted normal reference range, reported smaller, albeit significant, reductions in serum LDL (32). The LDL lowering effects of GH appear to be most marked in those with the highest baseline values.

The effects of GH on HDL levels are more controversial. The baseline serum levels of HDL in adulthood onset GHD have been reported to be normal (29) and reduced (220) in different studies. Most studies have reported no change in HDL levels with GH replacements in adults although small increases have been recorded by some investigators (35, 220, 221). Overall, the effect of GH on HDL levels appears to be marginal, if present at all. Clinical trials of GH replacement have failed to show a significant effect on serum triglyceride levels. In addition, the association between acromegaly and hypertriglyceridaemia, as well as the observation that pharmacological (high-dose) GH treatment provokes a rise in serum triglyceride, strongly suggest that GHD is not a cause of hypertriglyceridaemia (223, 224).

Lp (a) may also be affected by GH replacement. The data in this area is mixed, possibly due to assay variability (34). High dose GH replacement, in GHD and GH sufficient individuals results in a rise serum concentration of this atherogenic lipoprotein (35, 225). However, in keeping with the data on LDL lowering, the alterations in Lp(a) metabolism are less obvious at lower GH replacement doses (226).

Overt and subclinical hypothyroidism have been associated with an elevated serum concentration of Lp(a) (227, 228). The direct effect of thyroxine replacement on modulating serum Lp(a) level is a matter of debate. However, limited research supports a suppressive effect of thyroxine (229).

A decline in serum freeT4 levels (often accompanied by a rise in serum T3) is common following commencement of GH replacement in both adults and children (61). The alterations in thyroid hormone metabolism, provoked by GH replacement, have led previous investigators to speculate about the selective effects of GH and thyroid hormone on the changes observed in the lipid profile. Limited data from a study of children suggests that the improvement in the lipid profile, expected with GH supplementation, becomes attenuated when GH induces a fall in serum T4 (230). Data from the adult literature is mixed. Klose et al , in a study of hypopituitary adults receiving GH replacement, reported that changes in total and LDL cholesterol correlated negatively with changes in serum freeT4 levels, even after correction for changes in serum IGF-1 and GH dosage (215). In contrast, Porretti et al found only a transient reduction serum freeT4 in apparently euthyroid patients following high-dose GH replacement; however this was accompanied by a temporary reduction in total and LDL cholesterol (63). Finally, research from the UK reported no change in the lipid profile of patients who manifested central hypothyroidism after commencing GH replacement (62).

The conflicting results from different studies may be due to different GH dosing schedules, variable patient characteristics across different cohorts or the confounding effect of additional pituitary hormone deficits. Recent research data from animal studies has given new insights into the selective effects of GH and thyroid hormone on the serum lipid profile in hypopituitarism. Hypophysectomised rats displayed markedly increased intestinal cholesterol absorption which is normalized following replacement with thyroid hormone but not with cortisol or GH (231). However, there is no human data to support this finding. Overall, in view of the increased cardiovascular mortality reported in patients with hypopituitarism, the relative impact of GH/IGF-1 and thyroid hormone on the lipid alterations in observed following GH replacement warrants further investigation (4).

Bone turnover markers

Bone is a metabolically active tissue that undergoes a continuous cycle of remodelling. Bone formation by osteoblasts and bone resorption by osteoclasts are tightly coupled processes; normally the bone resorption phase, which last ten days, is followed by a longer period of bone formation, lasting up to three months. These integrated processes are critical to the maintenance of bone integrity and are usually regulated by systemic hormones (parathyroid hormone, Vitamin D) and local cytokines and growth factors in bone. Uncoupling of these processes can lead to changes in bone strength and mass. A variety of biochemical markers of bone turnover can be measured in serum and/or urine (Table 1.2). In general, they are classified according to the metabolic bone process they best reflect.

Table 1.2

Serum & urine bone turnover markers (BTMs) measured in routine clinical practice and research. They are classified according to the metabolic bone process they best reflect. BMTs are measured in the serum unless stated otherwise.

Bone formation markers	**Bone resorption markers**
Osteocalcin (OCI)	C-terminal type I collagen telopeptide (CTX-1)
N-terminal propeptide of type 1 procollagen (P1NP)	Aminoterminal crosslinked telopeptide of type I collagen (NTX-I)
C-terminal propeptide of type I procollagen (P1CP)	Urine and serum pyridinoline
Bone-specific alkaline phosphatase (BALP)	Urine and serum deoxypyridinoline
	Urine hydroxyproline
	Tartrate-resistant acid phosphatase

Serum markers of bone formation include bone-specific alkaline phosphatase (BALP), osteocalcin and N-terminal propeptide of type 1 procollagen (PINP). BALP is a membrane-bound enzyme of osteoblasts. Its exact physiological role is not clear although it appears to play an important role in osteoid formation. It has been commonly measured in serum for many decades as a marker of osteoblast activity and is a useful clinical marker of both mineralisation defects (osteomalacia) and abnormal remodelling (such as Paget's disease). However, assay interference has limited the utility of BALP with some assays displaying up to 20% cross-reactivity with liver-derived alkaline phosphatase, particularly in those with raised liver enzymes. More specific serum markers of bone formation are now available.

P1NP is derived from precursor of Type 1 collagen which account for 90% of bone matrix. Type 1 procollagen contains both N (amino) and C (carboxy) terminal extensions which are cleaved by proteases during the conversion to Type 1 collagen. Thus, measurement the N-terminal (P1NP) is a marker of collagen deposition and bone formation. Type 1 collagen propeptides may arise from other tissues including skin, dentine and blood vessels. However, the turnover of collagen in these tissues is much slower than bone such that they do not contribute significant quantities to the circulating pool of P1NP.

Osteocalcin is a bone-specific, non-collagen protein which is produced by osteoblasts. Its production is dependent on Vitamin K – it has three Vitamin K dependent gammacarboxyglutamic acid residues which are responsible for the calcium binding properties of the protein. After secretion by osteoblasts it is incorporated into bone matrix and secreted into the circulating plasma. Intact osteocalcin (1-49 amino acids) and the N-MID fragment (1-43 amino acids) circulate in the plasma. However, the intact molecule is unstable due to protein cleavage between amino acids 43 and 44. The N-MID fragment is more stable and amenable to in vitro measurement as a marker of osteoblast function and bone formation.

The majority of bone resorption markers are degradation products of bone collagen. During normal bone turnover mature type 1 collagen is degraded by osteoclasts and breakdown fragments are released into the circulation and excreted via the kidneys. Amongst these breakdown products are the cross linked telopeptides of type 1 collagen – carboxyterminal and aminoterminal telopeptides. Several immunoassays are now available for measurement of carboxyterminal telopeptides of type 1 collagen with antibodies directed against a variety of epitopes. An ELISA (termed β-CTX) recognises the C-terminal type I collagen telopeptide containing an isoaspartyl (β-aspartyl) peptide bond in its L-enantiomeric form. The β−L-aspartyl is believed to result mainly from the ageing of extracellular proteins. These isomerized telopeptides are highly specific for the degradation of type I collagen dominant in bone. Serum levels rise in patients with increased bone resorption and decline with anti-resorptive therapy.

The bone remodelling cycle in adults is sensitive to numerous hormonal stimuli; oestrogen, in particular, has powerful anabolic effects on bone metabolism by stimulating osteoblasts in premenopausal women. However, GH, thyroxine and glucocorticoids also have a significant impact on bone remodelling, emphasising the complex effects of hypopituitarism, and its treatment, on bone health.

Most of the data concerning the relationship between markers of bone turnover and thyroid hormone is derived from studies of hyperthyroid patients. Overt thyrotoxicosis has long been associated with enhanced bone remodelling, osteoporosis and an increased risk of fracture (232). Biochemical markers of bone formation (serum BALP & osteocalcin) and resorption (CTx1, urinary pyridinoline and hydroxyproline) are elevated although the net effect is clearly one of resorption (233, 234). Interestingly, the degree of elevation of bone turnover markers correlates with disease activity as reflected by serum thyroid hormone levels (234). Serum and urine concentration of bone turnover markers decline when a euthyroid state is restored.

Most studies in subclinical hyperthyroidism have reported a lower BMD but the data in respect of bone biomarkers is conflicting. Some investigators have reported similar findings to those in overt hyperthyroidism, with elevation of bone turnover markers and a good correlation between the markers (235, 236). However, other studies have not found any difference between bone biomarkers in patients with subclinical hyperthyroidism in comparison to controls (237, 238). This may relate to differences in the definition of subclinical hyperthyroidism.

Research in patients with primary hypothyroidism has confirmed the negative correlation between serum TSH and markers of bone turnover (239). Treatment of hypothyroidism increases BTMs; in contrast BTMs in urine and serum decline after withdrawal of thyroxine therapy.

Further evidence for the potential utility of bone biomarkers as markers of peripheral thyroid hormone action is provided by studies examining the effect of thyroid hormone therapy in athyrotic patients. Patients receiving suppressive doses of thyroxine, often following treatment of thyroid cancer, have higher serum/urine concentration of bone turnover markers than matched euthyroid controls. Research data shows that both done resorption markers (CTX, urine hydroxyproline) and bone formation markers (PINP, BALP, osteocalcin) increase when supraphysiological doses of thyroxine are used to suppress TSH (234, 240-243). One notable exception is the study by Marcocci et al where bone turnover markers were comparable to controls when the absolute minimal dose of thyroxine was used to suppress TSH (244). When thyroxine therapy is withdrawn there is a rapid decline in the concentration of bone turnover markers within a few weeks demonstrating that these markers are highly and rapidly responsive to fluctuations in serum thyroid hormone levels. However, in athyrotic patients taking thyroid hormone, changes in serum thyroid hormone concentration do not consistently correlate with fluctuations in the level bone turnover makers. Therefore, these markers of peripheral thyroid hormone action may be more useful in patients with endogenous hyper/hypothyroxinaemia.

Limited research has explored the clinical usefulness of bone turnover markers in the diagnosis and management of central hypothyroidism. Ferretti et al found that withdrawal of thyroxine replacement, in a cohort of patients with central hypothyroidism, led to a significant decrease in both a serum bone resorption (ICTP) and a marker of bone formation (bone GLA protein) when freeT4 levels declined below the reference range (128). The levels of serum bone biomarkers rose when the full replacement dose of thyroxine was restored. Furthermore, the investigators report a strong correlation between the serum level of bone turnover markers and serum freeT4 and or freeT3. Other pituitary hormone replacement was not altered during the study; in particular, subjects did not receive GH replacement. Persani et al also showed highly significant correlations between serum levels of carboxyterminal terminal telopeptide of Type 1 collagen (ICTP) and freeT4 and T3 in patients with central hypothyroidism (245). This study also highlighted the utility of bone turnover markers, as indices of thyroid hormone action, when investigating patients with inappropriate secretion of TSH. After ruling out laboratory assay interference, the clinical challenge is to differentiate patients with thyroid hormone resistance from patients with a TSH secreting pituitary adenoma. Data in this specialised field indicate that markers of bone turnover can help differentiate the two diseases, further emphasising the utility serum/urine bone indices as biomarkers of thyroid hormone action.

Hypopituitarism in adulthood is associated with osteopaenia; bone mineral density (BMD) at the lumbar spine and femoral neck is approximately 10% lower in hypopituitary patients than in age-matched controls. The relative effect of GHD, in comparison with hypogonadism and over replacement with thyroid hormone or glucocorticoids is controversial. However, a role for GH in maintaining bone mass is implied by the positive correlation recognised between bone density and serum IGF-I concentration (44, 246). In addition, ex vivo evidence supports the theory that GH stimulates osteoblast differentiation and proliferation in humans (247). Furthermore, serum markers of bone formation –BALP and osteocalcin – are lower

in adults in GHD (38, 248). There are inconsistencies in the data related to the risk of fracture; however several studies support an increased risk of nonvertebral and morphometric vertebral fracture in GHD patients, independent of the presence of additional pituitary hormone deficiencies (249-251).

The effect of GH on bone marker indices has been extensively studied. Evidence suggests that bone remodelling is increased by GH replacement in adulthood. Serum markers of bone formation – BALP, osteocalcin, P1NP - increase, as do markers of bone resorption – CTX1, pyridinoline, deoxypyridinoline and hydroxyproline (29, 45, 46, 252-254). The initial increase in bone turnover with GH replacement increases the bone remodelling space; bone resorption precedes formation leading to a transient reduction in BMD during the first few months of treatment (255). The original trials, demonstrating an increase in bone turnover markers with GH replacement, were conducting in patients with childhood-onset GHD using pharmacological doses of GH. However, more recent studies in patients with adult-onset GHD, have confirmed the effect of GH substitution on serum and urine markers of bone turnover using physiological doses.

Baum et al, in a 18 month prospective, placebo controlled study showed, on average, a doubling of serum osteocalcin and urine pyridinoline concentration in adults treated with GH compared with controls (46). This was accompanied by a 5% and 2.4% accrual of bone density in the lumbar spine and femoral neck respectively. These findings were confirmed by Hansen et al who showed a significant rise in serum bone formation markers BALP, OCI among adults receiving GH replacement when compared with placebo treated controls; bone resorption markers including serum CTX1 and urinary pyridinoline also increased significantly in the GH treated group (253). However, there was no clear correlation between the rise in serum IGF-1, induced by GH replacement, and the alteration in bone formation markers. Also, contemporary studies have also shown a sexually dimorphic effect of GH on bone mass in adults. In men, GH replacement clearly

improves BMD but in women the effect appears to be stabilise BMD despite similar increments in bone turnover markers.

Therefore, it is clear that bone turnover markers are sensitive to circulating changes in thyroid hormone and GH/IGF-1. However, the relative influence of GH and thyroxine replacement, in hypopituitary adults, on bone mass and bone turnover markers has been under-researched. In addition, bone biomarkers may provide useful insight into the clinical significance of the changes in the HPT axis following GH replacement.

1.7.2 Resting Energy Expenditure

Resting energy expenditure (REE) is the rate of fuel energy consumption by a resting individual without the effect of meal consumption or physical activity. In humans, it accounts for approximately 60% of total energy expenditure and metabolic processes such as maintenance of transmembrane ion gradients and resting cardiometabolic activity. Non-resting energy expenditure is mainly in the form of physical activity and accounts for approximately 30% of total energy expenditure. The remaining 10% of energy expenditure is associated with the thermic effect of feeding (256).

The effect of thyroid hormone on thermogenesis and metabolic rate was first described over a century ago (257). Since then, several theories have been explored to explain the stimulatory effect of thyroid hormone on resting energy expenditure (258). Na/K ATPase activity, which maintains the essential transmembrane ionic gradient, appears to be stimulated by T3 administration and this may account for part of the thermogenic effect of the hormone (259). Increased calcium cycling across the cell membrane of skeletal muscle cells is stimulated by T3; this process has high energy demands and may also contribute to the rise in

REE induced by thyroid hormone (260). Interactions between thyroid hormone and mitochondrial energy transfer, which have been extensively investigated, appear to have a less important influence on REE than originally expected (258). The augmentation of REE by thyroid hormone is, of course, clinically apparent in overt thyroid dysfunction. In particular, thyrotoxicosis is characterised by weight loss when an increase in metabolic rate stimulates several synthetic and catabolic pathways. Ultimately, an increased appetite is often insufficient to compensate for the increased fuel demands of an accelerated metabolic rate (261) . In contrast, profound hypothyroidism can result in a 25% reduction in REE in humans (138).

More subtle abnormalities in the circulating concentration of thyroid hormone can also have an impact on metabolic rate. Al-Adsani et al varied the dose of thyroxine replacement in patients with primary hypothyroidism and measured resting energy expenditure (REE) at various TSH levels (262). Serum free T4 remained within the normal reference range throughout – akin to subclinical thyroid dysfunction. The investigators showed a significant change in REE with alteration in the replacement dose of thyroxine and a negative correlation between serum TSH. However, there was no clear relationship between REE and induced changes in circulating T3 or T4. The authors speculated that the locally generated, tissue concentration of T3, generated by deiodination of T4, may be more important than the circulating concentration thyroid hormone in regulating energy expenditure. This theory is supported by animal data; in rodents, brown adipose tissue thermogenesis depends largely on locally generated T3 by DIO2 (263).

The relationship between REE and degree of thyroid dysfunction may be useful as a marker of peripheral thyroid hormone action. However, there is scant data on the performance of this biomarker in patients with central hypothyroidism. The relationship between TSH and REE appears to be more consistent than between serum T3/T4 and REE. Also, the confounding effect of other pituitary hormones, most notably GH, may present difficulties interpreting REE measurements in hypopituitary patients.

GH has undoubted stimulatory effects on REE independent of changes in lean body mass. A placebo controlled trial, in healthy subjects, reported an increase in REE after a five hour intravenous infusion of GH with concomitant use of a euglycaemic clamp (264). Furthermore, several lines of evidence confirm that resting energy expenditure is increased following GH replacement in GHD adults (265-268). Conversely, metabolic rate is increased in patients with acromegaly (269). The mechanism whereby GH increases REE is not fully understood although some authors have suggested that the GH induced changes in the HPT axis may, at least in part, explain the rise in REE (65, 67).

Several studies have demonstrated a rise in serum T3 with GH substitution in adults and children (63, 67, 270-273). In a six month study measuring the response of basal metabolic rate (BMR) to GH replacement, the rise in serum free T3 was positively correlated with BMR (67). However, in vivo experiments show that administration of T3 alone, to healthy subjects, does not fully mimic the GH induced rise in REE(274). This implies that GH has an independent effect on REE. Interestingly, the stimulation of REE may be an independent effect of GH; administration of IGF-1 alone leads to a less pronounced rise in REE in comparison to GH which may be due to the suppressive effect of IGF-1 on insulin (275). Some investigators have suggested that direct effects of GH on protein synthesis may account for the rise in REE (179). However, GH replacement also raises cardiac output; the consequent increased blood flow to skeletal muscle, among other organs, may well account for the stimulation of REE (276, 277).

The positive relationship between GH and REE is emphasised by research in acromegaly. Previous studies in human subjects have shown that REE is increased in active acromegaly in comparison with BMI-matched health controls (269). Furthermore, REE was elevated in proportion to serum IGF-1 and declined with successful treatment of acromegaly.

1.7.3 Cardiac structure and function

Thyroid hormone has both direct and indirect effects on the cardiovascular system. The hormone can stimulate cardiac contractility directly (278).The direct cardiac effects of thyroid hormone are mainly mediated through interaction of T3 with the thyroid hormone nuclear receptor. However, non-genomic mechanisms also appear to be important.

Recent animal studies have found that the alpha isoform of the thyroid hormone receptor (TR) predominate in the heart (279). Once the T3–occupied receptor binds to thyroid hormone responsive elements (TREs) of cardiac myocyte, DNA transcription of many T3-responsive myocardial genes ensues. The expression of the sarcoplasmic reticulum Ca^{2+}ATPase is induced by T3 in myocytes. The result is a more efficient removal of Ca2+ from the cytosol leading to enhanced systolic contractile function and faster diastolic relaxation in the hyperthyroid heart (280). Thyroid hormone, via its nuclear action, can alter the balance of contractile proteins including myosin. A healthy heart contains all three myosin isoforms - V1, V2 and V3 - whereas in hypothyroidism the V3 predominates (281). The myosin ATPase activity of V3 is lower than V1 and V2 resulting in a decreased velocity of contraction of hypothyroid heart muscle. Conversely, T3 promotes V1 myosin formation in the hyperthyroid heart leading to accelerated contraction.

Non-genomic effects of thyroid hormone also appear to be important in the myocardium. In particular, T3-induced changes in ion flux can be demonstrated within several minutes. The activity of plasma membrane Na+/K+ATPase, which extrudes Na^+ from the cell in exchange for extracellular K^+, is promoted by T3 which alters the electrochemical and mechanical performance of the myocardium (280).

T3 also acts directly on vascular smooth muscle to cause vasodilatation and a reduction in peripheral arterial vascular resistance (282). The direct consequence

of reduced systemic vascular resistance is an effective deficit in arterial filling; this leads to activation of the renin, angiotensin, aldosterone system with renal salt retention (283). Thyroid hormone also stimulates erythropoietin production (278). The combined effect results in an increase in blood volume and augmentation of cardiac output (284). Finally, conflicting evidence exists about the interaction between thyroid hormone and the sympathoadrenal system. However, the balance of evidence suggests that there is enhanced sympathetic sensitivity in hyperthyroidism which may be mediated through an increased number of β-adrenergic receptors on the myocardium (280).

The effect of thyroid hormone on systolic function has been quantified by previous researchers. Myocardial systolic time intervals have been shown to be particularly sensitive indicators of cardiac exposure to thyroid hormone. These can be measured non-invasively and are independent of heart rate (285). However, they can be affected by changes in blood pressure and some indices, in particular the myocardial performance index (see below) increases with age (286, 287). Historically, measurement of cardiac cycle time intervals was required the combined use of electrocardiography, phonocardiogram and pulse tracing (288). However, modern Doppler echocardiography can easily measure these time indices. This cardiac imaging technique is already in widespread clinical use; it is convenient, non-invasive and inexpensive for both patient and clinician.

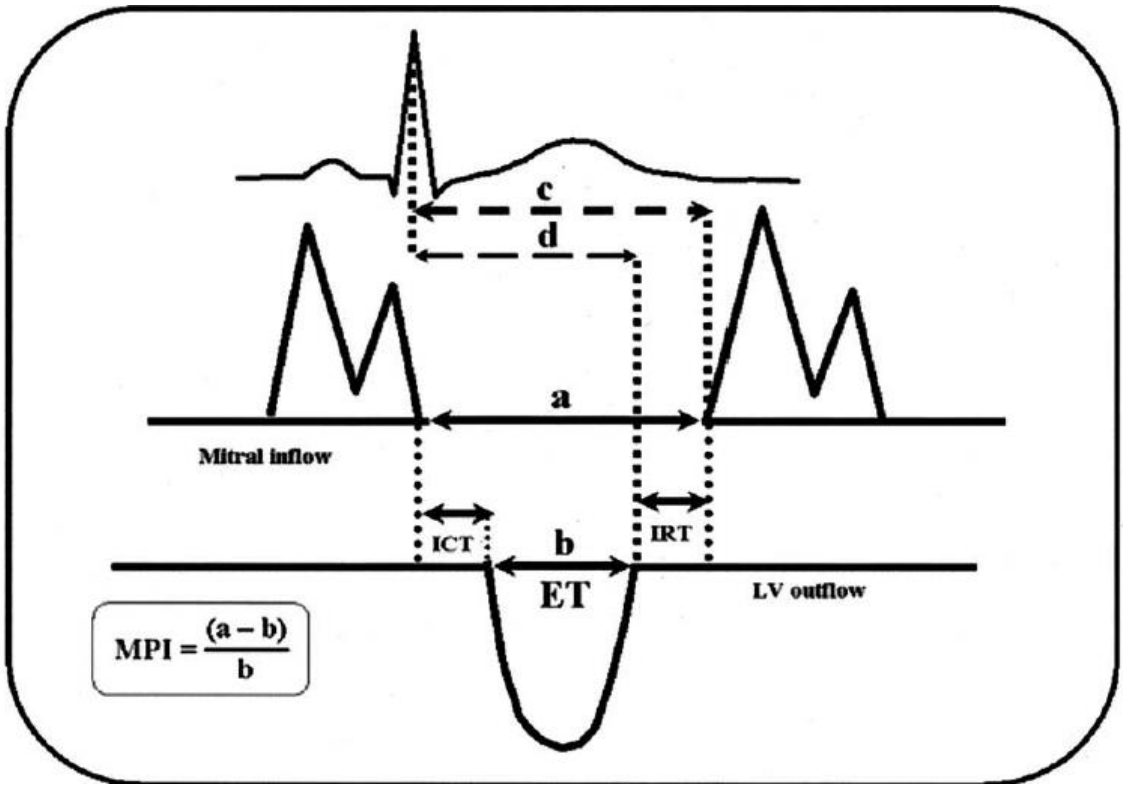

Figure 1.2

Cardiac time intervals on echocardiography and electrocardiogram.

ICT (isovolumetric contraction time). This is the time when the ventricles have begun to contract, but the volume of the chambers has not yet changed. It occurs immediately after the period of electromechanical delay, following electrical stimulation of the ventricles.

IRT (isovolumetric relaxation time). This occurs at the end of systole when the semi-lunar valves shut and the ventricles relax; this results in a fall in the intraventricular pressure, initially with no change in the chamber volume.

Figure 1.2 (contd)

ET (ejection time). After the semilunar valves open, the ventricles can eject the stroke volume into the systemic circulation during a period known as ET.

Myocardial performance index (MPI) is also known as the Tei index. It incorporates both systolic and diastolic time intervals in expressing global systolic and diastolic ventricular function. MPI = (ICT+IRT)/ET.

Interval a (from cessation to onset of mitral inflow) is equal to the sum of isovolumetric contraction time (ICT), ejection time (ET) and isovolumetric relaxation time (IRT).

Left ventricular ET (b) is the duration of left ventricular outflow velocity profile. Thus, the sum of ICT and IRT was obtained by subtracting the time interval b from a.

MPI was calculated as (a -b)/b.

IRT was measured by subtracting the interval d (time between the peak of the electrocardiographic R wave and cessation of LV outflow) from interval c (between R wave and onset of mitral inflow).

Isovolumetric contraction time (ICT) is obtained by subtracting IVRT from (a -b).

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Primary hypothyroidism and hyperthyroidism have been shown to have opposing effects on systolic time intervals. The isovolumetric contraction time (IVCT) is prolonged and MPI is increased in hypothyroidism and vice versa (290). The left ventricular ejection time (ET) is shortened in hypothyroidism with a resultant increase in ICT:ET ratio (290). These abnormalities are reversible with restoration of the euthyroid state. Studies in primary subclinical hypothyroidism have also confirmed the significant prolongation of systolic time intervals which are normalise in parallel with TSH following replacement with thyroxine (291-293).

Limited data suggests that measurement of systolic time intervals in patients with hypopituitarism may be useful for the diagnosis and management of central hypothyroidism. *Doin et al* showed that adult patients with overt central hypothyroidism (i.e. low serum free T4 concentration in presence of low or normal TSH level) had a significantly prolonged ICT with a higher MPI in comparison with controls. ICT correlated negatively with serum freeT4 levels. AIso, the change in ICT was proportional to the rise in serum freeT4 after thyroxine replacement was commenced or the dose adjusted (289). Using ROC curve analysis the authors found that IVCT > 53ms and ICT/ET ratio >0.18 had an accuracy, positive and negative predictive values of 90, 96 and 84% respectively. When this prediction tool was applied to patients with primary subclinical hypothyroidism, the serum TSH was in agreement with the systolic interval prediction model in 80% of cases. When the model was extended further to a group of patients with hypopituitarism, who had a serum freeT4 in the normal reference range, it was estimated that 56% had subclinical hypothyroidism.

The utility of systolic time intervals, as markers of peripheral thyroid hormone exposure, has been further emphasised in a study of children and adolescents with GH deficiency. *Martins and colleagues* showed that thyroxine replacement in children with central hypothyroidism resulted in shortening of the IVCT to a time comparable to control subjects (294). Interestingly, the investigators found an inverse correlation between serum total T3 and IVCT in children receiving GH

replacement; no such correlation was appreciable in GHD children not supplemented with GH. This led the authors to speculate that the GH improved the biological effects of thyroid hormone. However, prospective analysis of a seven children receiving GH replacement did not show a significant correlation between the shortening of IVCT and either the rise in serum totalT3, the fall in freeT4 or the rise in IGF-1.

GH has specific effects on myocardial structure and function. Experimental studies in animals found that found evidence IGF-I, as opposed to GH, augmented myocardial contractility by sensitizing myofilaments to calcium ions (295). Also, IGF-1 retards cardiac myocyte apoptosis (296). Clinically, the effect of GH on the heart is most apparent in acromegaly where excess GH causes a specific cardiomyopathy with initial hyperkinesis, left ventricular hypertrophy and diffuse interstitial fibrosis (41). If untreated, this progresses to diastolic dysfunction and congestive cardiac failure. In contrast, effect GHD on the structure and function of the human heart has been a matter of some controversy. Several cardiac parameters have been reported to be abnormal in patients with GHD including a reduced left ventricular mass (LVM), ejection fraction (EF) and fractional shortening (the degree of shortening of the left ventricular diameter between end-diastole and end-systole) (39, 40). However, these abnormalities have not been consistently reported and may be more marked in CO-GHD in comparison with AO-GHD. In addition, the abnormalities, appear to be subtle and are more easily detected with equilibrium radionuclide angiography than conventional echocardiography (297).

A recent meta-analysis examined the results of 16 studies which used echocardiogram to measure the effect of GH replacement in adults on a variety of indices of ventricular structure and function (298). The results confirmed and improvement in LVM, interventricular septal thickness, left ventricular end-diastolic diameter and stroke volume. Overall, there was no effect of GH replacement on either IRT or fractional shortening. However, studies of the effect of GH on the heart have not routinely assessed the impact on systolic time intervals which are known

to be sensitive biomarkers of thyroid hormone action. This approach may be useful to determine the selective effects of GH and thyroid hormone, on the heart, in hypopituitary patients.

1.7.4 Quality of life

Biochemical and echocardiographic measurements give little insight into patient symptoms or well-being. Modern healthcare places a greater focus on patient reported outcomes (PROs). Self-assessment of health status, using generic or disease-specific tools, provides insight into the quality of life (QOL) deficits in particular diseases. Furthermore, PROs provide a useful assessment of the impact of therapeutic interventions.

Previous research has demonstrated a convincing association between hypopituitarism and poor health-related quality of life (QOL) (299). Van der Kalaauw et al compared 403 pituitary patients with treated acromegaly (n=118), treated Cushing's disease (n=58), prolactinomas (n=128) and non-functioning pituitary adenoma (NFPA) (n=99) to 82 subjects with paraganglionoma and to 440 healthy controls from similar socio-economic backgrounds (300). All QOL subscales were reduced in patients compared to controls and the presence of hypopituitarism negatively influenced the total QOL score. When QOL measures in pituitary patients were compared to the paraganglionoma subgroup, but with no pituitary disease, there was no difference in overall QoL score. However, patients with pituitary adenomas experienced greater impairment in the following subscales of quality of life: role functioning due to emotional and physical problems on the Short Form 36 (SF 36), more pain and impairment in physical ability in the Nottingham Health Profile (NHP); the authors suggest that hypopituitarism and imperfect endocrine replacement may be a contributing factor to these altered QOL findings.

Nielsen et al examined 109 patients with a history of NFPA, 27% of whom were panhypopituitary and 46% with less severe hypopituitarism (301). They were unable to demonstrate any decrease in QOL compared to healthy controls, except in those who had undergone craniotomy. In contrast, Dekkers et al found multiple pituitary hormone deficiency to be an independent predictor of impaired QoL, especially with respect to social and physical functioning and increased general fatigue in a group of 99 subjects with a history of NFPA (302). The difference in findings between these studies may be due to the higher rate of hypopituitarism in Dekkers' study where 93% had some degree of hypopituitarism and 48% were panhypopituitary compared to 73% and 27% in Nielsen's group respectively.

In addition to the syndrome of panhypopituitarism and multiple pituitary hormone deficiencies, deficiency of individual pituitary hormones including ACTH, gonadotrophins, GH and TSH have been linked with impaired QOL. Therefore, the selective utility of QOL scores in assessing the clinical impact of alteration in thyroid and growth hormone exposure is controversial.

Impaired health-related QOL is a well-recognised feature of the syndrome of adult GHD (303). Many of the original, placebo-controlled studies of GH replacement demonstrated an improvement in QOL when assessed using a generic health status questionnaires (49, 51). More recent, open label studies, which used doses of GH now considered excessive, have also recorded an improvement in QOL (54, 304). Interestingly, there does not appear to be a clear dose response, in terms of QOL, with GH replacement. Subjects treated with high-dose GH – many of whom achieved a serum IGF-1 level exceeding the upper limit of the reference range - gained a similar improvement in well-being as subjects with a IGF-1 maintained within the normal range while receiving lower doses of GH (54). More recently, the disease specific tool, AGHDA, has shown a high degree of specificity and reproducibility in measuring well-being in GHD adults (55, 305). Contemporary practice in many countries mandates demonstration of poor QOL, using the AGHDA self-assessment tool, to warrant GH replacement in adulthood and clear evidence of

an improvement in QOL, after three to six months, to justify long-term prescription of GH. Therefore, the measurement of QOL is a key clinical parameter in the monitoring of GH replacement in adulthood. The possible confounding effect of other hormonal deficiencies in the measurement of QOL, in particular thyroid hormone deficiency, is worthy of consideration.

Primary hypothyroidism has a clear association with an impaired sense of well-being (306). There is clear data supporting an impaired quality of life in patients with overt hypothyroidism. However, the evidence in subclinical hypothyroidism is conflicting (307). This may be due to the differences in the populations studied and length of follow-up as well as a variable definition of subclinical hypothyroidism. Furthermore, generic health questionnaires may have a poor sensitivity for detecting QOL deficits in hypothyroidism as studies using variable doses of thyroxine to target different TSH values, within the normal reference range did not detect a difference in health-related QOL (308). Recently, some investigators have validated disease-specific questionnaires in attempt to further characterise deficits in QOL in patients with thyroid dysfunction (309, 310). Further complicating the relationship between thyroid hormone deficiency and QOL is the finding that a significant minority of patients “adequately” treated for primary hypothyroidism continue to report an impaired sense of well-being (311, 312).

Cast against this complex interaction between GH, thyroid hormone and QOL is a paucity of data in the field of central hypothyroidism. Slawick et al compared three doses of thyroid hormone replacement in patients with central hypothyroidism (n=29), not receiving concomitant GH replacement (125). Two body weight adapted (1.6µg/kg) replacement doses (one regimen of T4 only and one of combined T4 and T3) were compared to an empiric dose of T4. Overall, there was no difference in the QOL parameters measured using generic questionnaires including SF 36. Interestingly, serum T3 levels were supra-therapeutic in the regimen including T3 replacement; however, this did not appear to have an impact on self-reported health status.

Recent research on patient reported outcomes has shed new light on the clinical importance of changes in the thyroid axis induced by GH replacement. Agha et al described the unmasking of central hypothyroidism in 36% of adults, previously considered euthyroid after commencing GH replacement according to a standard clinical protocol (62). Patients who became hypothyroid after GH replacement had a higher AGHDA score (indicating poorer QOL) after three months than those who remained euthyroid. Following T4 replacement, AGHDA score were equivalent in both groups. This preliminary evidence suggests that self-assessed QOL – a key clinical metric in the evaluation of GH replacement in routine practice – is significantly affected by concurrent modulation of the HPT axis. Further research is, therefore, warranted to further elucidate the selective effects of GH and thyroid hormone on well-being and patient reported outcomes in hypopituitary subjects.

1.8 Aims of the thesis

The aims of this study are as follows:

1. Prospectively examine the impact of physiological GH replacement on changes in the HPT axis in hypopituitary adults

2. Define the mechanisms underlying changes the serum concentration of thyroid hormones, provoked by GH replacement, by measuring:
   - deiodinase enzyme activity in subcutaneous fat (adipose tissue was selected for tissue analysis due to ease of accessibility in human subjects
   - TSH, thyroid binding globulin and thyroglobulin levels.

3. Investigate the clinical importance of changes in the thyroid axis, during GH replacement in adults, by measuring the parallel response among a variety of peripheral biomarkers of thyroid hormone action including:
    - serum biomarkers derived from liver and bone
    - serum creatinine kinase
    - resting energy expenditure
    - cardiac time intervals on echocardiography
    - health-related quality of life

Chapter 2

Methodology

2.1 Identification and recruitment of study subjects

When eligible patients attended the pituitary out-patient clinic in Beaumont Hospital for a routine scheduled appointment, they were informed of the study aims and protocol. If they were willing to be assessed for eligibility then they were assessed for inclusion and exclusion criteria described below.

If the patient was deemed an appropriate candidate by inclusion and exclusion criteria described below they were given a detailed patient information leaflet and asked to consider participating in the study. Unless the subjects had denied permission to contact them again about the study, I telephoned them between five to seven days later to discuss their willingness to participate.

2.1.1 Inclusion criteria

- Severe growth hormone deficiency and suitable for replacement therapy as per usual clinical practice
- Adults between ages 18-80 years
- Stable on other hormone replacements (for at least three months) as per standard clinical practice for patients with pituitary hormone deficiencies

2.1.2 Exclusion criteria

- Unwilling to give consent
- Age < 18 years or > 80 years
- Pregnant women
- Lactating women
- Women who are planning to be pregnant during the study period
- Uncontrolled high blood pressure
- History or diagnosis of diabetes mellitus
- Significant kidney / renal impairment Creatinine > 150μmol/L
- Significant liver impairment ALT > 100IU/L
- Any abnormality in the complete blood count, specifically anaemia – as defined as haemoglobin level below the gender matched reference range.
- Conditions associated with altered bone turnover such as Paget's disease, osteoporosis or fracture within the previous 1 year
- Taking medications that significantly influence research results e.g. long term inhaled steroids for asthma
- History of cancer, significant heart disease or other conditions which significantly impact the person's ability to do either tests, or have a markedly reduced life expectancy

2.1.3 Definition of hormone abnormalities

All subjects had dynamic pituitary hormone testing at the time of their original diagnosis and as part of routine assessment and treatment of pituitary disease. In the event that patients had multiple treatments and repeat dynamic testing, the most recent results were used to define hormone abnormalities.

2.1.3.1 Anterior pituitary function

Severe GH deficiency was confirmed by a GH stimulation test, either insulin tolerance test (ITT) or glucagon stimulation test (GST); a serum GH peak less than 3 µg/L was taken as evidence of severe GH deficiency. ACTH deficiency was defined as a peak cortisol of less than 500 nmol/L during the ITT and less than 450 nmol/l during the GST.

In pre-menopausal women, hypogonadotrophic hypogonadism was defined as olgio/amennorhoea in the presence of low or normal serum gonadotrophin levels. In post-menopausal women, hypogonadotrophic hypogonadism was defined as serum FSH < 25u/L after at least one year since menopause. In men, hypogonadotrophic hypogonadism was defined by a low serum testosterone concentration in combination with inappropriately normal or low gonadotrophins. TSH deficiency was defined by low serum free T4 levels, with normal or low serum TSH level.

2.1.3.2 Posterior pituitary function

Cranial diabetes insipidus (CDI) was diagnosed on the basis of failure to concentrate urine to >600mOsm/kg, with a plasma osmolality >298mOsm/kg, following a water deprivation test. If formal WDT was not carried out, CDI was diagnosed in the appropriate clinical setting (post neurosurgery) in conjunction with hypernatraemia (Na >145mmol/l), polyuria >3.5litres/24hours and an early morning fasting urine osmolality <300mOsm/kg.

2.2 Study design

We performed a prospective, observational study of 20 adult hypopituitary patients with severe GH deficiency. GH replacement was offered as part of routine clinical care. If the patient had multiple pituitary hormone deficiencies (defined as deficiency in two or more pituitary hormones), other hormones were adequately replaced for at least three months prior to commencing GH replacement. Subjects with and without TSH deficiency were included.

Prior to commencing GH replacement, subjects attended for research studies (described below). All subjects then were commenced on recombinant GH at a starting dose of 0.3mg/day administered subcutaneously – Saizen® (somatropin (rDNA origin) for injection) Merck Serono Ltd. Serum IGF-1 was checked after one month and the dose was titrated to achieve an IGF-1 in the upper half of age-related reference range. Tests were repeated after 3-6 months on a stable dose of GH.

2.3 Study procedures

The subjects completed a 24 hour urine collection at home. Subjects were advised to discard the first early morning urine sample and collect all urine thereafter for 24 hours during this admission. Once the urine collection was completed, the total urine volume was recorded and three 5ml aliquots were preserved for storage at -80degrees until quantitative analysis for urine iodine excretion, urine copper concentration and protein oxidation could be performed.

The following day, after an overnight fast, they attended the Clinical Research Laboratory at the School of Health and Human Performance Dublin Clinical Research Centre. Resting energy expenditure (REE) was measured by open circuit indirect calorimetry using the Deltatrac® system. Detailed methodology is described below (section 2.4).

All subjects then had a percutaneous biopsy of subcutaneous fat, from the anterior abdominal wall, using a 6mm Bergström needle. The Bergström needle consists of an outer cannula with a small window at the side of the tip and an inner trocar with a cutting blade at the distal end. Under 2% lignocaine local anesthesia and aseptic conditions, the needle was advanced into the subcutaneous fat through an incision in the skin (<1cm) and fascia. Next, suction was applied to the inner trocar using a 50ml syringe and suction catheter; the outer trocar was pulled back and adipose tissue was drawn into the window of the outer cannula by the suction. The inner trocar was rapidly closed, cutting the fat tissue sample. The needle was rotated 90° and the cutting procedure was repeated. This process was repeated three to four times. This technique typically produced a fat sample of 150-200 mg from each subject.

A sterile, waterproof dressing was applied to the incision site. Subjects were able to resume their activities of daily living immediately. However, they were asked to abstain from vigorous physical activity for 72 hours. They were instructed to inspect the wound daily and take simple analgesia for discomfort as required.

Subjects were then transported by car to the RCSI, Clinical Research Facility at Beaumont Hospital. Venepunture was performed to measure baseline pituitary function, full blood counts, urea & electrolytes, serum TSH, free & total T4, free & total T3, rT3, thyroid binding globulin (TBG), anti-thyroid peroxidase (TPO) antibody titre, selenium, insulin like growth factor-1 (IGF-1), testosterone and creatinine kinase (CK). Liver and bone- derived biomarkers of thyroid hormone action were also measured (see below). Blood samples were centrifuged at 3000 rpm for 10 min at room temperature, and stored was stored at -80°C until the end of the study.

Weight (kg) and height (meters) were measured to calculate body mass index (BMI). A BMI of greater than 30 kg/m2 was considered representative of obesity. Blood pressure and heart rate were recorded while resting on three separate occasions during the morning of the research testing schedule. Subjects

were then given breakfast. After eating they were asked to complete quality of life questionnaires in a quiet, brightly lit room. Finally, each subject had an echocardiogram performed to measure cardiac time intervals. Echocardiograms were performed by an experienced cardiac technician and supervised by a Consultant Cardiologist.

Tests were repeated after 3-6 months on a stable dose of GH.

2.4 Resting Energy Expenditure

Resting energy expenditure (REE) was measured by open circuit indirect calorimetry using the Deltatrac® system. This measures the rate of O2 and CO2 exchange in the lungs and this can be used to estimate energy expenditure (313).

Subjects were asked to lie in a supine position immediately after arriving at the testing facility. They remained in this habituation period for one hour in a quiet, thermo neutral (22˚C) dimly lit room. After one hour, a plastic hood with two outlets was placed over the subjects head. Open circuit indirect calorimetry was conducted using a transparent canopy in the dilution testing mode. Ambient air was drawn through the hood by negative pressure created by the pump. The subject was supplied with room air via the inlet valve at a rate of 40 L/min, while extracts of the expired air was obtained via the outlet valve. The pump was set so that an appropriate amount of air will flow through the canopy and this was determined by the subject's minute ventilation. This was to ensure that dilute expired carbon dioxide (FEO2) is maintained within the correct range (0.5%-1.0%) (314). Expired air was analyzed by mixing chamber to establish VO2 and VCO2.

The canopy consisted of an inlet and an outlet valve. Both valves were connected to the calorimeter (Deltatrac II) via tubes. The Deltatrac II recorded the oxygen consumption (VO2) and carbon dioxide production (VCO2) from the inlet and outlet gas samples of the valves and calculates the oxygen consumption (VO2) and

carbon dioxide production (VCO2) in ml/min by differentiation. Values for all parameters were averaged over 1 minute intervals. Subsequently, these values were used to estimate REE and substrate utilization (% carbohydrate and % fat) using the Weir equation (315). In addition, 24 hour urine collection was obtained to account for protein oxidation and to complete the energy expenditure measurement.

Protein oxidation

Urea nitrogen excretion was measured by spectrophotometry and converted to urea nitrogen excretion in g/d. Assuming that urea nitrogen excretion is a constant proportion (85%) of total urinary nitrogen [Ref], protein intake was derived from the formula 6.253(urinary nitrogen + 2), as suggested by Isaksson (316).

2.5 Echocardiographic assessment

A complete, transthoracic, two-dimensional and Doppler echocardiogram was performed using a 2.0- to 2.5-MHz transducer according to standard technique. Two dimensional M-mode echocardiograms were recorded from parasternal short-axis view at mid-left ventricular (LV) level to determine LV dimensions. Left ventricular ejection fraction (EF) was obtained by biplane Simpson's method. Pulsed wave Doppler was used to measure cardiac time intervals by sequential recording of the mitral inflow (from the apical four chamber view) and the left ventricular outflow tract (from the apical long axis view). Myocardial performance index (MPI) was defined as sum of the isovolumetric contraction time (ICT) and isovolumetric relaxation time (IRT) divided by the left ventricular ejection time (ET).

Tissue Doppler imaging (TDI) measurements were made during the echocardiographic examination from an average of 5 consecutive heart beats. Time intervals were measured from mitral inflow and LV outflow velocity traces. Separate measurements were taken from the septal and lateral wall – see Figure 2.1. Interval

a (from cessation to onset of mitral inflow) is equal to the sum of isovolumetric contraction time (ICT), ejection time (ET) and isovolumetric relaxation time (IRT). Left ventricular ET (b) is the duration of left ventricular outflow velocity profile. Thus, the sum of ICT and IRT was obtained by subtracting the time interval b from a. MPI was calculated as (a -b)/b. IRT was measured by subtracting the interval d (time between the peak of the electrocardiographic R wave and cessation of LV outflow) from interval c (between R wave and onset of mitral inflow). Isovolumetric contraction time (IVCT) is obtained by subtracting IVRT from (a -b). The IVCT/ET ratio was also calculated.

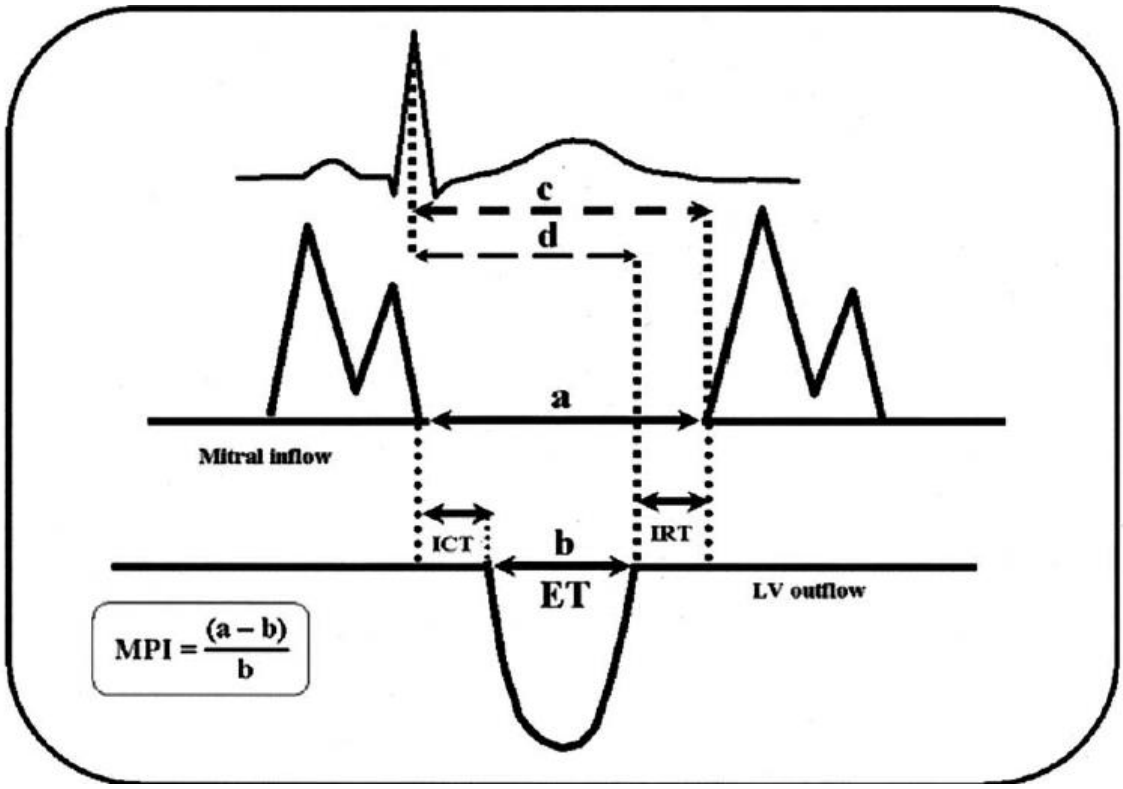

Figure 2.1

Cardiac time intervals on echocardiography and electrocardiogram.

ICT (isovolumetric contraction time). This is the time when the ventricles have begun to contract, but the volume of the chambers has not yet changed. It occurs immediately after the period of electromechanical delay, following electrical stimulation of the ventricles.
IRT (isovolumetric relaxation time). This occurs at the end of systole when the semi-lunar valves shut and the ventricles relax; this results in a fall in the intraventricular pressure, initially with no change in the chamber volume.
ET (ejection time). After the semilunar valves open, the ventricles can eject the stroke volume into the systemic circulation during a period known as ET.

Figure 2.1 (contd)

Myocardial performance index (MPI) is also known as the Tei index. It incorporates both systolic and diastolic time intervals in expressing global systolic and diastolic ventricular function. MPI = (ICT+IRT)/ET.

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2.6 Assessment of quality of life (QOL)

During each testing session subjects were administered the following health-related QOL questionnaires –

- Nottingham Health Profile (NHP)
- Short Form 36 (SF 36)
- Assessment of Growth Hormone Deficiency in Adults (AGHDA)

The Nottingham Health Profile (NHP) Questionnaire

The NHP contains 38 yes/no questions over 6 health domains; 1. energy (three items), 2. pain (eight items), 3. emotional reaction (nine items), 4. sleep (five items), 5. social isolation (five items) and physical mobility/functioning (eight items). Each question is weighted and the sum of each domain is 0-100 with higher scores indicating worse quality of life in this case (129) (Appendix 1)

The Short Form 36 (SF 36) Questionnaire

The SF 36 aims to assess general well-being over the preceding 4 weeks by evaluating responses to statements through 8 domains of health; 1. physical functioning, 2. social functioning, 3. role physical – limitations in role activities due to physical health problems, 4. role emotional – limitations in role activities because of emotional problems, 5. mental health, 6. vitality, 7. pain and 8. general health . Scores are coded and transformed to a scale of 0-100 with higher scores indicating a better quality of life (128) (Appendix 2).

Assessment of Growth Hormone Deficiency in Adults (AGHDA)

The AGHDA questionnaire consists of 25 questions, with YES/NO responses, derived from the symptoms most commonly reported by adults with severe GHD. A

score of 25/25 indicate the worst possible QOL score. Scores of 4/25 or less have been recorded in a healthy, general population. (Appendix 3)

2.7 Laboratory methods

2.7.1 Deiodinase isoenzyme activity

DIO1 and DIO2 activities were assayed in tissue sample homogenates prepared in 0.1 M potassium phosphate, 2 mM EDTA, and 2 mM dithiothreitol (DTT), pH 7.0. Protein concentration was determined by Bradford's method using BSA as a standard. Tissue samples were assayed in duplicates. DIO1 activity was measured using 25–50 µg protein in 100 µl reaction mixture, [125I]-rT3 (100 000 c.p.m./tube),400 nM rT3, and 2 mM DTT. Incubation was carried at 37°C for 1 h, with or without 1 mM propylthiouracil(PTU). Results are expressed in pmol/min per mg protein.

DIO2 activity was measured using 50 µg protein in 100 µl reaction mixture consisting of [125I]-T4 (200 000 c.p.m./tube), 2 nM T4 or 500 nM T4 (to inhibit DIO2 activity), 1 mM T3 (to inhibit DIO3 activity), 20 mM DTT, and 1 mM PTU for 2 h at 37 °C. Results are expressed in fmol/min per mg protein.
Before each assay, [125I]-T4 and [125I]-rT3 (Perkin Elmer, Billerica, MA, USA) were purified by dialysis as described previously (14). For DIO1 and DIO2 activity assays, reactions were stopped by adding 100 µl of cold 2% BSA and 800 µl of 10% trichloroacetic acid. After centrifugation at 2000 g for 10 min, 800 µl supernatant was applied to AG 50W-X2 columns (bed volume=1 ml) (Bio-Rad Laboratories) and eluted with 10% glacial acetic acid. The [125I] generated in the assay was counted in a gamma scintillation counter (Packard Cobra Gamma Counter, Perkin Elmer, Waltham, MA, USA). Blanks were performed in

quadruplicates by substituting the volume of tissue homogenates for assay buffer. Assay detection limit was calculated as the average of [125I] produced by blanks multiplied by 3.3 times SD.

DIO3 activity was measured as in accordance with the methodology described previously by *van Zeijl et al* (317). Adipose tissue (approximately 40 mg) was homogenized on ice in 500µl PE buffer (0.1 M sodium phosphate, 2 mM EDTA pH 7.2) containing 50 mM dithiothreitol (DTT) using a Polytron (Kinematica, Luzern, Switzerland). Protein concentration has been measured with the Bio-Rad protein assay (Bio-Rad Laboratories, Veenendaal, The Netherlands), according to manufacturer's instructions.

Samples were measured in duplicate using 75 µl homogenate in a final volume of 0.15 ml in the presence of 1nM T3 or 500nM T3 (tissue blank, 500 nM T3 saturates DIO3 activity) with the addition of approximately 1*105c.p.m. [3`-125I]T3 (NEX110X, Perkin Elmer) in PE buffer. Samples were incubated for 2h. The activity reactions were stopped by adding 0.15 ml ice-cold ethanol. After centrifugation, 0.125 ml of the supernatant was added to 0.125 ml 0.02 M ammonium acetate (pH 4), and 0.1 ml of the mixture was applied to 4.6 x 250 mm Symmetry C18 column connected to a Waters HPLC system (Model 600E pump, Model 717 WISP auto-sampler, Waters, Etten-Leur, The Netherlands). The activity of T3 and T2 in the eluate was measured online using a Radiomatic 150 TR flow scintillation analyser (Perkin Elmer, Waltham, MA, USA) D3 activity is expressed as fmol generated 3,3`T2 per minute per mg tissue. DIO3 activity when incubated with 1nM T3, minus the activity measured when incubated with 500nM T3 represents true D3 activity.

2.7.2 Biochemical indices

Pituitary and thyroid function

Total T3 was measured by a competitive binding protein, chemiluminescent immunoassay (UniCel DxI 800, Beckman Coulter). Reverse T3 (rT3) was measured by liquid chromatography/tandem mass spectrometry (Quest Diagnostics, San Juan Capistrano, CA). The remainder of the serum thyroid hormones (total T4, free T4 and free T3) and TSH were measured using fluoroimmunometric and sensitive "second generation" assays respectively (DELFIA, PerkinElmer Life Sciences). Thyroid binding globulin (TBG) was measured by immunoassay (Siemens Immunlite). Inter-assay and intra-assay coefficients of variation (CV) were less than 10% throughout.

Thyroglobulin was measured using a paramagnetic chemiluminescent immunoassay using 4 biotinylated mixture of 4 monoclonal anti thyroglobulin antibodies. Inter-assay co-efficient of variation was 5.6% and 2.5% at serum concentrations of 0.97 and 4.42 ug/L respectively. Anti-TPO IgG was quantified by fluorenzyme-immunoassay (Elia Phadia 250).

IGF-1 was measured in a chemiluminescent immunoassay using an acridinium labelled anti-IGF 1 antibody (IDS-iSYS). Inter-assay and intra-assay coefficients of variation (CV) were less than 10%.

Testosterone was measured using a liquid phase radioimmunoassay (Spectria, Cisbio Bioassays). The assay is a liquid phase radioimmunoassay using antibody-coated tubes. ^{125}I-labelled testosterone competes with testosterone in the patient sample for a fixed number of antibody binding sites in the tube. At the end of the incubation period, unbound material is removed by decanting and washing. The

amount of testosterone in the patient's serum is inversely proportional to the amount of radioactivity in the coated tube.

Bone-derived serum biomarkers

Bone formation markers

The concentration of bone-specific alkaline phosphatase (BALP), a marker of both bone mineralisation and maturation, were measured by an immunoenzymatic assay (Ostase, Immunodiagnostic Systems Ltd, Bolton, UK) on an automated ELISA platform with inter-assay and intra-assay coefficients of variation (CV) values of 5.8% (at 8.4µg/L) and 6.5% (at 7.4µg/L) respectively. The minimum detectable concentration is estimated to be 0.7µg/L.

The concentrations of total procollagen type I N-propeptide (PINP) were by electrochemiluminescence immunoassay measured on the Elecsys 2010 analyser (Roche Diagnostics) with intra-assay CV values of 2.6%, 1.8% and 1.3% and inter-assay CV of 4.1%, 2.3% and 2.2% at concentration values of 12.8, 57.2 and 527µg/L respectively. Lower limit of detection was 5µg/L.

The concentrations of osteocalcin 1–49 (OCI) were measured using the same analyser (Roche Diagnostics) with intra-assay CV of 1.4%, 1.1% and 1.7% and inter-assay CV values of 3.1%, 3.1% and 3.0% at concentration values of 6.1, 12.2 and 35.6µg/L respectively and a lower limit of detection of 0.5µg/l.

Bone resorption markers

The concentrations of C-terminal cross-linking telopeptide (CTX-I) were measured using an electrochemiluminescence immunoassay on the Elecsys 2010 analyser (Roche Diagnostics) with intra-assay CV values of 3.5%, 2.1% and 2.0%

and inter-assay CV values of 8.4%, 3.8% and 2.8% at concentration values of 0.051, 0.488 and 2.35µg/l respectively. The lower detection limit was 0.07µg/l.

Liver-derived serum biomarkers

Sex hormone binding globulin

Quantitative measurement of sex hormone binding globulin (SHBG) was performed using a two-step chemiluminescent microparticle immunoassay (ACHITECT iSystem, Abbott). Intra-assay CV was 4.78%, 4.8% and 5.24% and inter-assay CV values of 9.54%, 5.65% and 7.55% at concentration values of 8.8, 24.5 and 152.8nmol/L respectively; analytical sensitivity was calculated to be 0.02nmol/L.

Ferritin

The Ferritin assay is a two-site chemiluminescent, immunoenzymatic (“sandwich”) assay (Beckman DxI 800 Series). Subject sample was added to a reaction vessel with goat anti-ferritin-alkaline phosphatase conjugate and paramagnetic particles coated with goat anti-mouse:mouse anti-ferritin complexes. Serum ferritin binds to the immobilised monoclonal anti-ferritin on the solid phase, while the goat anti-ferritin enzyme conjugate reacts with different antigenic sites on the ferritin molecules. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of ferritin in the sample. Inter-assay co-efficient of variation was 7%.

Serum iron

Iron (Fe) was analysed by a spectrophotometric assay (Beckman AU Series Analyser). The automated analyser utilises TPTZ [2,4,6-Tri-(2-pyridyl)-5-triazine] as the chromogen. In an acidic medium, transferrin-bound iron dissociates into free ferric ions and apo-transferrin. Hydrochloric acid and sodium ascorbate reduce the ferric ions to the ferrous state. The ferrous ions then react with TPTZ to form a blue coloured complex which can be measured bichromatically at 600/800 nm. The increase in absorbance is directly proportional to the amount of iron present. Inter-assay CV was 2.4%.

Total iron binding capacity (TIBC)

Total iron binding capacity (TIBC) (was calculated based upon the total iron present and the unbound iron binding capacity or UIBC) UIBC was estimated by a spectrophotmetric assay (Beckman AU Series Analyser). Fe^{2+} from reagent 1 reacts with Nitroso-PSAP from reagent 2 to form an intense green complex. When sample is added a part, or all of the iron ions bind specifically with transferrin at unsaturated iron binding sites at alkaline pH. They are thus not available for the colour reaction with Nitroso-PSAP. The difference between the resulting changes in the measured absorbance's with or without samples is equivalent to the iron quantity bound to transferrin. This is the unsaturated iron binding capacity (UIBC). Inter-assay CV was 3%.

Caeruloplasmin

Serum caeruloplasmin was measured using an automated immunoassay (Beckman AU Series Analyser). Subject sample mixed with buffer and antiserum. Human caeruloplasmin reacts specifically with the anti-human caeruloplasmin antibodies to yield insoluble aggregates. The absorbance of these aggregates is proportional to the caeruloplasmin concentration in the sample. Inter-assay CV was 4%.

Trace elements

Serum selenium (Se) and copper (Cu) levels were determined by inductively coupled plasma mass spectrometry (ICP-MS), on an Elan 6100 DRC plus (SCIEX Perkin-Elmer, Beaconsfield). For each analyte, samples were run against matrix matched calibration curve, spiked with the standard solution. The elements were measured individually against calibrations standards of up 3.0µmolL-1 for selenium (Selenium standard solution 1000ppm, Fisher Chemicals), 50µmolL-1 for plasma copper and 20µmolL-1 for urine copper (Copper standard solution 1000ppm, Fisher Chemicals). For serum copper, intra-assay CV was estimated at 1.1%, 1.4% & 0.9% and inter-assay CV 5.0%, 2.9% & 3.2% at 7.5, 15 & 21µmol/L respectively. For urine copper intra-assay CV was estimated 8.1% at 0.2µmol/L and 1.4% at 0.9µmol/; inter-assay CV 13.7% at 0.2µmol/L and 7.7% at 0.9µmol/L. For selenium intra-assay CV was estimated at 3.7%, 3.5% & 5.2% and inter-assay CV 7.9%, 8.2% & 6.8% at concentrations of 0.6, 1.2 & 2.2µmol/L respectively.

Lipoproteins

Total cholesterol, triglyceride (Trig) and HDL cholesterol (HDL-C) were measured using standard laboratory techniques (CV<5%). LDL cholesterol (LDL-C) was calculated using the Friedewald equation.

LDL and HDL subfractions were separated using the Quantimetrix Lipoprint system (Redondo Beach, CA) (318). The Lipoprint System uses non-denaturing, linear polyacrylamide gel electrophoresis to separate and measure the lipoprotein fractions. High-resolution 3% polyacrylamide gel tubes were used for electrophoresis. Twenty-five microlitres of sample were mixed with 200 microlitres of Lipoprint loading gel, which contained Sudan Black B dye to stain the lipoproteins. This was placed on the upper part of 3% polyacrylamide gel. After 30 min of photopolymerization at room temperature, samples were electrophoresed for

60 min with 3 mA for each gel tube. The electrophoresis was followed by resting the tubes in the dark for 1 hour before performing densitometry. Densitometry was performed at 610 nm.

Raw data from the densitometer were imported into a Microsoft Excel© spreadsheet. Using a computerized method that was developed for the Quantimetrix Lipoprint system using NIH image program version 1.62 (Bethesda, MD), and subfractions were identified and quantified.

The Lipophpor system (Quantimetrix) was used as quality control. Each chamber had two quality controls. Very low-density lipoprotein (VLDL), seven LDL, and ten HDL subclasses were quantified and further classified as large, intermediate, and small subfractions. Using a weighted scoring system developed by the manufacturers, LDL scores were also calculated. On the basis of LDL migration rates on the scan, LDL phenotypes A (predominantly large, buoyant LDL) or non-A(predominantly small, dense LDL) were assigned to scores of less than 5.5 for phenotype A and greater than 5.5 for non-A.

Miscellaneous

Cretainine Kinase (CK)

CK was measured by a spectrophotometric assay (Beckman AU Series Analyser). CK reversibly catalyses the transfer of a phosphate group from creatine phosphate to adenosine diphosphate (ADP) to give creatine and adenosine triphosphate (ATP) as products. The ATP formed is used to produce glucose-6-phosphate and ADP from glucose. This reaction is catalysed by hexokinase (HK) which requires magnesium ions for maximum activity. The glucose-6-phosphate is oxidised by the action of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) with simultaneous reduction of the coenzyme nicotinamide adenine

dinucleotide (NADP) to give NADPH and 6-phosphogluconate. The rate of increase of absorbance at 340/660 nm due to the formation of NADPH is directly proportional to the activity of CK in the sample. Inter-assay CV was 1.5%

Urinary iodine

Iodine levels were determined by inductively coupled plasma mass spectrometry (ICP-MS), on an Elan 6100 DRC plus (SCIEX Perkin-Elmer, Beaconsfield. For each analyte, samples were run against matrix matched calibration curve, spiked with the standard solution. The elements were measured individually against calibrations standards of up to 10µmolL-1 (Potassium iodide, BDH, Poole). Sample volumes for the calibration, test and quality control, were diluted quantitatively (from 1in 15 to 1in 50) with a diluent containing 0.3% ammonia (BDH, Poole). Inter-assay coefficient of variation was 9.7% at 0.65µmol/L and 6.2% at 2.4µmol/L.

2.8 Statistical analysis

Biochemical indices were analysed for normality using the D'Agostino–Pearson normality test. Mean and standard error of the mean (SEM) were determined for normally distributed continuous data and median (and range) was used for data not normally distributed.

Paired continuous variables were analysed by paired t-test for normally distributed data and by Wilcoxon rank sum test for data not normally distributed. Chi square test was used to compare paired categorical variables. Correlation between continuous variables was determined by calculating the Pearson correlation coefficient for data sampled from a Gaussian distribution and Spearman coefficient for data with a non-Gaussian distribution. Significance was defined for p-values less than 0.05.

Statistical analysis was performed using GraphPad Prism 5 software (GraphPad Inc., La Jolla, CA, 2010).

2.9 Ethics

Informed consent was obtained from all subjects to participate in this study which was approved by Beaumont Hospital and Dublin City University Ethics (Medical Research) Committee.

Chapter 3

Alterations in the hypothalamic-pituitary-thyroid axis following growth hormone replacement

3.1 Introduction

Complex alterations in the hypothalamic-pituitary-thyroid (HPT) axis have been reported following growth hormone (GH) administration in both adults and children, with and without growth hormone deficiency. The most consistent effect on the HPT axis, reported by previous investigators, is a reduction in serum free thyroxine (free T4) (62, 63, 66, 270, 271, 319, 320). In hypopituitary patients, GH replacement has been reported to lead to a decline in serum freeT4 to the subnormal range in 36%-47% of patients previously considered euthyroid (61). However, some investigators have reported no change in free T4 (64, 321).

Serum tri-iodothyronine (T3) concentration has been observed to rise (63, 67, 69) or remain unchanged (64, 66, 70, 322, 323). Similarly, reductions in serum thyroid stimulating hormone (TSH) secretion, following GH supplementation, have been reported in some but not all studies (67, 69, 322). A small number of studies have measured the hormone by-product, reverse T3 which usually declines following GH replacement (63, 67). Furthermore, some researchers have reported changes in the HPT axis following GH replacement to be transient and, therefore, of less clinical significance (270, 324). The inconsistencies in the literature about these perturbations may be due to different study populations and design, thyroid hormone assay imprecision or lack of purity of cadaveric GH, used in older studies, which was occasionally contaminated with TSH.

The aim of this part of the study was to prospectively define the impact of physiological GH replacement on a cohort of adult hypopituitary patients treated with a standard clinical protocol.

3.2 Methods

Subjects

We performed a prospective, observational study of 20 adult hypopituitary patients with severe GH deficiency. GH replacement was offered as part of routine clinical care. If the patient had multiple pituitary hormone deficiencies (MPHD), other hormones were adequately replaced for at least three months prior to commencing GH replacement. Subjects with and without TSH deficiency were included. Informed consent was obtained from all subjects to participate in this study which was approved by Beaumont Hospital Medical Research (Ethics) Committee.

Baseline tests performed before GH supplementation included serum TSH, free & total T4, free & total T3, rT3, anti-thyroid peroxidase (TPO) antibody titre and insulin like growth factor-1 (IGF-1). All serum samples were collected after an overnight fast. Weight (kg) and height (metres) were measured to calculate body mass index (BMI).

All subjects were commenced on recombinant GH at a starting dose of 0.3mg/day administered subcutaneously – Saizen® (somatropin (rDNA origin) for injection) Merck Serono Ltd. Serum IGF-1 was checked after one month and the dose was titrated to achieve an IGF-1 in the upper half of age-related reference range. Tests were repeated after 3-6 months on a stable dose of GH.

Biochemical indices

Total T3 was measured by a competitive binding protein, chemiluminescent immunoassay (UniCel DxI 800, Beckman Coulter). rT3 was measured by liquid chromatography/tandem mass spectrometry (Quest Diagnostics, San Juan Capistrano, CA). The remainder of the serum thyroid hormones (total T4, free T4 and free T3) and TSH were measured using fluoroimmunometric and sensitive "second generation" assays respectively (DELFIA, PerkinElmer Life Sciences). Anti-TPO IgG was quantified by fluorenzyme-immunoassay (Elia Phadia 250). IGF-1 was measured in a chemiluminescent immunoassay using an acridinium labelled anti-IGF 1 antibody (IDS-iSYS).

3.3 Results

Baseline characteristics are outlined in Table 3.1. Fourteen subjects were treated for pituitary adenomas (10 non-functioning, 4 lactotroph tumours), one had a treated germinoma, one a craniopharyngioma and four had idiopathic hypopituitarism. Seventeen (85%) had multiple pituitary hormone deficiencies (MPHD); three patients had isolated GH deficiency (in the latter cases GH deficiency was confirmed by two dynamic tests). Subjects with isolated GH deficiency comprised two patients with previously treated pituitary adenomas and one with isolated idiopathic GH deficiency.

Table 3.1

Baseline characteristics of the study cohort. Demographic data is also displayed for the sub-groups with and without central hypothyroidism (CH). All patients with CH were prescribed thyroxine replacement. Data for age and BMI displayed as median (range)

GHD growth hormone deficiency.

a Comparison of patients with and without CH.

	All patients	**Patients with CH**	**Patients without CH**	**p value**
n	20	13	7	
Male/Female	20/0	13/0	7/0	
Aetiology of GHD				
NFPA	10	6	4	
Prolactinoma	4	3	1	
Other	6	4	2	
MPHD n (%)	17 (85)	13(100)	4(57)	
Age (range)	53.1 (22.3-69.3)	42.51 (22.3-69.3)	57.26 (35.1-68.2)	0.05[a]
BMI kg/m^2 (range)	31.3 (19.2-49.7)	33.9 (19.2-49.6)	30.5 (23.6-37.6)	0.42[a]

Serum testosterone levels did not change during the course of the study (14.20±11.95nmol/L vs. 14.95±10.40nmol/L, p=0.45). Three patients had cranial diabetes insipidus. Serum electrolyte levels were recorded at each visit; there were no abnormalities suggestive of over- or under-replacement with ddAVP. All patients had serum freeT4 and free T3 levels within the normal reference range prior to commencing GH replacement (Table 3.2).

The mean daily dose of growth hormone was 0.34±0.11 mg (range 0.15 - 0.5mg) administered subcutaneously. Serum IGF-1 levels rose significantly, as expected, during the course of the study (+114.4±12.3µg/L, p<0.0001). Following GH replacement, freeT4 levels declined (-1.09±1.99 pmol/L, p=0.02). Total T4 levels also fell (-9.61± 4.25 nmol/, p=0.035) (Table 3.2). There was a strong positive correlation between the change in total and free T4 suggesting a parallel decline in the free and protein-bound form of the hormone (r 0.7620, p=0.0001). Reverse T3 levels also fell (-3.44±1.42 ng/dL, p=0.03) and freeT3 levels increased (+0.34±0.15 pmol/l, p=0.03) after GH replacement. In addition, the serum T3/T4 and fT3/rT3 ratio rose significantly (Figure 3.1). The rise in serum IGF-1 correlated with the fall in freeT4 (r -0.46; 95% CI -0.755 to -0.006, p=0.048) while there was a trend towards a significant correlation with the rise in freeT3 and IGF-1 (r 0.442; 95% CI -0.0291 to 0.753, p=0.058) (Figure 3.2).

All changes in circulating thyroid hormone levels occurred within the normal reference range; no study subject required adjustment of or commencement of thyroxine replacement during the time course of the study.

There was no significant change in early morning, fasting serum TSH levels during the study period. Sub-group analysis of subjects with a detectable TSH level confirmed stable, baseline TSH levels throughout. Similarly, serum concentration of thyroglobulin and TBG were unchanged by growth hormone therapy (Table 3.2).

Table 3.2

Alteration in circulating thyroid hormones following replacement of growth hormone. Data is reported as mean±SEM where variable is normally distributed (TT4, fT3, rT3) and median±SD where variable is not normally distributed (fT4 & TT3). Only those with a detectable pre-treatment TSH level (n=13) were included in this analysis – reported as median (range). * p<0.05. GH Growth Hormone.

	Pre GH	Post GH	Δ	Ref. range	P value	95% CI
FreeT4 (pmol/L)	12.9±4.0	12.1±3.2	-1.09±1.99*	9-20	0.02	-2.24 to -0.32
Total T4 (nmol/L)	109±4.9	99.5±4.5	-9.6±4.25*	69-141	0.04	-18.5 to -0.72
Free T3 (pmol/L)	5.4±0.2	5.7±0.2	+0.34±0.15*	3.0-7.5	0.03	0.03 to 0.65
Total T3 (nmol/L)	1.72±0.4	1.62±0.4	-0.02±0.32	1-3	0.76	-0.1 to 0.19
Reverse T3 (ng/dL)	17.6±1.4	14.2±0.8	-3.44±1.42*	8-25	0.03	-6.44 to -0.45
TSH (mU/L)	1.1 (0.4-5.1)	1.1 (0.2-3.3)	-0.1±1.09	0.4-4.0	0.31	-0.87 to 0.17

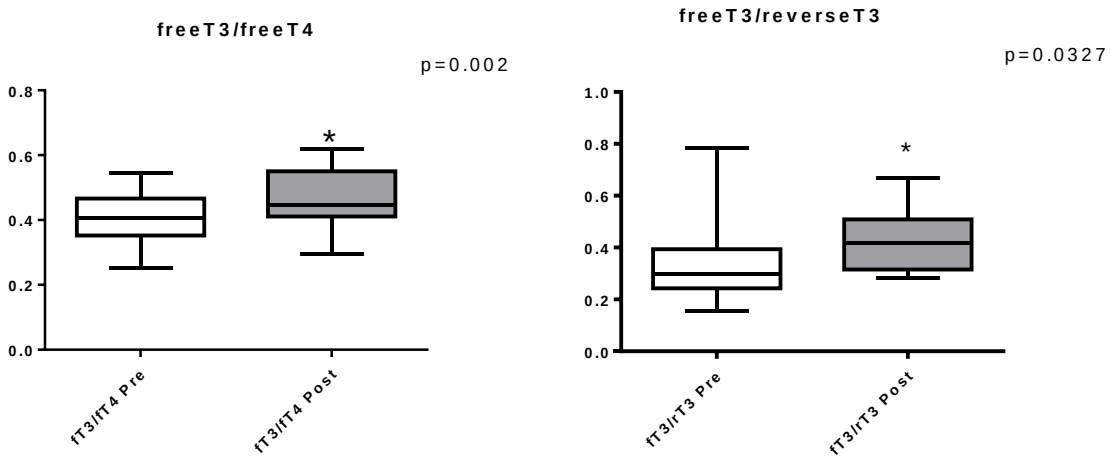

Figure 3.1.

Ratio of freeT3/freeT4 and freeT3/reverse T3 before (white boxes) and after (grey boxes) GH supplementation. * p<0.05.

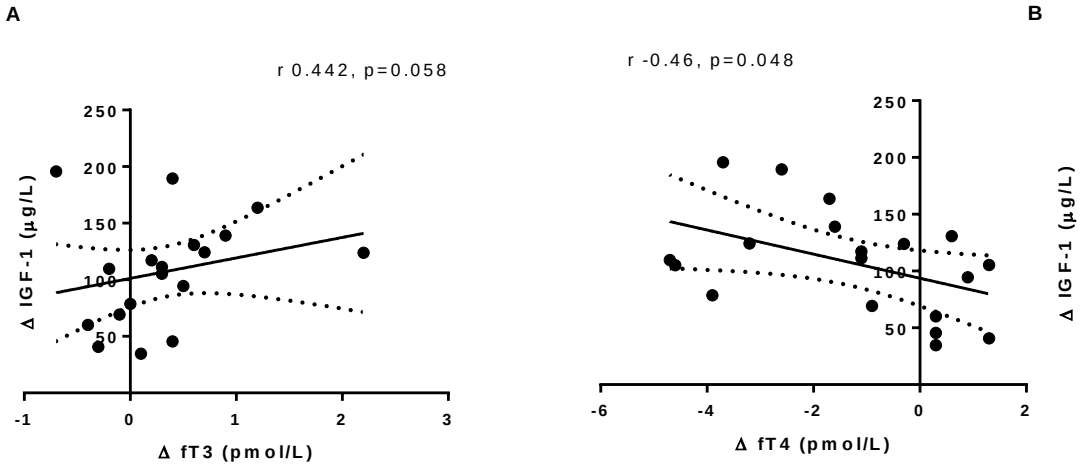

Figure 3.2

Correlation between rise in serum IGF-1 concentration (ΔIGF-1; µg/L) and change in serum freeT3 (ΔfT3; pmol/L) (A) and freeT4 (ΔfT4; pmol/L) (B) following growth hormone replacement.

Body mass index (BMI) did not change during the study; 31.38±1.649 kg/m 2 vs. 31.07±1.679kg/m2, p=0.26. The initial BMI did not correlate with the baseline T4 or T3 levels. However, subjects with a higher BMI before commencing GH replacement had a more significant rise in free T3 during the study (Figure3.3). Age of the subjects did not influence the changes observed in the circulating concentration of thyroid hormones.

Two subjects had an elevated titre of anti-TPO antibodies; exclusion of these subjects from the analysis did not significantly alter the changes observed in circulating thyroid hormones. No subject developed anti-TPO antibodies during the course of the study.

Subgroup analysis was performed on patients with central hypothyroidism i.e. TSH deficiency. This subgroup consisted of patients (n=13) with more profound hypopituitarism i.e. MPHD including TSH deficiency; all were taking thyroxine replacement. The subgroup was formed after the exclusion of three patients with isolated GHD, two with GHD and gonadotrophin deficiency and two with GHD and ACTH deficiency. All patients in this subgroup had an organic cause of hypopituitarism. Baseline characteristics are shown in table 3.3. There was no difference in the baseline values of free & total T3, free and total T4, reverse T3, TSH or IGF-1 in those with or without TSH deficiency.

When patients with TSH deficiency were analysed separately, a similar pattern of biochemical changes was observed (Table 3.4). GH replacement induced a fall in free T4 (-2.15 ±1.89pmol/L; 95% CI -3.39 to -0.98, p=0.004) and total T4 (-14.07 ± 4.3nmol/L; 95% CI -23.45 to -4.69, p=0.007). Reverse T3 also declined (-4.15 ±1.72ng/dL; 95% CI -7.94 to 0.39, p=0.03). However, the rise in free and total T3 did not reach statistical significance. Serum IGF-1 concentration rose as expected (+134.7 ±14.6µg/L; 95%CI 102.9 to 166.5, p<0.001).

Table 3.3

Alteration in circulating thyroid hormones following GH replacement in subjects with TSH deficiency (n=13). Data is reported as mean±SEM where variable is normally distributed (TT4, fT3, TT3, rT3) and median±SD where variable is not normally distributed (fT4). * p<0.05. GH Growth Hormone.

	Pre GH	Post GH	Δ	Ref. range	P value	95% CI
FreeT4 (pmol/L)	13.5 ±4.59	12.20 ±3.66	-2.15 ±1.89*	9-20	0.004	-3.39 to -0.98
Total T4 (nmol/L)	116.7 ±4.58	102.6 ±4.3	-14.07±4.3*	69-141	0.007	-23.45 to -4.69
Free T3 (pmol/L)	5.312 ±0.23	5.74 ±0.28	+0.43±0.22	3.0-7.5	0.085	-0.07 to 0.22
Total T3 (nmol/L)	1.65 ±0.11	1.83 ±0.12	+0.18±0.08	1-3	0.05	0.005 to 0.36
Reverse T3 (ng/dL)	19.17 ±1.54	15.00 ±0.77	-4.15±1.72*	8-25	0.03	-7.94 to -0.39

3.4 Discussion

In this prospective clinical study, we describe the impact of GH replacement on alterations in the hypothalamo-pituitary-thyroid axis in adult patients with hypopituitarism. Our results demonstrate a decreased circulating concentration of free and totalT4 in conjunction with an increase in freeT3 following initiation of GH replacement. These alterations occurred despite TSH levels remaining unchanged.

Previous research in this field has produced divergent results. Much of the data concerning patients with GHD is derived from small studies of children using high doses of GH. Some studies have not demonstrated any significant changes in the thyroid hormone levels. Rubio et al, in a study of 12 GHD children in the 1970's, found no change in the concentration of serum TSH, thyroid hormones or thyroidal response to exogenous TSH following six months GH replacement (325). In contrast, more contemporary studies have demonstrated a fall in T4 and reverse T3, accompanied by a rise in T3, following GH replacement in children (270, 271, 320). However, new development or unmasking of central hypothyroidism was rare in these studies, leading some authors to question the clinical significance of these alterations. Furthermore, previous studies in GHD children have also suggested that changes in the HPT axis may be transient; in one study they resolved after 12 months (324) and after 24 months in another study (270). However, the children in these studies had isolated idiopathic GHD and, by definition, a normal thyroid axis. This may explain the milder and transient changes in the HPT axis in these studies,

A diversity of findings is also apparent in studies of adults with GHD. Amato et al, in a small study of nine adults, with mostly childhood onset GHD, showed no change in thyroid parameters following GH substitution (64). A larger study of 21 adults by Monson et al reported a fall in serum freeT4 concentration in the euthyroid group only (273). Serum T3 levels rose in the whole group but this appeared to be a transient phenomenon. One patient developed a freeT4 level below the normal rang

after commencing GH treatment; however this subject had a normal serum T3 and rT3 with no clinical signs of central hypothyroidism.

The discrepancies among the results of previous studies are likely due to several factors. Most have been small studies with a variation in whether the patients had isolated idiopathic GHD or MPHD. In the latter situation the HPT axis will be intact and pehaps less subject to manipulation by GH replacement. Also different assays have been used to measure the serum concentration of thyroid hormone with variable precision and reproducibility. Furthermore, a variety of GH dosing schedules have been employed in different studies; also older studies used cadaveric GH which was occasionally contaminated with TSH.

Despite the inconsistencies in the published literature, the pattern of change in circulating thyroid hormones that we report is largely in keeping with recent studies of larger cohorts of patients with organic pituitary disease (62, 63, 66, 68). Porretti et al studied 66 patients with MPHD receiving GH replacement and reported a significant fall in T4 and rT3 in addition to a transient rise in T3 (63). Also, the authors described the development of central hypothyroidism in 47% of previously euthyroid subjects; 18% of those with treated hypothyroidism required an increase in thyroxine dose.

In the largest adult study in the field to date, Agha et al evaluated the effect of standard clinical replacement of GH 243 patients, of whom 159 patients had previously diagnosed central hypothyroidism and were appropriately treated with thyroxine prior to commencing GH replacement. GH dose was titrated to achieve a target serum IGF-1 level in the upper half of the age-related reference range. In the euthyroid group (n=84), serum free T4 concentration declined to below the reference range in 36% of subjects necessitating thyroxine replacement at three to six months after GH substitution. No further changes were seen after nine to 12 months of GH replacement. The main predictor for exposing post-GH central hypothyroidism in this group was the presence of multiple pituitary hormone

deficiencies. Sixteen per cent of the treated hypothyroid group had a reduction in serum free T4 level requiring increased thyroxine dose with no further changes seen after 6 months of GH replacement.

In our current study, all changes in thyroid hormone concentration occurred within the normal reference range. This may be due to increasing awareness of the GH-induced fall in T4, resulting in a tendency to optimise T4 replacement prior to initiating GH by keeping the serum freeT4 concentration in the upper half of the normal range.

Subgroup analysis of patients with central hypothyroidism, taking thyroxine replacement, showed a more marked decline in serum free and total T4 in comparison to the whole study group. However, changes in T3 in this subgroup were more mixed; the rise in free T3 was more modest whereas the increase in total T3 was greater than the full study cohort. The small number in this subgroup (n=13) make it difficult to draw firm conclusions about the impact of thyroxine replacement on GH-induced changes in the HPT axis. Furthermore, previous studies in this field have reported conflicting results when comparing the results of GH replacement in those with and without a previous diagnosis of central hypothyroidism. Similar to our results, Jørgensen et al, in a placebo controlled study, found that the decline circulating T4 levels was more accentuated in those with central hypothyroidism in comparison to patients not taking thyroid hormone supplementation. In contrast, Agha et al reported less marked changes in the HPT axis of patients treated for central hypothyroidism in comparison to the apparently euthyroid subjects. Thyroid hormone assay methodology and a variable study design may explain the diversity of these research findings.

Suppression of serum TSH, both basal and TRH – stimulated levels, has previously been reported in some studies of GH replacement (69, 322, 326). However, the data is inconsistent. The secretion of TSH demonstrates diurnal variation and is characterised by a nocturnal surge. GH replacement has been

shown, in a previous small study, to inhibit this nocturnal rise, possibly through increased somatostatinergic tone (67). In our study, we evaluated basal TSH levels after an overnight fast. Patients with previously diagnosed central hypothyroidism took thyroxine replacement in the evening for two weeks prior to the research study visits. This dosing schedule was designed to ensure stable serum thyroid hormone levels during the morning of the study visits. Seven patients (all taking thyroxine replacement) had a suppressed serum TSH throughout the study. This constitutes 54% (7/13) of patients with central hypothyroidism. Previous research has confirmed that suppression of TSH is common in patients with central hypothyroidism. An audit of all patients with central hypothyroidism at a tertiary referral centre in the USA revealed that 64% had a subnormal serum TSH concentration. Indeed, some investigators have suggested that a serum TSH concentration >1.0mU/L is a reflection of inadequate thyroxine supplementation in central hypothyroidism. However, this is complicated by the fact that some patients with genuine central hypothyroidism have a high serum TSH. This is believed to be due to abnormal post-translational modification of the molecule leading to highly immunoreactive but biologically inactive TSH (114, 327). In our study, considering all patients with a detectable serum TSH, GH replacement, at a standard clinical dose, did not induce changes in serum TSH concentration. This is in keeping with comparable studies of adult patients with hypopituitarism (62, 63, 68).

Alterations in the HPT axis have also been reported in non-GHD subjects treated with GH. Taken together the results of these studies are also variable, for much the same reasons discussed above. Nonetheless, the overall trend in biochemical changes in TSH and thyroid hormone are similar to those observed in subjects with GHD. In a placebo-controlled study in obese healthy women, GH treatment resulted in a reduction in freeT4, an increase in serum T3 but no change in TSH (328). In contrast, Moller et al found no change in freeT4 but a consistent rise in serum T3 after fourteen days of high dose GH (4mg/day), in comparison to placebo, in healthy individuals (65).

The findings of this study, confirm the significant impact of GH replacement on the HPT axis. These effects were observed during routine clinical practice using low dose GH supplementation and aiming to maintain the serum IGF-1 in the upper half of the age-related reference range. The decline in T4 and rise in T3 serum concentrations, that we observed, are largely in keeping with previous studies in similar populations. The mechanism underlying these changes is not fully understood. The rise in freeT3/freeT4 ratio is suggestive of an enhanced peripheral activation of the T4 prohormone. However, no direct human evidence for this theory exists. Also, other potential mechanism may influence the changes in the HPT axis including the effect of GH replacement on TSH dynamics. Finally, clinical importance of a fall in T4, particularly a rising serum T3 concentration (the biologically active form of the hormone) is worthy of further investigation.

Chapter 4

Potential mechanisms responsible for changes in the thyroid axis induced by growth hormone replacement

4.1 Introduction

Administration of exogenous growth hormone (GH) may provoke changes in the hypothalamo-pituitary-thyroid axis in both children and adults (61). This is apparent in both hypopituitary and healthy subjects after commencing GH supplementation (62, 65, 68, 328). In Chapter 3, I described the alterations in the serum concentration of circulating thyroid hormones after administration of GH to a cohort of men with severe GHD. We observed a significant fall in free & total T4. Reverse T3 also declined and freeT3 concentration increased. TSH, in those with a detectable level, did not change during the study. While previous data in adults suggests that GH replacement can unmask central hypothyroidism in approximately 40% of adults with pituitary disease (63), all changes in thyroid hormone concentration, in our study, occurred within the normal reference range.

Several theories exist about the mechanisms which could mediates these changes in the thyroid axis. These include derangement of TSH dynamics, alteration in serum concentration of thyroid hormone binding proteins and a direct effect on hormone synthesis in the thyroid gland. However, the changing pattern of circulating thyroid hormone concentrations, with a fall in serum T4 often accompanied by a rising T3, is suggestive of an alteration in the peripheral interconversion of thyroid hormone subtypes.

T4, secreted by the thyroid, is a prohormone and must be activated into T3 in peripheral tissues, by removal of an iodine moiety from the outer (phenolic) ring (Figure 4.1). Reverse T3 (rT3), an inactive hormone by-product, can also be produced peripherally, by removal of the inner (tyrosyl) ring. It has been argued that GH affects deiodination of thyroid hormones in peripheral tissue. These reactions are catalysed by iodothyronine deiodinase enzymes (72). There are 3 subtypes of deiodinase enzymes - DIO1, DIO2 and DIO3 - which are differentially expressed depending on the tissue type. For example, DIO1 is highly expressed in liver and kidney while DIO2 is expressed more avidly in brain, pituitary and brown adipose tissue (329). T4 is converted into the biologically active hormone T3 by outer ring deiodination – a reaction catalysed by DIO2 and DIO1. DIO3 converts T4 into the biologically inactive hormone rT3.

The hypothesis, that an effect on deiodinase activity is the mechanism for GH-induced changes in thyroid hormone metabolism, is supported by the findings of some but not all studies. Some investigators have observed a fall in T4 levels without a parallel rise in T3 or changes in reverse T3 following GH treatment (66, 330). Ultimately, however, there is no conclusive evidence from human studies, to date, in support of the hypothesis that GH affects the peripheral deiodination of thyroid hormone.

The primary aim of this study was to prospectively examine the relationship between changes in the serum concentration of thyroid hormones and deiodinase activity in subcutaneous fat, following GH replacement, in hypopituitary subjects. Adipose tissue was selected for analysis due to ease of accessibility in human subjects. Secondary aims included assessment of the effect of GH therapy on circulating thyroid hormones, TSH, thyroid binding globulin and thyroglobulin levels.

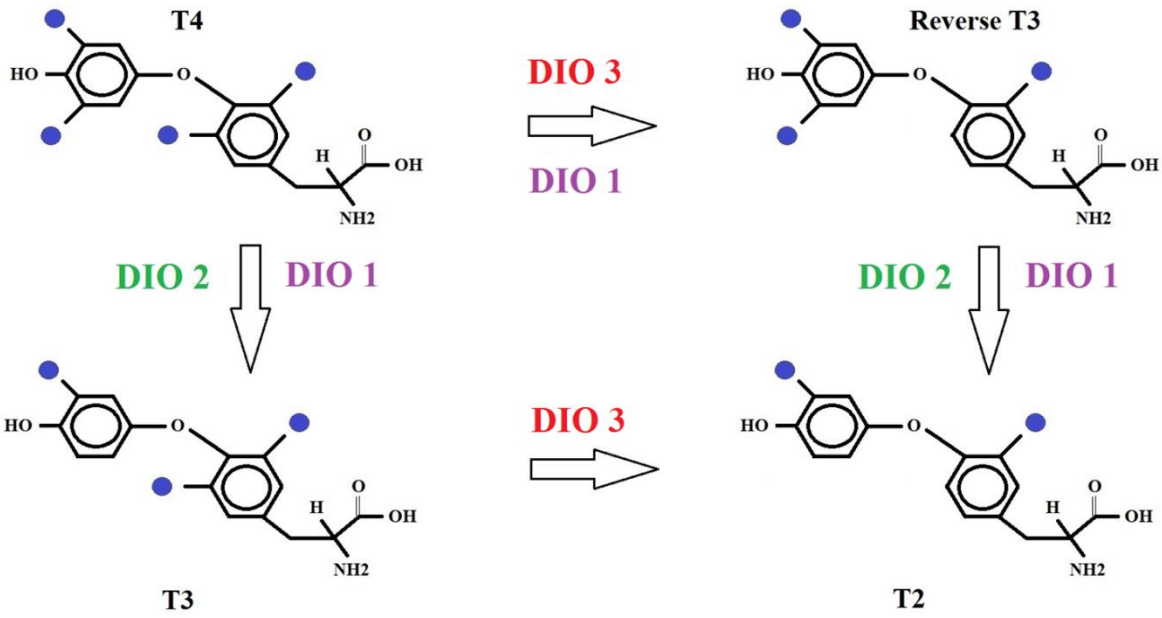

Figure 4.1

Peripheral metabolism of iodothyronines by deiodinase isoenzymes (DIO1, DIO2, DIO3). Removal of an iodine moiety (blue circles) from the outer ring of T4 (deiodination) produces the active hormone T3 which can interact with the thyroid hormone nuclear receptor. Alternatively, removal of an iodine moiety from the inner ring of T4 deactivates the compound into reverse T3 (rT3). T3 can be deactivated, by undergoing further deiodination with DIO3, producing the inactive T2 molecule which is rapidly metabolised.

4.2 Methods

4.2.1 Subjects

We performed a prospective, observational study of 20 adult hypopituitary patients with severe GH deficiency. GH replacement was offered as part of routine clinical care. Diagnosis of GHD and pituitary hormone deficiencies is defined in Chapter 2. If the patient had multiple pituitary hormone deficiencies (defined as deficiency in two or more pituitary hormones), other hormones were adequately replaced for at least three months prior to commencing GH replacement. Subjects with and without TSH deficiency were included. Informed consent was obtained from all subjects to participate in this study which was approved by the local hospital Medical Research (Ethics) Committee.

Prior to commencing GH replacement, a biopsy of subcutaneous fat was also performed on all subjects. Under aseptic conditions and local anaesthesia with 2% lignocaine, a Bergström biopsy needle was used extract a sample of subcutaneous fat from the anterior abdominal wall. Samples were snap frozen in liquid nitrogen and stored at −80°C until analysis.

Baseline tests performed before GH supplementation included serum TSH, free & total T4, free & total T3, rT3, TBG, thyroglobulin, anti-thyroid peroxidase (TPO) antibody titre, selenium, insulin like growth factor-1 (IGF-1), testosterone and electrolytes. A 24 hour urine collection was analysed for iodine concentration. All serum samples were collected after an overnight fast. Weight (kg) and height (metres) were measured to calculate body mass index (BMI).

All subjects were commenced on recombinant GH at a starting dose of 0.3mg/day administered subcutaneously – Saizen® (somatropin (rDNA origin) for injection) Merck Serono Ltd. Serum IGF-1 was checked after one month and the

dose was titrated to achieve an IGF-1 in the upper half of age-related reference range. Tests were repeated after 3-6 months on a stable dose of GH.

4.2.2 Biochemical indices

Total T3 was measured by a competitive binding protein, chemiluminescent immunoassay (UniCel DxI 800, Beckman Coulter). Reverse T3 was measured by liquid chromatography/tandem mass spectrometry (Quest Diagnostics, San Juan Capistrano, CA). The remainder of the serum thyroid hormones (total T4, free T4 and free T3) and TSH were measured using fluoroimmunometric and sensitive "second generation" assays respectively (DELFIA, PerkinElmer Life Sciences). TBG was measured by immunoassay (Siemens Immunlite). Thyroglobulin was measured by chemiluminescent immunoassay (Beckman Coulter Access 2). Anti-TPO IgG was quantified by fluorenzyme-immunoassay (Elia Phadia 250).

IGF-1 was measured in a chemiluminescent immunoassay using an acridinium labelled anti-IGF 1 antibody (IDS-iSYS). Testosterone was measured using a liquid phase radioimmunoassay (Spectria, Cisbio Bioassays). Serum selenium and urinary iodine levels were determined by inductively coupled plasma mass spectrometry (ICP-MS), on an Elan 6100 DRC plus (SCIEX Perkin-Elmer, Beaconsfield). Inter-assay and intra-assay coefficients of variation were less than 10% throughout.

4.2.3 Deiodinase isoenzyme activity

DIO1 and DIO2 activities were assayed in tissue sample homogenates prepared in 0.1 M potassium phosphate, 2 mM EDTA, and 2 mM dithiothreitol (DTT), pH 7.0. Protein concentration was determined by Bradford's method using BSA as a standard. Tissue samples were assayed in duplicates. DIO1 activity was measured using 25–50 µg protein in 100 µl reaction mixture, [125I]-rT3 (100 000 c.p.m./tube),400 nM rT3, and 2 mM DTT. Incubation was carried at 37°C for 1 h,

with or without 1 mM propylthiouracil(PTU). Results are expressed in pmol/min per mg protein.

DIO2 activity was measured using 50 µg protein in 100 µl reaction mixture consisting of [125I]-T4 (200 000 c.p.m./tube), 2 nM T4 or 500 nM T4 (to inhibit DIO2 activity), 1 mM T3 (to inhibit DIO3 activity), 20 mM DTT, and 1 mM PTU for 2 h at 37 °C. Results are expressed in fmol/min per mg protein.

Before each assay, [125I]-T4 and [125I]-rT3 (Perkin Elmer, Billerica, MA, USA) were purified by dialysis as described previously (14). For DIO1 and DIO2 activity assays, reactions were stopped by adding 100 µl of cold 2% BSA and 800 µl of 10% trichloroacetic acid. After centrifugation at 2000 g for 10 min, 800 µl supernatant was applied to AG 50W-X2 columns (bed volume=1 ml) (Bio-Rad Laboratories) and eluted with 10% glacial acetic acid. The [125I] generated in the assay was counted in a gamma scintillation counter (Packard Cobra Gamma Counter, Perkin Elmer, Waltham, MA, USA). Blanks were performed in quadruplicates by substituting the volume of tissue homogenates for assay buffer. Assay detection limit was calculated as the average of [125I] produced by blanks multiplied by 3.3 times SD.

DIO3 activity was measured as described previously by van Zeijl et al (317). In brief, adipose tissue (approximately 40 mg) was homogenized on ice in 500µl PE buffer (0.1 M sodium phosphate, 2 mM EDTA pH 7.2) containing 50 mM dithiothreitol (DTT) using a Polytron (Kinematica, Luzern, Switzerland). Protein concentration has been measured with the Bio-Rad protein assay (Bio-Rad Laboratories, Veenendaal, The Netherlands), according to manufacturer's instructions.

Samples were measured in duplicate using 75 µl homogenate in a final volume of 0.15 ml in the presence of 1nM T3 or 500nM T3 (tissue blank, 500 nM T3 saturates DIO3 activity) with the addition of approximately 1*105c.p.m. [3`-125I]T3 (NEX110X, Perkin Elmer) in PE buffer. Samples were incubated for 2h. The activity reactions

were stopped by adding 0.15 ml ice-cold ethanol. After centrifugation, 0.125 ml of the supernatant was added to 0.125 ml 0.02 M ammonium acetate (pH 4), and 0.1 ml of the mixture was applied to 4.6 x 250 mm Symmetry C18 column connected to a Waters HPLC system (Model 600E pump, Model 717 WISP auto-sampler, Waters, Etten-Leur, The Netherlands). The activity of T3 and T2 in the eluate was measured online using a Radiomatic 150 TR flow scintillation analyser (Perkin Elmer, Waltham, MA, USA) D3 activity is expressed as fmol generated 3,3`T2 per minute per mg tissue. DIO3 activity when incubated with 1nM T3, minus the activity measured when incubated with 500nM T3 represents true D3 activity.

4.3 Results

Baseline characteristics are outlined in Table 4.1. All patients had serum freeT4 and free T3 levels within the normal reference range prior to commencing GH replacement (Table 4.2). The mean daily dose of growth hormone was 0.34±0.11 mg (range 0.15 - 0.5mg) administered subcutaneously. Serum IGF-1 levels rose significantly, as expected, during the course of the study (+114.4±12.3µg/L, $p<0.0001$). Body mass index (BMI) did not change during the study; 31.38±1.649 kg/m 2 vs. 31.07±1.679kg/m2, $p=0.26$. Serum testosterone levels did not change during the course of the study (14.20±11.95nmol/L vs. 14.95±10.40nmol/L, $p=0.45$).

Table 4.1

Baseline demographic features for the full study cohort.
MPHD Multiple Pituitary Hormone Deficiencies. GHD Growth Hormone Deficiency.
NFPA Non-functioning pituitary adenoma.

Male/Female	20/0	
Aetiology of GHD n(%)		
NFPA	10(50)	
Prolactinoma	4(20)	
Other	6(30)	
MPHD n(%)	17(85)	
On Thyroxine n(%)	13(65)	
Age (median)	53.1 years	range 22.3-69.3
BMI (median)	31.3 kg/m^2	range 19.2-49.7

4.3.1 Changes in serum thyroid hormones, TSH and thyroglobulin concentrations

Following GH replacement, freeT4 levels declined (-1.09±1.99 pmol/L, p=0.02). Reverse T3 levels also fell (-3.44±1.42 ng/dL, p=0.03) and freeT3 levels increased (+0.34±0.15 pmol/l, p=0.03) (Table 4.2). In addition, the serum T3/T4 and fT3/rT3 ratio rose significantly (Figure 4.2). All changes in circulating thyroid hormone levels occurred within the normal reference range; no study subject required adjustment of or commencement of thyroxine replacement during the time course of the study.

There was no significant change in early morning, fasting serum TSH levels during the study period. Sub-group analysis of subjects with a detectable TSH level confirmed stable, baseline TSH levels throughout. Similarly, serum concentration of thyroglobulin and TBG were unchanged by growth hormone therapy (Table 4.3).

Two subjects had an elevated titre of anti-TPO antibodies; exclusion of these subjects from analysis did not significantly alter the changes observed in circulating thyroid hormones. No subject developed anti-TPO antibodies during the course of the study.

Table 4.2

Alteration in circulating thyroid hormones following replacement of growth hormone. Data is reported as mean±SEM where variable is normally distributed (TT4, fT3, rT3) and median±SD where variable is not normally distributed (fT4 & TT3). Only those with a detectable pre-treatment TSH level (n=13) were included in this analysis – reported as median (range). * p<0.05. GH Growth Hormone.

	Pre GH	Post GH	Δ	Ref. range	P value	95% CI
FreeT4 (pmol/L)	12.9±4.0	12.1±3.2	-1.09±1.99*	9-20	0.02	-2.24 to -0.32
Total T4 (nmol/L)	109±4.9	99.5±4.5	-9.6±4.25*	69-141	0.04	-18.5 to -0.72
Free T3 (pmol/L)	5.4±0.2	5.7±0.2	+0.34±0.15*	3.0-7.5	0.03	0.03 to 0.65
Total T3 (nmol/L)	1.72±0.4	1.62±0.4	-0.02±0.32	1-3	0.76	-0.1 to 0.19
Reverse T3 (ng/dL)	17.6±1.4	14.2±0.8	-3.44±1.42*	8-25	0.03	-6.44 to -0.45
TSH (mU/L)	1.1 (0.4-5.1)	1.1 (0.2-3.3)	-0.1±1.09	0.4-4.0	0.31	-0.87 to 0.17

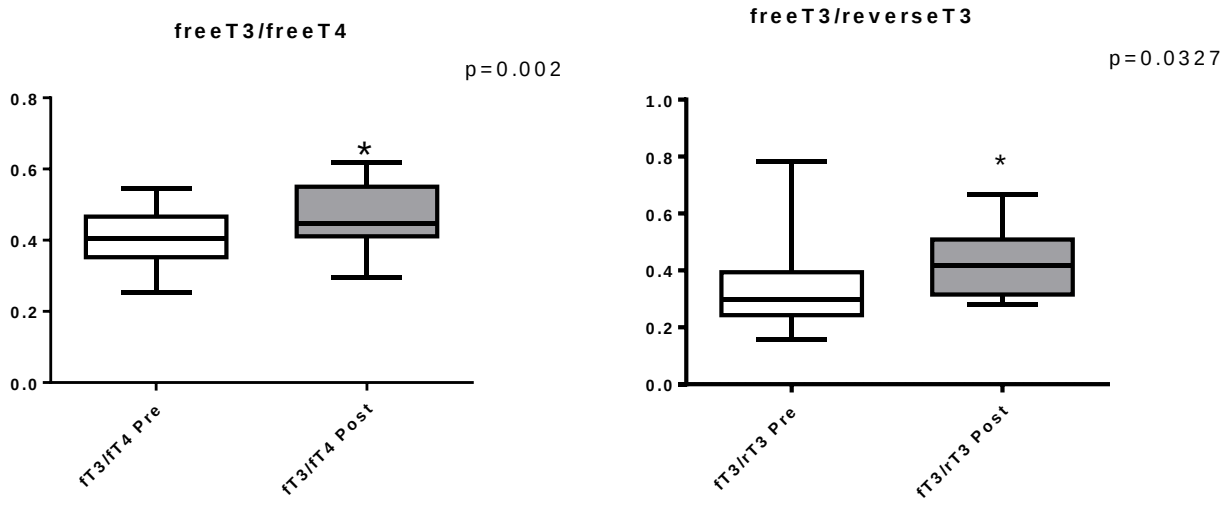

Figure 4.2

Ratio of freeT3/freeT4 and freeT3/reverse T3 before (white boxes) and after (grey boxes) GH supplementation. * $p<0.05$.

Table 4.3

The effect of GH replacement on serum thyroid stimulating hormone (TSH), thyroid binging globulin (TBG) and thyroglobulin (Tg). Only those with a detectable pre-treatment TSH level (n=13) were included in this analysis – (TSH range 0.41-5.1mU/L). Changes in TBG and Tg were analysed in all patients (n=20). Δ median difference.

	Pre GH median (range)	**Post GH** median (range)	**Δ**	**P value**	**95% CI**
TSH (mU/L)	1.1 (0.4-5.1)	1.1 (0.2-3.3)	-0.1±1.09	0.31	-0.87 to 0.17
TBG (µg/mL)	14.1 (8.9-22)	14.1 (9.1-24)	+1.1±0.83	0.18	-0.59 to 2.88
Tg (µg/L)	6.8 (1.8-47)	6.5 (3.1-39)	-0.65±4.89	0.17	-1.50 to 0.0

4.3.2 Changes in deiodinases activity in subcutaneous adipose tissue

In subcutaneous fat, DIO2 isoenzyme activity declined (-11.08 fmol/mg per min; 95% CI -28.95 to 0.19, p=0.036). The decline in DIO2 activity was greater after exclusion of outliers (n=3): -12.62 fmol/mg per min; 95% CI -25.40 to -4.96, p=0.002). DIO1 and DIO3 isoenzyme activity remained unchanged following GH substitution (Figure 4.4). The decline in DIO2 activity correlated with the fall in serum freeT4 and totalT4 concentration but not the changes in T3 or reverse T3 (Figure 4.5). There was no significant correlation between the fall in DIO 2 activity and the rise in serum IGF-1 level.

Deiodinase enzymes are selenoproteins and their activity can be affected by severe selenium deficiency or marked alteration in selenium exposure.(16, 17) In our study, all subjects were replete in selenium (normal population reference range 0.8 – 2.0 Umol/L). Furthermore, serum selenium levels were not altered following GH replacement (1.24±0.04umol/L vs. 1.24±0.04 umol/L; 95% CI -0.096 to 0.088, p=0.93).

The study was conducted in Dublin, Ireland. This region is classified as mildly iodine deficient based on subnational data (331). However, no patient had severe iodine deficiency (defined as urinary iodine concentration <0.16 µmol/L) prior to commencing GH. Median urinary iodine concentration was unchanged before and after GH replacement – 0.84 µmol/L (range 0.17 - 2.81) vs 0.75 µmol/L (range 0.15-3.63); 95% CI -0.27 to 0.18, p=0.6215. Similarly, median 24 hour urinary excretion of iodine, a reflection of iodine nutritional status, remained stable throughout the study - 1.19 µmol/24hr (range 0.32-3.37) vs. 1.26 µmol/24hr (range 0.38-5.26); 95% CI -0.73 to 0.42, p=0.42).

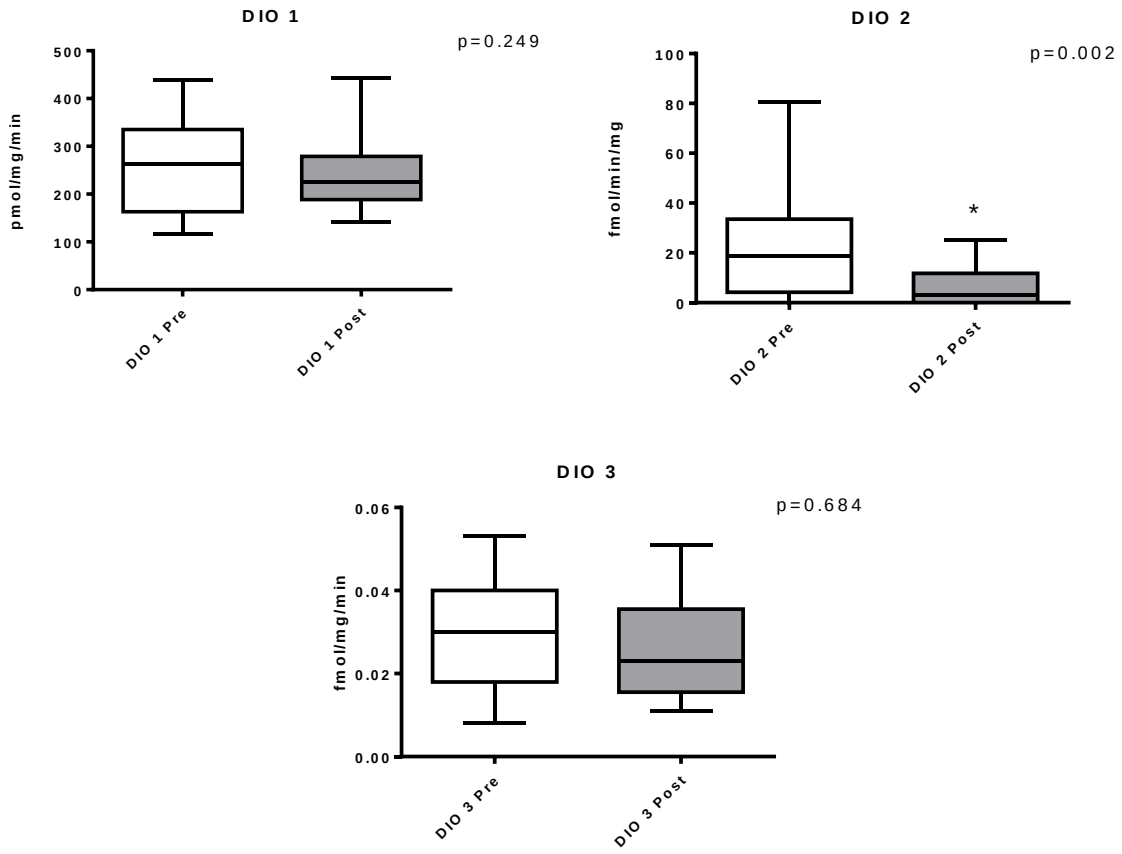

Figure 4.3

Deiodinase isoenzyme activity in subcutaneous fat following GH replacement. Data for DIO2 is shown following removal of significant outliers (n=3). DIO1 activity expressed as pmol/min per mg tissue; DIO3 & DIO2 expressed as fmol/min per mg tissue.

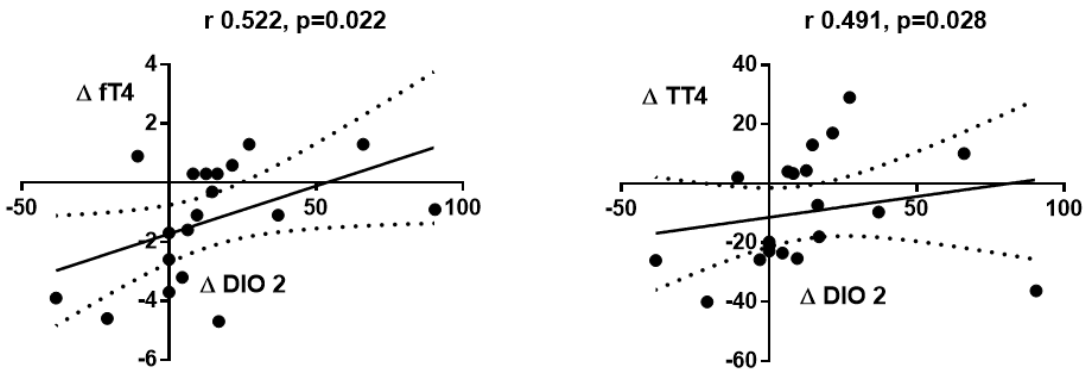

Figure 4.4

Correlation between fall in DIO2 activity (ΔDIO2; horizontal axis) and decline in serum T4 concentration following growth hormone supplementation.

4.4 Discussion

In this prospective clinical study, we attempt to shed light on the mechanisms underlying the alterations in the hypothalamo-pituitary-thyroid axis following GH replacement. Novel studies, including in vitro analysis of human serum and adipose tissue, have revealed the complex metabolic processes which occur during GH treatment. Our results demonstrate a decreased circulating concentration of freeT4 in conjunction with an increase in freeT3 following initiation of GH replacement. These alterations occurred despite TSH levels remaining unchanged. There are inconsistencies in the published literature to date; however, the pattern of change in circulating thyroid hormones that we report is largely in keeping with previous studies of large cohorts of patients with organic pituitary disease (62, 63, 66, 272, 320).

In our study, all changes in thyroid hormone concentration occurred within the normal reference range. This may be due to increasing awareness of the GH-induced fall in T4, resulting in a tendency to optimise T4 replacement prior to initiating GH by keeping the serum freeT4 concentration in the upper half of the normal range. While the underlying mechanism responsible for these alterations remains speculative, the rise in T3 in parallel with a fall in T4 levels is suggestive of increased peripheral conversion of T4 to T3.

We adopted a novel approach to investigate this potential mechanism through in vitro analysis of subcutaneous adipose tissue from adults, before and after exposure to recombinant GH. However, direct measurement of DIO2 activity, the principal deiodinase isoenzyme responsible for T4 to T3 conversion in peripheral tissue, did not reveal changes in DIO2 that would explain the alterations observed in the serum levels of thyroid hormones: paradoxically, DIO2 activity, in subcutaneous fat, declined following GH exposure. The activity of DIO1, which can also generate T3 in peripheral tissue, was unchanged by GH replacement. Serum freeT3 concentration rose during our study which could also be explained by

reduced deactivation of T3 - a reaction principally catalysed by DIO3; however, the activity of this isoenzyme, in subcutaneous fat, was unaltered by GH therapy.

Iodothyronine deiodinase enzymes are selenoproteins responsible for the local activation and inactivation of thyroid hormones (72, 73). In healthy humans, they maintain T3 homeostasis, both in the serum and in peripheral tissue, by controlling the formation and degradation of T3. In euthyroid human subjects, 80% of circulating T3 is formed by deiodination of T4 in peripheral tissue (73). We speculated that a fall in T4, accompanied by a rise in T3, following GH supplementation was due to increased DIO2 activity; however, the opposite effect on DIO2 was observed.

The explanation for this unexpected observation is unclear but we suggest a number of possible mechanisms. Firstly, previous animal research has demonstrated that hypophysectomised rats have an enhanced expression of DIO2 in brown adipose tissue (332). The investigators hypothesised that pituitary hormones exert tonic inhibition of DIO2 activity in peripheral tissue; only GH prevented the rise in DIO2 activity when hypophysectomised animals were given replacement pituitary hormones. This provides preliminary evidence that GH may have a suppressive effect on DIO2 activity in some tissues. Alternatively, the increased circulating and/or locally generated T3 may have caused the decline in DIO2 activity in an attempt to maintain tissue T3 homeostasis. Previous research has confirmed that T3 itself can suppress tissue DIO2 mRNA expression and activity in various animal tissues (333, 334). Also, GH is well recognised to cause lipolysis and a reduction in DIO2 activity could be an attempt to protect the fat tissue from the additional lipolytic action of T3 (261, 335, 336).

The rise in T3 level could also be explained by reduced clearance of T3 as a consequence of attenuated activity of DIO3. This is supported by limited animal research. Injection of GH into chicken embryos suppresses of hepatic DIO3 activity and is associated with a rise in serum T3 levels (337). In our study, DIO3 activity in

subcutaneous fat was not affected by GH replacement. However, our deiodinase assays measured maximum in vitro activity. These enzymes may not work at maximum activity in vivo and small, but clinically significant, changes in sub-maximum tissue activity may not have been detected by our assay. Ultimately, deiodinase enzyme activity was quite low in subcutaneous adipose tissue of our study subjects. The activity we observed, before and after GH exposure, may not necessarily reflect activity in other organs, such as liver, where the enzymes are expressed at a considerably higher level. To our knowledge, there are no previous human studies, directly examining the effect of GH replacement on tissue deiodinase activity, against which we could compare our findings.

Despite the discordance between the serum and tissue findings in our study, modulation of peripheral deiodination of thyroid hormones, by GH, remains a likely explanation for the changes in circulating thyroid hormone levels. Thyroid hormone influences gene expression in virtually every organ in humans and deiodinase enzymes are differentially expressed in a wide variety of tissues (78). DIO1 and/or DIO2 activity in other organs, such as the liver, skeletal muscles or kidney, may make a greater contribution to the circulating pool of T3 in patients with hypopituitarism. DIO3 activity was only measured at low levels in subcutaneous fat, in contrast to other tissue, such as skeletal muscle and liver, where it is often expressed at much higher levels (76, 338). DIO3 activity in other organs may be more sensitive to inhibition by GH and this may account for the rise in free T3 concentration.

Previous researchers have suggested alternative explanations for GH-induced alterations in thyroid hormone levels. The vast majority of circulating T4 is bound to TBG. A reduced binding capacity of TBG has been demonstrated following administration of high dose cadaveric GH (71). However, these findings were not reflected by any change in circulating thyroid hormone levels in non-hypopituitary subjects. Also, the decline in TBG occurred in parallel with a fall in serum albumin and total protein. The results of this study may be explained by fluid retention which

can occur with high-dose GH administration. In our prospective analysis, there was a parallel change in total and free T4 levels and also a rise in free T3 which do not support a possible change in TBG binding capacity. Also, in keeping with previous research, direct measurement of TBG serum concentration, before and after exposure to recombinant GH for at least three months, did not reveal any significant changes (63).

An alteration of TSH dynamics has also been postulated as a mechanism to explain the fall in T4 levels observed in many studies following GH replacement. Previous studies in adults have demonstrated a more pronounced reduction in T4 levels among hypopituitary subjects with a measureable serum TSH level (62). Porter et al, in a study of GHD adolescents reported a lower basal and TRH-stimulated TSH following GH replacement (339). However, this study was conducted at a time when cadaveric GH - which was occasionally contaminated with TSH - was used. Root et al demonstrated a decline in thyroidal I-131 uptake following GH replacement in children (340). This occurred without a change in iodine exposure and appeared to be reversed by exogenous TSH administration. TSH is usually secreted in a pulsatile manner with diurnal variation. Also, Jorgensen et al suggested that mean 24 hour secretion of TSH is reduced by GH therapy; in particular the nocturnal surge of TSH is attenuated (67). We did not measure TSH secretion profiles or the effect of TRH stimulation in our study. However, we found that subgroup analysis of subjects with a detectable baseline serum TSH revealed less marked changes in circulating thyroid hormone levels. Nonetheless, a direct effect of GH on TSH dynamics remains one possible explanation for some of the alterations in serum thyroid hormone levels following GH replacement.

Another possible underlying mechanism is a direct effect of GH on the thyroid gland, suppressing T4 production or promoting intrathyroidal conversion of T4 to T3 – DIO1 and DIO2 are expressed in the thyroid. However, serum thyroglobulin levels, a surrogate marker of thyroid hormone synthesis, as well as iodine exposure, were unchanged throughout our study.

This study extends the findings of previous studies which have examined the impact of GH replacement on thyroid hormone metabolism. This is the first prospective study, to our knowledge, to attempt to unravel the effects of GH on peripheral deiodination of T4 and T3, in hypopituitary subjects, by directly examining peripheral human tissue. We selected subcutaneous adipose tissue as it is a well-known peripheral tissue target of GH and easily accessible in human subjects (336). In vitro analysis of the activity of all three selenodeiodinase isoenzymes in subcutaneous fat did not explain the serum changes in thyroid hormone. However, it remains possible that differential regulation of deiodinase activity in other tissues (e.g. liver) explains the fluctuations in thyroid hormones.

The study has some limitations. The study cohort was relatively small and all were men. The gender imbalance occurred by chance during open unbiased recruitment. However, this may have reduced the confounding effect of oestrogen which has been shown to influence deiodinase activity in animal models (341, 342). Data from a healthy, match control group would have added to the findings of this study. However, this study was designed as a longitudinal observational cohort study during routine clinical treatment with each subject acting as their own control. The possible impact of altered TSH dynamics, including the response to TRH, was not assessed in this study; however, only seven subjects (35%) were TSH sufficient. In vitro experiments were limited to subcutaneous fat due to the relative ease of tissue accessibility. Analysis of other tissue (such as liver and skeletal muscle) may have confirmed a tissue-specific regulation of deiodinase activity by GH. Finally, we did not measure deiodinase mRNA expression in peripheral issue which may have given further insights into the peripheral modulation of thyroid hormone metabolism by GH (72).

In conclusion, DIO2 activity in subcutaneous adipose tissue is suppressed, while DIO1 and DIO3 activity are unchanged, following growth hormone replacement, in adults with hypopituitarism. This does not explain either the

decrease in circulating concentration of freeT4 and reverseT3 or the rise in freeT3 induced by GH replacement.

Chapter 5

Clinical consequences of changes induced in the hypothalamic-pituitary-thyroid axis following growth hormone replacement

5.1 Introduction

Central hypothyroidism, a rare cause of thyroid hormone deficiency, is challenging to diagnose and treat. Typical symptoms of hypothyroidism may be obscured by clinical features of co-existing pituitary hormone deficits such as GH or ACTH deficiency. In addition, there is no reliable marker of central hypothyroidism and the diagnosis is largely biochemical, based on the finding of a low free serum thyroid hormone concentration with inappropriately low serum TSH (110). The diagnosis is further complicated by the fact that a serum T4 concentration within the population reference range, similar to primary hypothyroidism, does not exclude central hypothyroidism. Research evidence suggest that patients with central hypothyroidism, in comparison to those with primary thyroid failure, are at risk of under-replacement with thyroxine (126). This has largely been attributed to difficulties in diagnosing and monitoring central hypothyroidism.

Most patients with central hypothyroidism have co-existing growth hormone deficiency (GHD) (100). Previous research has demonstrated that GH replacement modulates peripheral thyroid hormone metabolism. The most predictable alteration is a reduction in circulating free T4 levels with between 36-47% of previously euthyroid subjects requiring thyroxine replacement (61). However, many prospective studies in adult cohorts have also shown a concomitant rise in T3 levels. The clinical significance of a fall in T4, in the face of a rising T3 level (the biologically active form of thyroid hormone) is worthy of consideration.

Serum concentration of specific proteins and trace elements, derived from the liver and bone, are influenced by minor alterations in circulating thyroid hormone levels. In addition, recent evidence suggests that cardiac time intervals (recorded on echocardiography) and resting energy expenditure (REE) may be reliable biological markers of thyroid hormone action in central hypothyroidism (294). These measurements may be useful in the assessment of central hypothyroidism and may give new insight in to the clinical relevance of fluctuations in thyroid hormone levels observed following growth hormone replacement.

The aim of this study was to examine the relationship between changes in serum concentration of thyroid hormones and known biological markers of thyroid hormone action following growth hormone supplementation in adults.

5.2 Methods

5.2.1 Subjects

We performed a prospective, observational study of 20 adult hypopituitary patients with severe GH deficiency. GH replacement was offered as part of routine clinical care. Severe GH deficiency was confirmed by a GH stimulation test, either insulin tolerance test (ITT) or glucagon stimulation test (GST); a serum GH peak less than 3 µg/L was taken as evidence of severe GH deficiency. Additional pituitary hormone deficiencies were defined as described in Chapter 2. If the patient had multiple pituitary hormone deficiencies (defined as deficiency in two or more pituitary hormones), other hormones were adequately replaced for at least three months prior to commencing GH replacement.

Baseline tests performed before GH supplementation included serum TSH, free & total T4, free & total T3, rT3, insulin like growth factor-1 (IGF-1), testosterone and creatinine kinase (CK). Liver and bone- derived biomarkers of thyroid hormone

action were measured in serum and 24 hour urine collections. All serum samples were collected after an overnight fast. Resting energy expenditure (REE) was also measured in the fasting state. In addition, an echocardiogram was performed to measure cardiac time intervals. Weight (kg) and height (metres) were measured to calculate body mass index (BMI). Blood pressure and heart rate were recorded while resting on three separate occasions during the morning of the research testing schedule.

All subjects were commenced on recombinant GH at a starting dose of 0.3mg/day administered subcutaneously – Saizen® (somatropin (rDNA origin) for injection) Merck Serono Ltd. Serum IGF-1 was checked after one month and the dose was titrated to achieve an IGF-1 in the upper half of age-related reference range.

Tests were repeated after 3-6 months on a stable dose of GH.

5.2.2 Laboratory methods

Biochemical indices

Total T3 was measured by a competitive binding protein, chemiluminescent immunoassay (UniCel DxI 800, Beckman Coulter). rT3 was measured by liquid chromatography/tandem mass spectrometry (Quest Diagnostics, San Juan Capistrano, CA). The remainder of the serum thyroid hormones (total T4, free T4 and free T3) and TSH were measured using fluoroimmunometric and sensitive "second generation" assays respectively (DELFIA, PerkinElmer Life Sciences). TBG was measured by immunoassay (Siemens Immunlite).

IGF-1 was measured in a chemiluminescent immunoassay using an acridinium labelled anti-IGF 1 antibody (IDS-iSYS). Testosterone was measured using a liquid phase radioimmunoassay (Spectria, Cisbio Bioassays).

Bone-derived serum biomarkers

The concentration of bone-specific alkaline phosphatase (BALP), a marker of both bone mineralisation and maturation, was measured by an immunoenzymatic assay (Ostase, Immunodiagnostic Systems Ltd, Bolton, UK) on an automated ELISA platform. The serum concentrations of total procollagen type 1 N-propeptide (P1NP), osteocalcin 1–49 (OCI) and C-terminal cross-linking telopeptide (CTX-I) were measured by electrochemiluminescence immunoassay measured on the Elecsys 2010 analyser (Roche Diagnostics).

Liver-derived serum biomarkers

Quantitative measurement of sex hormone binding globulin (SHBG) was performed using a two-step chemiluminescent microparticle immunoassay (ACHITECT iSystem, Abbott). Ferritin was estimated by a chemiluminescent assay (Beckman DxI 800 Series). Caeruloplasmin was measured by immunoassay (Beckman AU Series Analyser)

Serum lipids, LDL and HDL subfractions were measured using a polyacrylamide gel tube electrophoresis method (LipoprintTM LDL System; Quantimetrix, Redondo Beach, CA). The Lipoprint System uses non-denaturing, linear polyacrylamide gel electrophoresis to separate and measure the lipoprotein fractions. The test uses a lipophilic dye that binds to the cholesterol in the lipoprotein particle prior to electrophoresis. The electrophoresed gels are scanned to determine the relative area of each lipoprotein subfraction which is multiplied by the total cholesterol of the sample to calculate the amount of cholesterol in each subfraction.

Inter-assay and intra-assay coefficients of variation (CV) were less than 10% throughout.

CK was measured by a spectrophotometric assay (Beckman AU Series Analyser). Inter-assay CV 1.5%

Serum selenium as well as serum and urine copper concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS), on an Elan 6100 DRC plus (SCIEX Perkin-Elmer, Beaconsfield).

5.2.3 Resting Energy Expenditure

Resting energy expenditure (REE) was measured by open circuit indirect calorimetry conducted using a transparent canopy in the dilution testing mode. (Deltatrac®). The canopy consisted of an inlet and an outlet valve. Both valves were connected to the calorimeter (Deltatrac II) via tubes. This measures the rate of O2 and CO2 exchange in the lungs and this can be used to estimate energy expenditure. Subsequently, these values were used to estimate REE and substrate utilization (% carbohydrate and % fat) using the Weir equation (Weir 1949). In addition, 24 hour urine collection was obtained to account for protein oxidation and to complete the energy expenditure measurement.

5.2.4 Echocardiographic assessment

A complete two-dimensional and Doppler echocardiogram was performed using a 2.0- to 2.5-MHz transducer according to standard technique. Two dimensional M-mode echocardiograms were recorded from parasternal short-axis view at mid-left ventricular (LV) level to determine LV dimensions. Pulsed wave Doppler was used to measure cardiac time intervals by sequential recording of the mitral inflow. Tissue doppler imaging (TDI) measurements were also made from an average of 5 consecutive heart beats. Time intervals were measured from mitral inflow and LV

outflow velocity traces. Separate measurements were taken from septal and lateral wall.

Cardiac intervals of interest are shown in Figure 5.1. ICT (isovolumetric contraction time) is the time when the ventricles have begun to contract, but the volume of the chambers has not yet changed. It occurs immediately after the period of electromechanical delay, following electrical stimulation of the ventricles. IRT (isovolumetric relaxation time) occurs at the end of systole when the semi-lunar valves shut and the ventricles relax; this results in a fall in the intraventricular pressure, initially with no change in the chamber volume. After the semilunar valves open, the ventricles can eject the stroke volume into the systemic circulation during a period known as ejection time (ET). Myocardial performance index (MPI) was defined as sum of the isovolumetric contraction time (ICT) and isovolumetric relaxation time (IRT) divided by the left ventricular ejection time (ET).

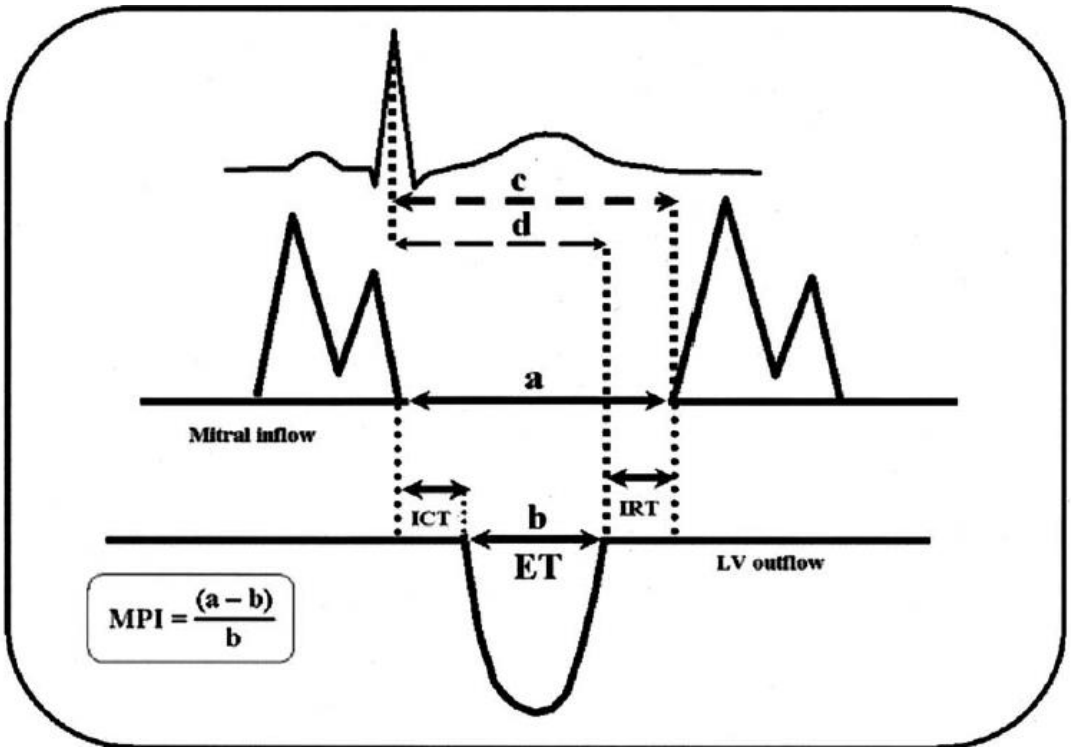

Figure 5.1

ICT (isovolumetric contraction time). This is the time when the ventricles have begun to contract, but the volume of the chambers has not yet changed. It occurs immediately after the period of electromechanical delay, following electrical stimulation of the ventricles.

IRT (isovolumetric relaxation time). This occurs at the end of systole when the semi-lunar valves shut and the ventricles relax; this results in a fall in the intraventricular pressure, initially with no change in the chamber volume.

ET (ejection time). After the semilunar valves open, the ventricles can eject the stroke volume into the systemic circulation during a period known as ET.

Figure 5.1 (contd)

Myocardial performance index (MPI) is also known as the Tei index. It incorporates both systolic and diastolic time intervals in expressing global systolic and diastolic ventricular function. MPI = (ICT+IRT)/ET.

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5.3 Results

Baseline clinical and hormonal characteristics are outlined in Table 5.1. Seventeen (85%) had multiple pituitary hormone deficiencies (MPHD) defined as two or more pituitary hormone deficiencies; three patients had isolated GH deficiency. Data is shown comparing subjects with and without central hypothyroidism (TSH deficiency). Median replacement dose of hydrocortisone for those with ACTH deficiency was 20mg/day (range 10-25). Hypertension was defined as prescribed anti-hypertensive medication. Hyperlipidaemia was defined as prescription of HMG CoA reductase inhibitor (statin). Fasting glucose was higher in the group without central hypothyroidism compared to the group with preserved TSH function. However, the former group included two patients with diabetes mellitus (2/7) whereas the latter group contained only one with diabetes (1/13).

Table 5.1

Baseline clinical characteristics of the study cohort. Data also shown for those with central hypothyroidism (CH) (taking thyroxine) and those with preserved TSH reserve (not taking thyroxine). Hypertension was defined as prescribed anti-hypertensive medication. Hyperlipidaemia was defined as prescribed HMG CoA reductase inhibitor. Data for continuous variables are displayed as median (range). GHD growth hormone deficiency.

*p<0.05. ° Comparison of patients with and without CH.

	All patients	**Patients with CH**	**Patients without CH**	**p value°**
n	20	13	7	
Male/Female	20/0	13/0	7/0	
MPHD n (%)	17 (85)	13 (100)	4 (57)	
ACTH deficient n (%)	11 (55)	9 (69)	2 (29)	
FSH/LH deficient n (%)	14 (70)	11 (85)	3 (43)	
Hypertension n (%)	5 (25)	3 (23)	2 (29)	
Hyperlipidaemia n (%)	6 (30)	3 (23)	3 (43)	

Table 5.1 (contd)

	All patients	Patients with CH	Patients without CH	p value°
BMI (kg/m^2)	31.3 (19.2-49.7)	33.9 (19.2-49.6)	30.5 (23.6-37.6)	0.42
Systolic BP (mmHg)	119 (95-158)	116 (95- 138)	120 (112-158)	0.39
Diastolic BP (mmHg)	73 (54-81)	71 (54-79)	80 (70-81)	0.15
Fasting glucose (mmol/L)	4.75 (3.6-8.0)	4.6 (3.6-5.6)	5.0 (4.7-8.0)	0.03*
Testosterone (nmol/L)	14.2 (5.6-50.0)	15.6 (6.3-50.0)	12.3 (5.6-21.2)	0.08
IGF-1 (baseline) (µg/dL)	126.5 (24.2-199.6)	100.1 (24.2-178.7)	142.2 (36.39-199.6)	0.33

5.3.1 Hypothalamic-pituitary-thyroid axis

As described in Chapter 3, the mean daily dose of growth hormone was 0.34±0.11 mg (range 0.15 - 0.5mg) administered subcutaneously. Following GH replacement, serum IGF-1 levels rose significantly, as expected, during the course of the study (+114.4±12.3µg/L, p<0.0001). Body mass index (BMI) did not change during the study; 31.38±1.649 kg/m 2 vs. 31.07±1.679kg/m2, p=0.26. FreeT4 levels declined (-1.09±1.99 pmol/L, p=0.02). Reverse T3 levels also fell (-3.44±1.42 ng/dL, p=0.03) and freeT3 levels increased (+0.34±0.15 pmol/l, p=0.03) (Table 5.2).

There was no significant change in early morning, fasting serum TSH levels during the study period. Sub-group analysis of subjects with a detectable TSH level confirmed stable, baseline TSH levels throughout. When patients with TSH deficiency (n=13) were considered separately, a similar pattern of biochemical pattern of changes was observed for free/total T4 and reverse T3 (Table 5.3). However, the rise in free T3 did not reach statistical significance in this group.

Table 5.2

Alteration in circulating thyroid hormones following replacement of growth hormone. Data is reported as mean±SEM where variable is normally distributed (TT4, fT3, rT3) and median±SD where variable is not normally distributed (fT4 & TT3). Only those with a detectable pre-treatment TSH level (n=13) were included in this analysis – reported as median (range). * p<0.05. GH Growth Hormone.

	Pre GH	Post GH	Δ	Ref. range	P value	95% CI
FreeT4 (pmol/L)	12.9±4.0	12.1±3.2	-1.09±1.99*	9-20	0.02	-2.24 to -0.32
Total T4 (nmol/L)	109±4.9	99.5±4.5	-9.6±4.25*	69-141	0.04	-18.5 to -0.72
Free T3 (pmol/L)	5.4±0.2	5.7±0.2	+0.34±0.15*	3.0-7.5	0.03	0.03 to 0.65
Total T3 (nmol/L)	1.72±0.4	1.62±0.4	-0.02±0.32	1-3	0.76	-0.1 to 0.19
Reverse T3 (ng/dL)	17.6±1.4	14.2±0.8	-3.44±1.42*	8-25	0.03	-6.44 to -0.45
TSH (mU/L)	1.1 (0.4-5.1)	1.1 (0.2-3.3)	-0.1±1.09	0.4-4.0	0.31	-0.87 to 0.17

Table 5.3

Alteration in circulating thyroid hormones following GH replacement in subjects with TSH deficiency (n=13). Data is reported as mean±SEM where variable is normally distributed (TT4, fT3, TT3, rT3) and median±SD where variable is not normally distributed (fT4). GH Growth Hormone.

	Pre GH	Post GH	Δ	Ref. range	P value	95% CI
FreeT4 (pmol/L)	13.5 ±4.59	12.20 ±3.66	-2.15 ±1.89*	9-20	0.004	-3.39 to -0.98
Total T4 (nmol/L)	116.7 ±4.58	102.6 ±4.3	-14.07±4.3*	69-141	0.007	-23.45 to -4.69
Free T3 (pmol/L)	5.312 ±0.23	5.74 ±0.28	+0.43±0.22	3.0-7.5	0.085	-0.07 to 0.22
Total T3 (nmol/L)	1.65 ±0.11	1.83 ±0.12	+0.18±0.08	1-3	0.05	0.005 to 0.36
Reverse T3 (ng/dL)	19.17 ±1.54	15.00 ±0.77	-4.15±1.72*	8-25	0.03	-7.94 to 0.39

5.3.2 Hepatic and muscle biomarkers

Changes in serum ferritin, SHBG and CK are shown in table 5.4. Significant changes in serum ferritin were not accompanied by changes in serum iron (14.40±1.188 μmol/L vs. 15.53±1.24μmol/L; 95% CI -1.1 to 3.37, p=0.3) or total iron binding capacity (42.59±2.89μmol/L vs. 45.17±2.51μmol/L; 95% CI -0.15 to 5.31, p=0.06). Serum ferritin correlated positively with serum free and total T4 before and after GH replacement (Figure 5.2). No such correlation was observed between IGF-1 and ferritin. The decline in serum ferritin during the study also correlated significantly with the fall in serum free & total T4; there was no significant correlation between the decline in ferritin and either the rise in freeT3 or IGF-1 (Figure 5.3).

Patients with TSH deficiency were analysed separately and a similar pattern of change in serum ferritin concentration was observed (100.4±66.70 vs 61.00± 59.64; 95% CI -65.06 to -14.14, p=0.003). No significant changes were observed in SHBG or CK in the subgroup analysis.

Table 5.4

Changes observed in serum biomarkers of thyroid hormone action following growth hormone (GH) replacement. Data shown for all subjects (n=20) is expressed as mean (SEM) for normally distributed data (CK) and median (SD) for data not normally distributed (SHBG, Ferritin).
CK creatinine kinase SHBG sex hormone binding globulin Δ change in serum concentration.
*p<0.05.

	Pre GH	Post GH	Δ	Ref. range	P value	95% CI
Ferritin ng/ml	83.30 (63.66)	48.65 (54.11)	-26.6 ± 36.09	16-215	0.005*	-44.83 to -8.94
CK IU/L	154.8 (26.77)	149.5 (18.81)	-4.5 ± 60.43	0-210	0.681	-25.00 to 11.00
SHBG nmol/L	35.85 (4.26)	34.09 (4.12)	-1.77 ± 2.0	13 -56	0.389	-5.96 to 2.43

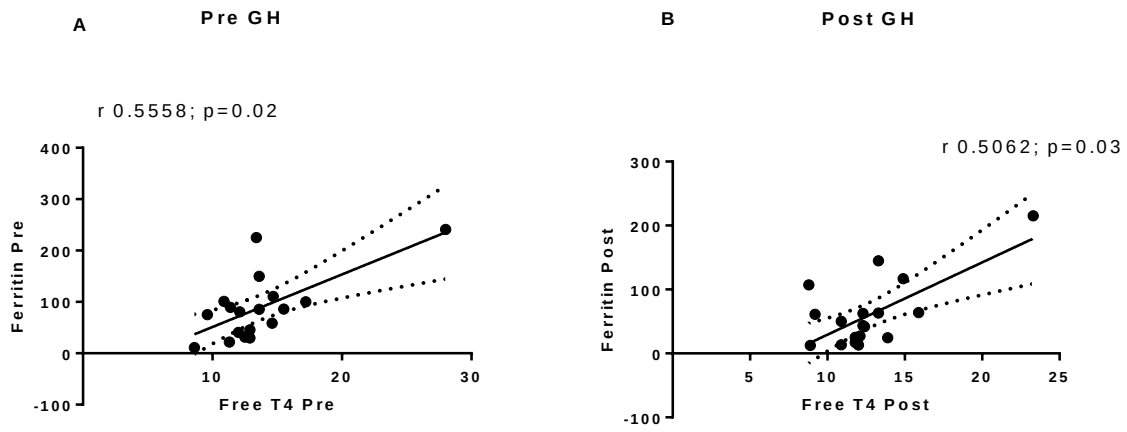

Figure 5.2

Correlation between serum ferritin and free T4 concentration for the full cohort. Previous researchers have shown that serum ferritin levels are sensitive to circulating thyroid hormone concentration. There was a significant positive correlation between serum ferritin correlated positively with serum free T4 before and after GH replacement.

Units: Ferritin ng/ml, free T4 pmol/L.

Δ delta – median change during the study period.

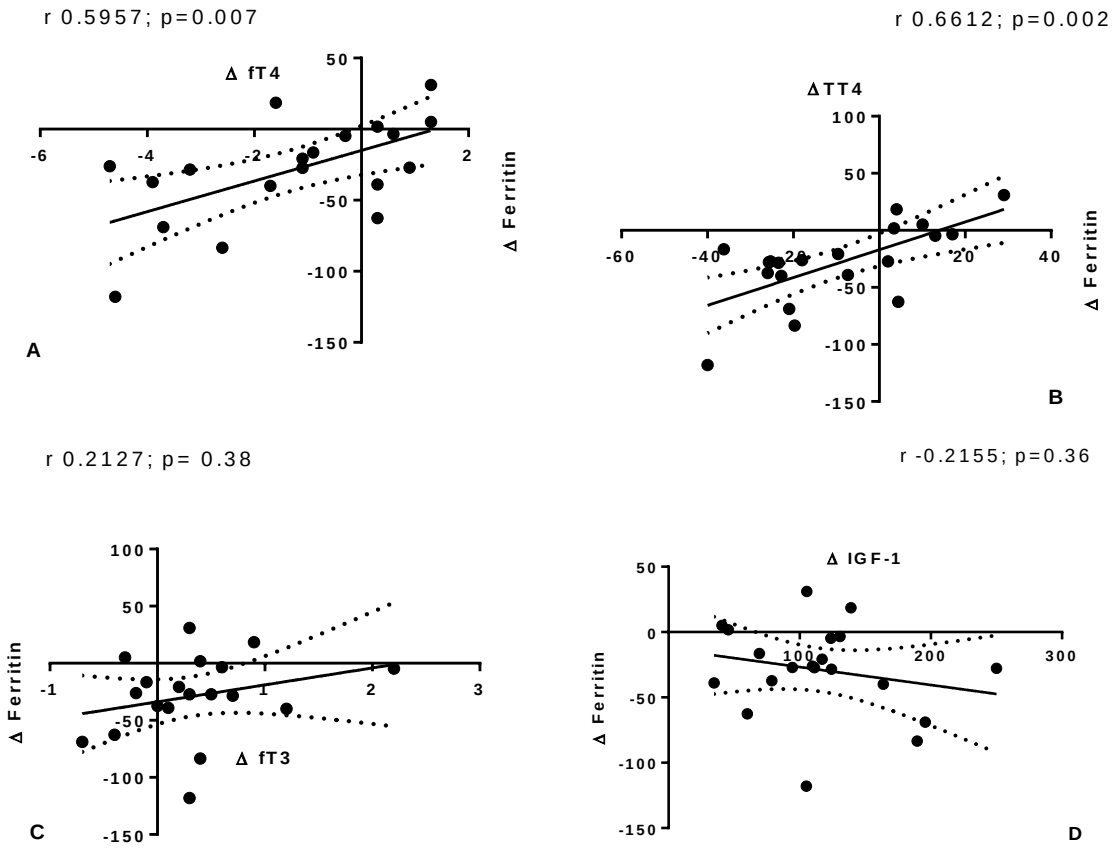

Figure 5.3

Correlation between the fall in serum ferritin and changes in free T4 (A), total T4 (B), free T3 (C) and insulin-like growth factor- 1(D) during the study period. The decline in serum ferritin during the study also correlated significantly with the fall in serum free & total T4; there was no significant correlation between the decline in ferritin and either the rise in freeT3 or IGF-1.

Units: Ferritin ng/ml, IGF-1 µg/L, free T3 pmol/L, free T4 pmol/L, total T4 nmol/L.

Δ delta – median change during the study period.

Analysis of copper metabolism, in the entire study group, did not show any significant changes in serum (or urine) copper or serum caeruloplasmin concentration. However, subgroup analysis of subjects with TSH deficiency (n=13), revealed that serum caeruloplasmin declined significantly following GH replacement while the decline of serum copper (Cu) was of borderline significance (Table 5.5). Urine copper excretion was not affected. Within this subgroup, serum Cu and caeruloplasmin correlated positively with serum freeT4 before, but not after, GH substitution.

There was no correlation between serum or urine biomarkers of copper metabolism and serum IGF-1. A weak correlation was seen between the decline in serum freeT4 and serum Cu; however this did not reach statistical significance (Figure 5.4). The change in caeruloplasmin did not correlate with either the fall in freeT4 or the rise in IGF-1.

Table 5.5

Serum and urine markers of copper metabolism before and after growth hormone replacement. Data shown only for those with TSH deficiency (n=13). All continuous variables were normally distributed and are reported as mean(SEM).
Cu copper GH growth hormone Caerulo. Caeruloplasmin. *p<0.05.

	Pre GH	Post GH	Δ	Ref. range	P value	95% CI
Copper µmol/L	16.97 (0.97)	15.35 (0.93)	-1.62 (±0.73)	12-25	0.05	-3.2 to -0.03
Caerulo. g/L	0.26 (0.01)	0.23 (0.02)	-0.03 (±0.01)	0.2-0.6	0.036*	-0.054 to -0.004
Urine Cu µmol/L	0.18 (0.04)	0.18 (0.03)	+0.004 (±0.03)		0.89	-0.056 to 0.063
24hr Urine Cu excretion µmol/24 hours	0.26 (0.05)	0.25 (0.03)	-0.003 (±0.05)	<0.9	0.96	-0.103 to 0.098

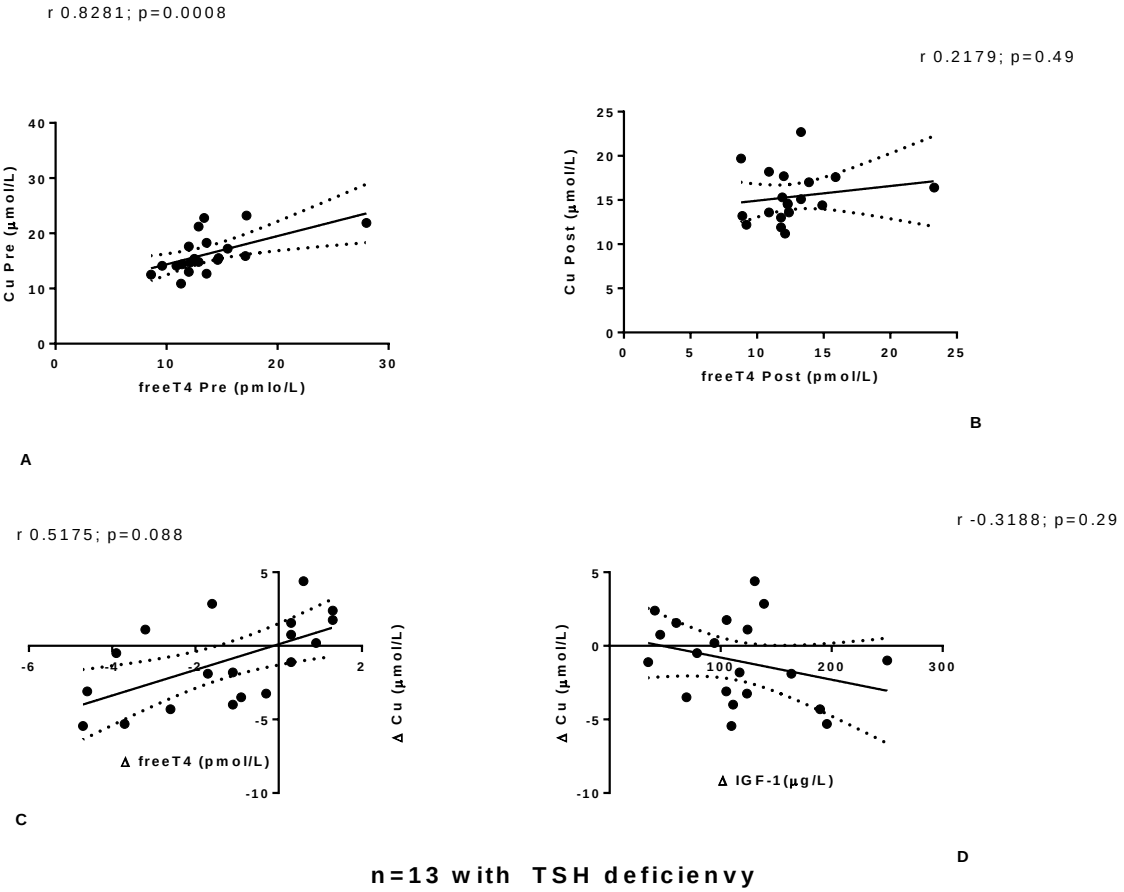

Figure 5.4

Previous researchers have shown that hepatic copper metabolism can be influenced by exposure to thyroid hormone.

A & B: Correlation between serum copper (Cu) concentration and free T4 before and after GH replacement.

C & D: Correlation between the decline in serum copper (ΔCu) and the fall in free T4 (Δ free T4) and rise IGF-1 (Δ IGF-1).

Data is shown only for subjects with central hypothyroidism (n=13).

There was no significant change in serum total cholesterol, HDL-C or LDL-C concentration following GH substitution (Table 5.6). Intermediate density lipoprotein (IDL) subclass C declined during the study (p=0.004). This reduction correlated with the rise in serum freeT3 (Figure 5.5). There were no differences in LDL particle subclasses expressed as absolute values or percentage change over the course of the study. Also, mean LDL size did not change (270.5±2.7Å vs 270.5± 2.2Å; p= 0.2893).

There was a trend towards an increase in the proportion of large HDL lipoprotein particles during the study (Table 5.6). More detailed analysis of individual subclasses revealed significant increases in the percentage of HDL-2 and HDL-3 particles in the serum following GH replacement (Figure 5.5). However, the percentage of HDL-1 particles, the other component of the “large HDL” particle subgroup, did not change significantly during the study. Similar results were found when large HDL particles were analysed by absolute change in serum concentration.

There was a negative correlation between the rise in IGF-1 and the rise large HDL particles, particularly HDL-2 (Figure 5.6). However, no correlation was found between the changes in thyroid hormone concentration and rise in large HDL subgroups. Lp(a) level rose significantly following GH exposure (p=0.002). However, neither the rise in IGF-1 nor fluctuations in thyroid hormone levels correlated with the rise in Lp (a).

Table 5.6.

Alteration in serum lipid parameters following replacement of growth hormone. Data is reported as mean±SEM where variable is normally distributed and median±SD where variable is not normally distributed (Tg, small dense LDL, Mean LDL size and Lp (a)). GH Growth Hormone TC Total Cholesterol HDL-C High density lipoprotein cholesterol LDL-C Low density lipoprotein cholesterol Trig. Triglyceride VLDL Very low density lipoprotein Intr. Intermediate. * $p<0.05$. ° $\chi 2$.

	Pre GH	**Post GH**	**Δ**	**P value**	**95% CI**
TC mg/dL	187.4± 9.54	192.9± 9.21	+5.55 ±7.77	0.48	-10.72 to 21.8
LDL-C mg/dL	100.3 ±6.66	103.4 ±6.52	+3.05 ±5.23	0.57	-7.91 to 14.01
HDL-C mg/dL	47.35 ±2.35	47.15 ±1.75	-0.2 ±1.66	0.91	-3.67 to 3.27
TC/HDL	3.644 ±0.18	3.718 ±0.18	+0.07 ±0.18	0.69	-0.31 to 0.45
Tg mmol/L	1.51 ±0.15	1.49 ±0.14	-0.01±0.16	0.92	-0.35 to 0.32

Table 5.6 (contd)

	Pre GH	Post GH	Δ	P value	95% CI
VLDL (%)	18.42 ± 0.72	19.03 ± 0.65	+0.61 ±0.72	0.41	-0.91 to 2.12
Large HDL (HDL 1-3) (%)	22.04 ± 2.29	25.17 ± 2.25	+3.13 ±1.62	0.07	-0.27 to 6.52
Inter. HDL (HDL 4-7) %	56.95 ± 1.38	56.39 ± 1.25	-0.56 ±1.01	0.59	-2.68 to 1.56
Small HDL (HDL 8-10) (%)	20.93 ±1.67	18.44 ± 1.43	-2.49 ±1.5	0.11	-5.64 to 0.66
ILD-A (%)	6.83 ±0.34	7.4 ±0.59	+0.57 ±0.47	0.23	-0.41 to 1.55
IDL-B (%)	5.18± 0.23	5.56 ± 0.3	+0.38 ±0.3	0.22	-0.25 to 1.01
IDL-C (%)	11±0.33	10 ±0.24	-0.98 ±0.29	0.004*	-1.6 to -0.36

Table 5.6 (contd)

	Pre GH	Post GH	Δ	P value	95% CI
Large LDL (LDL 1+2) (%)	28.48 ±0.74	29.00 ± 0.98	+0.53 ±0.58	0.37	-0.68 to 1.73
Small Dense LDL (LDL3-7) (%)	0.7 ±1.43	0.6 ±0.75	0.0 ±1.35	0.36	-1.0 to 0.27
Mean LDL size (Å)	270.5 ±2.69	270.5 ±2.22	+0.5 ±2.9	0.29	-0.66 to 2.06
Non-A LDL pattern (%)	20	5		0.34°	
Lp(a) nmol/L	27.4 ±52.17	34.25 ±64.9	+2.1 ±21.07	0.002*	2.64 to 22.35

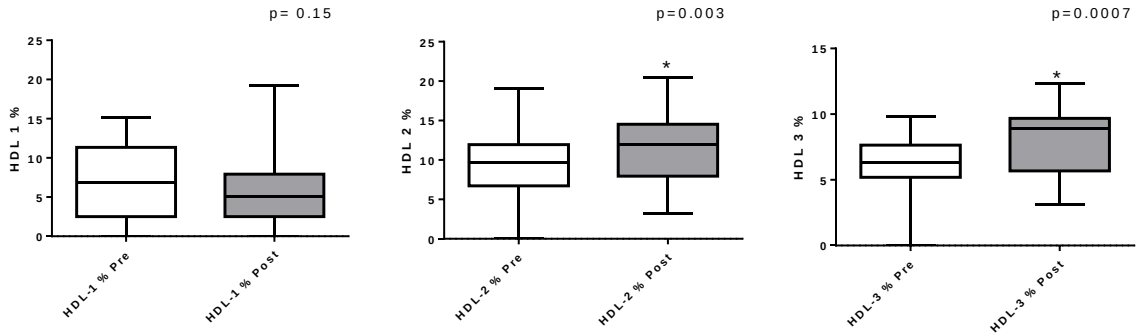

Figure 5.5

Percentage change in large HDL subclasses during the study. A significant rise was seen in percentage of HDL-2 and HDL-3 during the study.

*p<0.05.

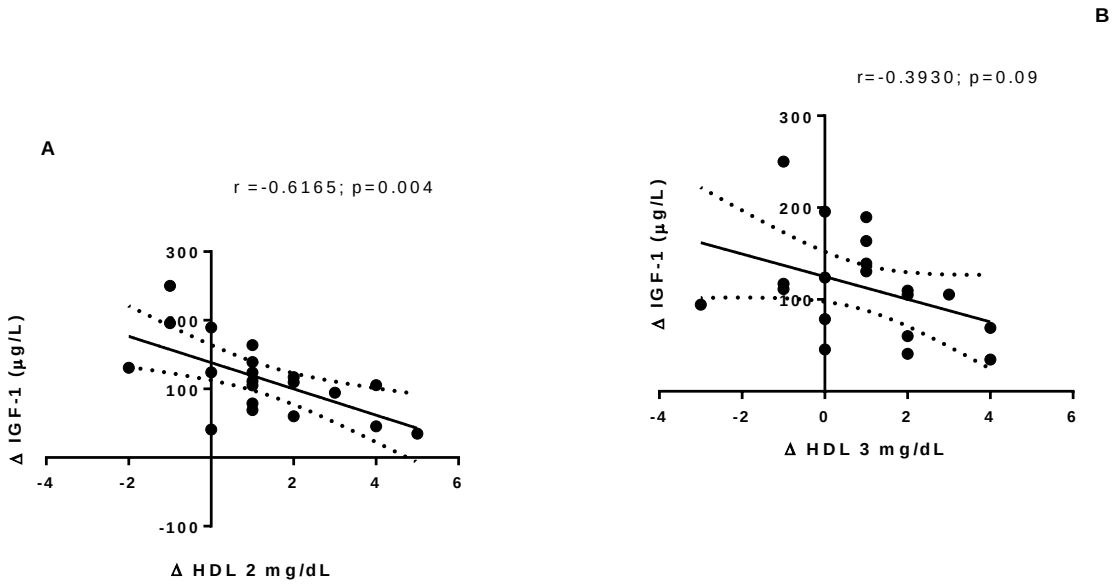

Figure 5.6

Change in the serum concentration of HDL 2 and HDL 3 in proportion to the change in IGF-1 during the study.

Subgroup analysis of subjects with TSH deficiency (n=13) revealed similar, minor changes in the serum lipid profile. There was no overall change in total cholesterol, LDL-C or HDL-C. Once again, percentage IDL-C declined; concentration of HDL 2 & 3 subparticles increased. There was a trend towards a significant reduction in small dense LDL (1.1% vs 0.5%; 95% CI -1.28 to -0.03, p=0.05).

Serum testosterone levels did not change during the course of the study (14.20±11.95nmol/L vs. 14.95±10.40nmol/L, p=0.45). All patients prescribed a HMG CoA reductase inhibitor remained on a stable dose throughout the study.

5.3.3 Bone biomarkers

Prior to GH replacement there was no significant correlation between bone turnover markers and either IGF-1 or thyroid hormone serum concentrations. Serum concentration of total procollagen type 1 amino-terminal propeptide (PINP) rose during the study (median rise +57.4%; p=0.0009) while the concentration of bone-specific alkaline phosphatase (BAP) remained unchanged. (Figure 5.7, Table 5.7). Serum osteocalcin (OCI) also rose (median rise +48.6%; p=0.0007). Isomerized C-terminal telopeptides of type I collagen (CTX-1) – a serum marker of bone resorption increased (median rise +73.7%; p=0.002) as outlined in Table 5.7 & Figure 5.7.

Table 5.7

The changes observed in circulating concentration of bone turnover markers during the study period. Significant increases were observed in markers of bone formation (most notable P1NP and OCI) as well as markers of bone resorption (CTX-1) SD standard deviation, Δ median difference.

	Pre GH median (SD)	**Post GH** median (SD)	**Δ**	**P value**	**95% CI**
P1NP (µg/L)	46.96 (21.56)	89.79 (59.79)	+26.94 ±61.58	0.0009	15.35 to 72.99
BAP (µg/L)	10.35 (3.94)	9.67 (4.767)	+0.45 ±4.41	0.75	-1.33 to 2.80
CTx-1 (µg/L)	0.30 (0.22)	0.56 (0.35)	+0.22 ±0.45	0.002	0.08 to 0.5
	Mean (SEM)	Mean (SEM)	Mean difference		
OCI (µg/L)	16.81 (1.44)	28.84 (3.03)	12.03±2.9	0.0007	5.77 to 18.29

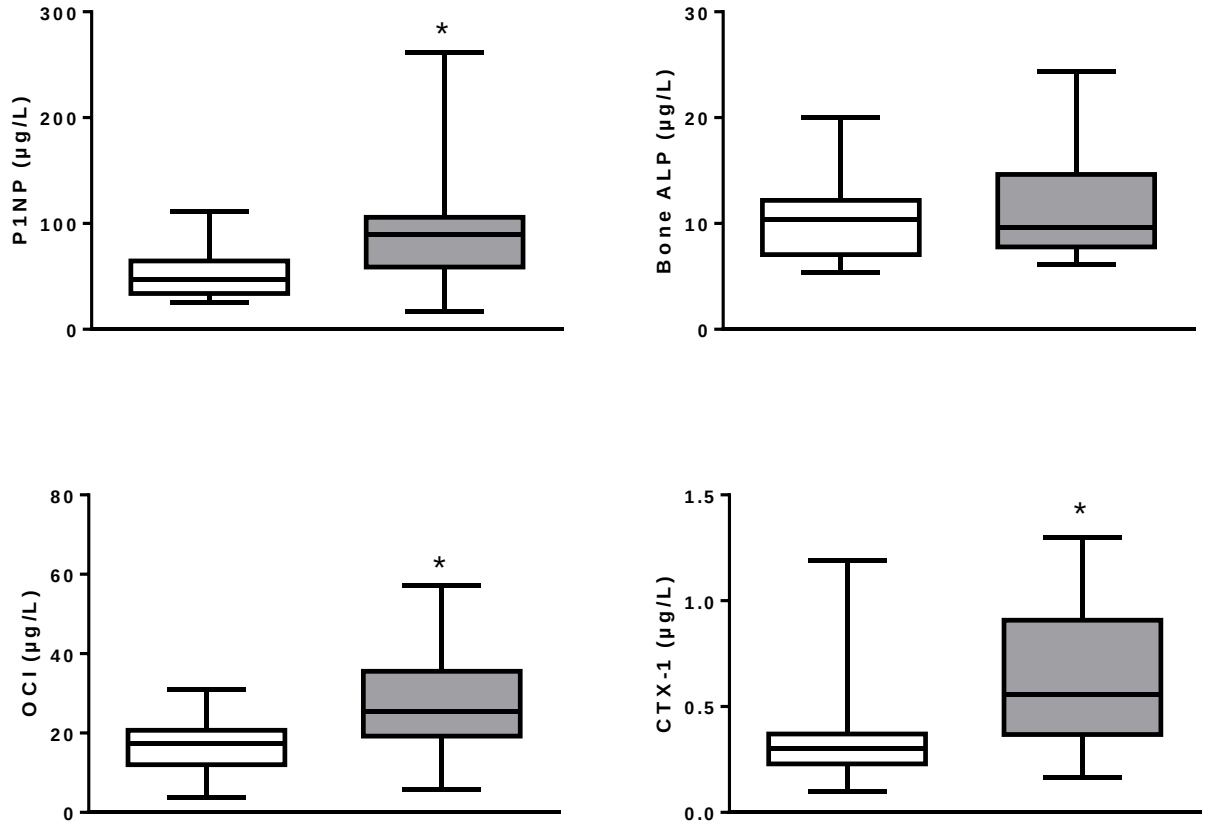

Figure 5.7

The changes observed in circulating concentration of bone turnover markers before (white boxes) and after (grey boxes) GH replacement. Significant increases were observed in markers of bone formation (most notably P1NP and OCI) as well as markers of bone resorption (CTX-1).

P1NP procollagen type 1 amino-terminal propeptide.

OCI osteocalcin.

BALP Bone alkaline phosphatase.

CTX-1 C-terminal telopeptides of type I collagen.

There was a strong correlation between the rise in OCI, P1NP and CTX-1 (Figure 5.8). However, the PINP:CTX-I ratio, an approximation of bone remodelling balance, was unchanged (160.3 versus 156.2; p= 0.4980).

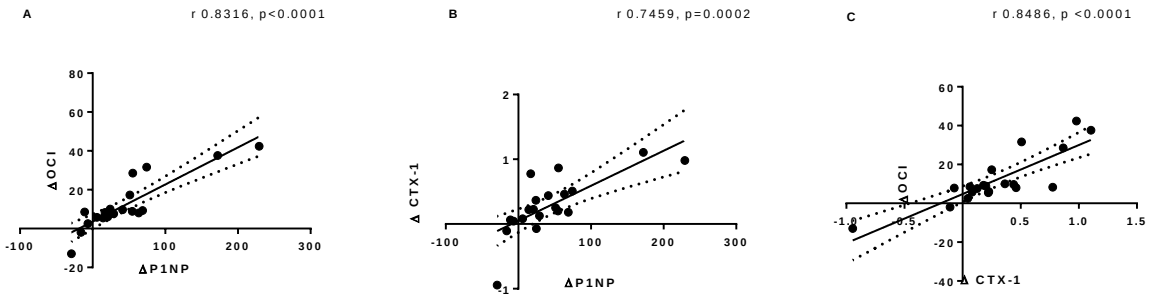

Figure 5.8

Correlation between changes in bone turnover markers during the study.

There was a strong positive correlation between the medial change Δ in OCI, PINP and CTX-1 indicating a true increase in bone turnover.

Units – µg/L for all bone turnover markers.

The rise in osteocalcin, a marker of bone formation, was proportional to the rise in in serum IGF-1. However, this relationship did not reach statistical significance. The increase in serum osteocalcin was more strongly correlated with the rise in the freeT3/free T4 ratio (Figure 5.9).

The rise in P1NP was also strongly associated with the rise in the ratio of fT3/fT4. However, a weak correlation was also observed between the rise in P1NP and serum IGF-1 (Figure 5.10). Increases in bone resorption markers, in particular the rise in CTX-1, were not associated with the rise in IGF-1 or changes in thyroid hormone concentrations.

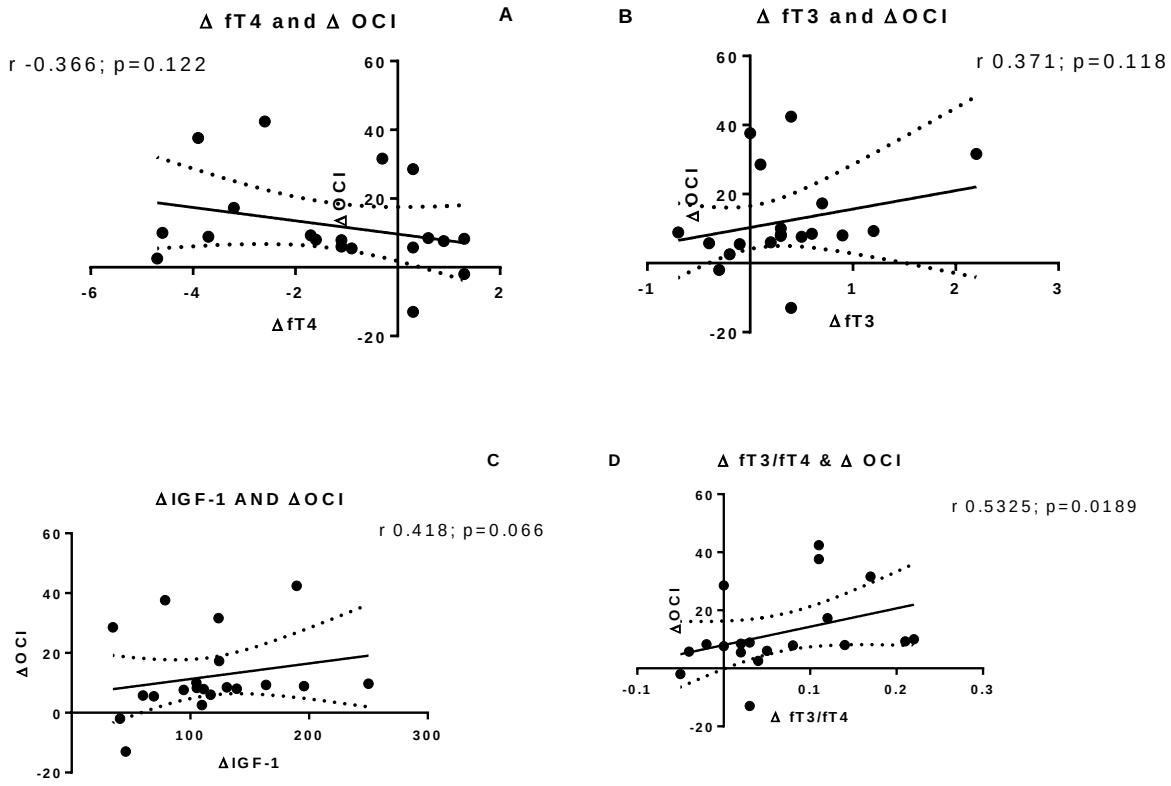

Figure 5.9

Change in serum osteocalcin (OCI) in proportion to change in circulating concentration of free T4 (A), freeT3 (B), IGF-1 (C) and the change in the ratio of fT3/fT4 (D)following growth hormone supplementation.

Units: OCI µg/L, free T4 pmol/L, free T3 pmol/L, IGF-1 µg/L.

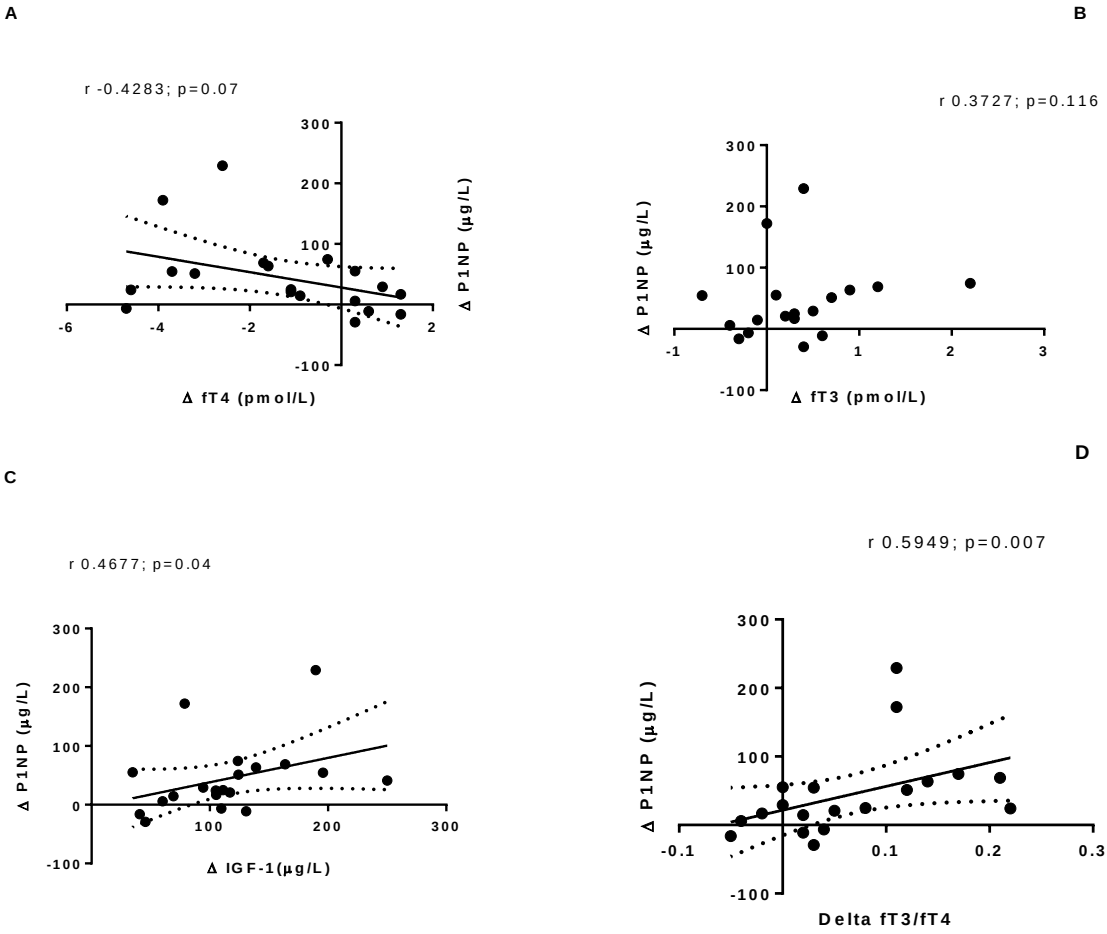

Figure 5.10

Change in serum total procollagen type 1 amino-terminal propeptide (PINP) in proportion to change in circulating concentration of free T4 (A), freeT3 (B), IGF-1 (C) and the change in the ratio of fT3/fT4 (D) following growth hormone supplementation.

When the subgroup with TSH deficiency (n=13) were analysed separately, highly significant increases were again observed in PINP and OCI; +113%, p=0.007 and +49%, p=0.001 respectively. However, in this subgroup analysis, BALP levels also increased significantly, +19%, p=0.01. Bone resorption marker, CTX-1, also increased significantly (+123%, p=0.001). However, the rise in bone turnover markers did not correlate with either the rise in serum IGF-1 or the fall in freeT4.

5.3.4 Metabolic rate measurement

Resting energy expenditure (REE), measured by indirect calorimetry in 19/20 study subjects, was not affected by GH replacement (2319± 110.5 kcal.min vs. 2368± 104.4 kcal.min; p= 0.41). Subgroup analysis of subjects with TSH deficiency (n=13) suggested that REE increased during the study; however, the rise in REE was not statistically significant (2353±141.1 kcal.min vs. 2477± 125.2 kcal.min; p= 0.11.) Neither circulating thyroid hormone levels nor serum IGF-1 concentration correlated with REE before or after GH replacement.

5.3.5 Cardiac time intervals

Heart rate and blood pressure did not change during the study. In addition, left ventricular ejection fraction (EF) was not affected by GH substitution. Measurements of cardiac time intervals are outlined in Table 5.8. Overall, there was no significant change in cardiac time intervals during the study. Systolic time intervals did not correlate with either the serum level of thyroid hormone or IGF-1 before or after GH exposure.

The subgroup with TSH deficiency was analysed separately (n=13). In this group, ICT appeared to be shortened following GH replacement; however, this change in ICT did not reach statistical significance (85.45± 13.83ms vs. 55.82±9.74ms; p=0.08.) In addition, the shortening of ICT, as measured by pulsed

wave doppler, was not replicated in tissue doppler measurements of this systolic interval.

Table 5.8

Echocardiographic assessment of cardiac time intervals measured by both pulse wave doppler and tissue doppler imaging (see Figure 5.1). Data is shown for all subjects and is expressed as mean (SEM). ICT/ET ratio and TDI-ICT-lateral wall are not normally distributed and are expressed as median(SD).

ICT Isovolumetric contraction time, IRT Isovolumetric relaxation time, ET Ejection time, MPI Myocardial performance index, MPI = (ICT+IRT)/ET, TDI tissue Doppler imaging, GH growth hormone, MS milliseconds.

	Pre GH	Post GH	Δ	P value	95% CI
ICT (ms)	73.56 (11.23)	64.50 (9.21)	-9.06±13.01	0.5	-36.79 to 18.67
IRT (ms)	76.56 (7.06)	70.00 (9.06)	-6.56±11.34	0.57	-30.73 to 17.61
ICT/ET	0.24 (0.2)	0.23 (0.15)	0.01±0.23	0.75	-0.17 to 0.07

Table 5.8 (contd)

	Pre GH	Post GH	Δ	P value	95% CI
MPI	0.58 (0.05)	0.5 (0.05)	-0.07±0.05	0.19	-0.19 to 0.04
TDI – ICT Lateral wall (ms)	81.00 (33.86)	78.00 (24.58)	-1.0±34.71	0.67	-24.69 to 13.75
TDI – ICT Septal wall (ms)	94.64 (10.67)	84.93 (5.539)	-9.71±10.79	0.38	-33.03 to 13.61

5.4 Discussion

This study prospectively assessed the clinical importance of alterations in the hypothalamic-pituitary-thyroid axis following GH replacement in hypopituitary men. We have demonstrated clear alterations in well-established, clinical biomarkers of thyroid hormone action. These occurred in line with fluctuations in the circulating concentration of thyroid hormone induced by GH substitution. Our results demonstrate that the fall in serum freeT4 and/or rise in T3, induced by GH, resulted in complex alterations in peripheral tissue metabolism. In particular, the fluctuations in thyroid hormone were reflected in liver and bone metabolism. Interestingly, alterations in peripheral biomarkers occurred despite thyroid hormone concentrations remaining within the normal reference range and in the presence of stable serum TSH levels.

Serum ferritin, a well-recognised marker of hepatic thyroid hormone exposure, declined in line with the fall in serum freeT4 concentration. Hepatic copper metabolism is also under the influence of thyroid hormone. The liver derived protein, caeruloplasmin, declined during the study, most notably in patients with more severe hypopituitarism including TSH deficiency. GH replacement also appeared to disrupt the clear relationship between serum freeT4 and caeruloplasmin concentration. Taken together, these findings suggest that the alterations in circulating thyroid hormone concentration, induced by GH therapy, may result in a more hypothyroid hepatic phenotype. SHBG, another hepatic protein under the influence of thyroid hormone, did not change during the study. However, GH, itself, can influence SHBG production (143, 145). The confounding effect of GH may render SHBG an unreliable peripheral biomarker, when assessing the clinical impact of fluctuations in serum thyroid hormone levels during GH replacement. CK, another serum marker of thyroid hormone action, did not change during the study.

Hepatic exposure to thyroid hormone could also influence serum lipoproteins. However, alterations observed in the lipid profile were complex. Lipid parameters

measured in routine clinical practice, including total cholesterol, HDL-C and LDL-C did not change during the study. A significant decline in the concentration of the IDL-C sub-particle was associated with the rise in serum freeT3. In addition, the increase in the percentage of large HDL lipoproteins appeared to be influenced by growth hormone status. Overall, the changes in the lipid profile were minor and of doubtful clinical significance. The confounding effect of GH, which can also influence the serum lipid profile, is likely to limit the utility of lipoprotein particles as markers of thyroid hormone action in this clinical setting.

This study also demonstrated that serum markers of bone turnover can serve as useful biomarkers of thyroid hormone action following GH replacement. The parallel rise in markers of bone formation (P1NP and OCI) and resorption (CTX1), without a change in the P1NP:CTx1 ratio indicates an increase in bone turnover without a disturbance of the normal bone remodelling cycle. Both GH and thyroid hormone can affect bone turnover; however, our results suggest that the selective effects of thyroid hormone were more important in determining the serum concentration of bone turnover markers.

Physiological replacement of both growth hormone and thyroid hormone has previously been shown to stimulate resting energy expenditure (REE) (262, 266-268). Surprisingly, REE did not change in our study. This may have been due to the decline in serum free T4 causing a degree of peripheral tissue hypothyroidism and attenuating the expected rise in REE with GH substitution. However, a trend towards a rise in REE was observed in the subgroup with TSH deficiency. This group was more likely to have multiple pituitary hormone deficiencies and, therefore, more profound GH deficiency. Some previous studies in this field have suggested that GH-induced changes in the thyroid axis are more marked and permanent in patients with more profound hypopituitarism (62).

Cardiac time intervals, which are sensitive to changes in the circulating concentration of thyroid hormone, did not change during the study. A trend towards

shortening of ICT was seen in the subgroup with TSH deficiency suggesting an improved biological action of thyroid hormone on the heart. The overall group may not have been of sufficient size to detect subtle changes in cardiac time intervals or REE.

The clinical consequences of changes in HPT axis provoked by GH replacement are easier to define in children. Previous studies of subjects with childhood-onset GHD have shown that a decline in serum T4, following commencement of GH therapy, was associated with a reduction in height velocity particularly when T4 declined below the normal reference range (70, 320, 322). Height velocity was generally restored when thyroxine supplementation was commenced or optimised to normalise serum T4 concentration. However, this has not been a universal finding and the clinical impact of changes in the HPT axis appears to be related to the severity of GHD (320). Children with idiopathic isolated GHD were more likely to develop minor, often transient, alterations in serum T4 and T3 within the reference range which did not appear to impact on growth. In contrast, children with MPHD often experienced more profound and permanent fluctuations in circulating thyroid hormone levels.

Characterising the clinical impact of GH-induced changes in the HPT axis in adult patients is more challenging. In hypopituitarism, serum free T4, similar to primary hypothyroidism, is a sensitive but not a specific marker of central hypothyroidism. Ferretti et al found no change in serum ferritin but a significant decline in serum SHBG and increase in serum CK after inducing hypothyroidism (by withdrawal of thyroid hormone leading to a low freeT4 in all subjects) in a cohort of 37 adults with previously diagnosed central hypothyroidism (128). SHBG and CK reverted to pre-treatment levels once freeT4 levels were restored on thyroxine replacement. In a blinded crossover study, Slawik et al compared the effect of various doses of levothyroxine (T4) and liothyronine (T3) replacement on metabolic parameters (125). A body weight-adapted dose of either T4 or a combination of T4 and T3 was associated with a healthier metabolic phenotype in comparison to

empiric dosing. Serum CK levels were significantly lower while subjects were taking a body weight-adapted dose. The less marked fluctuations in serum T3 and T4 in our study (all changes occurred within the normal reference range) may explain the divergent findings in respect of the utility of SHBG, CK and ferritin in central hypothyroidism.

To our knowledge, the impact of thyroxine replacement, or changes in the HPT axis following GH treatment, has not previously been compared to serum indices of copper metabolism. Animal studies have highlighted the positive correlation between serum T4 and both serum Cu and its transport protein caeruloplasmin (185, 186). This has been confirmed in human studies of patients with primary thyroid disease (187). Unlike many other markers of peripheral thyroid hormone action, GH appears to have little impact on copper metabolism. Therefore indices of peripheral Cu metabolism may be useful reflection of the selective effects if thyroid hormone in patients receiving GH replacement (197, 198). Our findings of a positive relationship between the alterations in free T4 and caeruloplasmin, which was most pronounced in subjects with central hypothyroidism, suggest a possible role for caeruloplasmin as a marker of hepatic thyroid hormone action.

The utility of serum lipoproteins as a marker of hepatic thyroid hormone action, in patients taking GH replacement, is matter of debate. Previous research, in relatively large cohorts of adults receiving GH replacement, did not show significant short term changes in routinely measured lipid parameters despite a fall in serum free T4 and the unmasking of central hypothyroidism in 36-47% of subjects (62, 63). However, studies in subjects with central hypothyroidism in whom the dose of levothyroxine has been adjusted have shown a significant impact of serum lipoproteins. Previous prospective research in adults with central hypothyroidism has shown that a body weight-adapted dose of T4 (or combined T4/T3), in comparison to an empiric dose, is associated with a lower total cholesterol, HDL-C and LDL-C (125). VDL and triglyceride levels were unchanged by different dosing schedules. Furthermore, temporary withdrawal of thyroxine replacement, for a 60

day period, in subjects treated for central hypothyroidism resulted in a significant increase in both total and LDL cholesterol (128). No changes were observed in HDL-C or triglycerides. We have measured several lipid sub particles to investigate if less dramatic changes in serum thyroid hormone concentration resulted in clinically significant alterations in lipid metabolism. Our results reveal only minor changes in lipid subparticles which are of uncertain clinical significance. In addition, these alterations were variably associated with thyroid and GH status. In particular, it was not clear whether the rise in the atherogenic Lp(a) particle was due to changes in the thyroid hormone, IGF-1 or both. Previous research has shown that both GH replacement and hypothyroidism (overt and subclinical) can lead to a rise in serum Lp(a) (225, 226, 343, 344). This may be due to either increased secretion or reduced clearance of the lipoprotein particle.

Previous researchers who have assessed the response of the HPT axis to GH replacement have not measured the concomitant response among bone turnover markers. However, there is limited data concerning bone biochemistry in patients with central hypothyroidism. In a study of 37 subjects with central hypothyroidism, not receiving GH replacement, the investigators measured the response of bone turnover markers after temporary withdrawal of thyroid hormone (128). The authors measured serum concentration of bone GLA protein - a maker of bone formation - and carboxyterminal telopeptide of type 1 collagen (ICTP) - a bone resorption marker. The former is a breakdown product of osteocalcin; the large N-MID fragment of osteocalcin was measured in my study. ICTP is largely equivalent to CTx-1, measured in the current study, although the ICTP assay detects a different epitope of carboxyterminal telopeptide of type 1 collagen. The results were comparable to my study - both bone GLA protein and ICTP declined significantly during withdrawal of thyroid hormone and increased when euthyroid state was restored. The authors also demonstrated strong correlations between serum free T3 and both serum bone GLA protein and ICTP.

The clinical utility of bone turnover markers, as biomarkers of peripheral thyroid hormone exposure will require careful consideration due to the potential interference from other pituitary hormone replacement. As mentioned previously, GH can increase bone turnover (45) (46, 252, 253). In addition, bone turnover can be suppressed while taking replacement glucocorticoid at a dose that many clinicians consider routine (345). Furthermore, sex steroids, in particular oestrogen, can stimulate the production of bone turnover markers (346, 347). In this current study, all patients were male and serum testosterone concentration did not change significantly during the study. Eleven (55%) had treated ACTH deficiency – median daily dose of hydrocortisone 20mg (range 10 -25). The dose of hydrocortisone did not change during the study period.

Resting energy expenditure (REE) typically rises with GH replacement although the underlying mechanism is still unclear (65, 267). Some authors have suggested that the rise in REE following GH substitution may be related to consequent rise in serum T3 concentration. Jørgensen et al, in a six month study of GH replacement in ten adults, demonstrated a positive correlation between the rise in REE and that of serum free T3 following GH replacement (67). A significant number of subjects (7/10) had free T3 levels in the subnormal range (despite a normal serum T4 concentration) before starting GH; this may have influenced the findings. While discordance between circulating T3 and T4 concentrations has been reported in some cohorts with central hypothyroidism, we did not observe it in the current study cohort. Martins et al, in a study of children with GHD, showed a correlation between serum total T3 and REE in subjects established on GH replacement; no such relationship was found in those not receiving GH. When GH-naïve children were given GH replacement, in the rise in REE was proportional to the rise in serum total T3. The rise in REE also correlated with the increase in IGF-1; however this was only of borderline significance.

In my study, REE did not rise as expected following GH replacement. In addition, REE did not correlate with either serum IGF-1 or thyroid hormone before

or after GH replacement. The divergence between my results and those of other investigators may be related to the study populations (children versus adults), variable doses of GH and thyroxine replacement as well methodological differences in estimation of REE.

Cardiac time intervals, in particular the systolic time interval – isovolumetric contraction time (ICT) – have been shown to be highly sensitive to changes in the circulating concentration of thyroid hormone (290, 291). ICT is the time when the ventricles have begun to contract, but the volume of the chambers has not yet changed. It occurs immediately after the period of electromechanical delay, following electrical stimulation of the ventricles. ICT is typically prolonged in hypothyroidism. This study found that ICT decreased during GH replacement. This was most apparent in those with central hypothyroidism; however this did not reach statistical significance. Previous research has suggested that ICT may be a useful marker of tissue hypothyroidism during GH therapy. In children with GHD, an inverse correlation between serum total T3 concentration and ICT was only observed in those receiving GH replacement leading the authors to suggest that GH improves the biological actions of thyroid hormone (294). When GH was administered to GH naïve children (n=7) the subsequent rise in total T3 only had a weak correlation with the rise in ICT. However, the small number of study subjects may have led to an underestimation of the utility of ICT as marker of thyroid hormone action on the heart.

Further research by Doin et al, has helped to validate the clinical use of ICT as biological marker of central hypothyroidism. In a controlled study of adults with pituitary disease and primary hypothyroidism, ICT had a high diagnostic accuracy in the identification of insufficient T4 replacement or of clinically occult central hypothyroidism in patients with pituitary disorders (289). After adjustment or commencement of thyroxine replacement the shortening of ICT correlated with the rise in serum free T4 concentration.

The main limitation of this study is its relatively small size. Differentiating the selective effect of thyroid hormone and GH/IGF-1 on tissue biomarkers was challenging in this small cohort. Also, the study cohort was exclusively male and results do not necessarily extend to female patients. The gender disparity occurred by chance, during open, unbiased study recruitment. However, it is likely the effect of oestrogen, in premenopausal women, would have had a significant impact on the bone and lipid parameters. Concurrent treatment for hypertension and hyperlipidaemia may have influenced the echocardiogram and lipid data respectively. The study did not include a health, matched control group which may have shed further light on our finding. However, this prospective study was designed as a longitudinal observational cohort study during routine clinical treatment with each subject acting as their own control.

In conclusion, we have prospectively demonstrated that alterations in the thyroid axis following GH replacement are associated with significant alterations in peripheral markers of thyroid hormone action. Fluctuations in thyroid hormone led to complex, tissue specific effects. Hepatic-derived peptides declined under the influence of thyroid hormone suggesting a more hypothyroid phenotype. In contrast, other tissues displayed, an enhanced biological action of thyroid hormone following GH substitution. In particular, the rise in serum T3:T4 ratio led to a marked increase in bone turnover. The small changes in thyroid hormone concentration, all of which occurred within the normal reference range, were not reflected in cardiac and skeletal muscle markers of thyroid hormone action. Therefore, bone and liver biomarkers may prove useful tools in assessing the clinical impact of alterations in the thyroid axis following GH replacement.

Chapter 6

The interaction between GH-induced changes in the thyroid axis on health-related quality of life

6.1 Introduction

In previous chapters, I have described the changes in the HPT axis following GH replacement. However, all changes in serum thyroid hormone concentration occurred within the normal reference range. In addition, the variability in these changes, reported in previous studies in the literature, has led many investigators to question the clinical significance of these alterations.

In children with GHD, retardation of growth velocity has been described in line with a fall in serum T4 concentration (320, 322). However, this finding is not universal in the literature. In Chapter 5, I described the impact of alterations in the HPT axis on biological markers of thyroid hormone action derived from a variety of peripheral tissues in adult patients. However, the clinical impact on patient symptoms, disability and quality of life (QOL) can only be assessed with patient reported outcomes including questionnaires.

Overt and subclinical hypothyroidism, are associated with impaired QOL when assessed by patient reported outcomes; however, the data in the latter condition is more inconsistent (348-350). This may be due to methodological differences in key studies including a variable definition of subclinical hypothyroidism. Studies using patient questionnaires have revealed that QOL does not always normalise after thyroxine replacement (311, 312, 351). This suggests that the peripheral tissue concentration of thyroid hormone is not always reflected

by the serum concentration and that formal assessment of QOL may have an important role in the clinical assessment of patients with hypothyroidism (352).

Similarly, a deficiency in health-related QOL is a well-recognised feature of the syndrome of adult growth hormone deficiency. Indeed, this is one of the principal indications for GH replacement in adulthood (353). Furthermore, in some jurisdictions, the prescription of GH is restricted to those with significantly impaired QOL who demonstrate an improvement after receiving substitution. There is preliminary evidence, among adults receiving GH replacement, that patients who experience a fall in serum T4 below the reference range (i.e. central hypothyroidism is unmasked) are less likely to experience an improvement in health-related QOL (62). The importance of QOL assessment adults in receiving GH replacement is, therefore, complicated by the interaction between GH and the thyroid axis. The selective effects of thyroid hormone and GH on QOL merits further consideration.

The aim of this study was to prospectively examine the association between changes in generic health-related and disease-specific QOL and changes in circulating thyroid hormone in adults receiving GH replacement.

6.2 Methods

Twenty adult men with severe GHD, who received GH replacement as part of routine clinical care were prospectively evaluated before, and 3-6 months after, commencing treatment. Subjects were recruited from the Pituitary Clinic in Beaumont Hospital.

If the patient had multiple pituitary hormone deficiencies (defined as deficiency in two or more pituitary hormones), other hormones were adequately replaced for at least three months prior to commencing GH replacement. The diagnosis of GHD and definition of other pituitary hormone deficiencies including diabetes insipidus is outlined in Chapter 2.

Baseline tests performed before GH supplementation included serum TSH, free & total T4, free & total T3, reverseT3, insulin like growth factor-1 (IGF-1), testosterone and electrolytes. Subjects were administered generic health related QOL questionnaires - the Short Form 36 (SF36) (Appendix 1) and the Nottingham Health Profile (NHP) (Appendix 2). They also completed the GHD disease-specific Assessment of Growth Hormone Deficiency in Adulthood (AGHDA) questionnaire (Appendix 3). Detailed description of these questionnaires is provided in Chapter 2.

All subjects were commenced on recombinant GH at a starting dose of 0.3mg/day administered subcutaneously – Saizen® (somatropin (rDNA origin) for injection) Merck Serono Ltd. Serum IGF-1 was checked after one month and the dose was titrated to achieve an IGF-1 in the upper half of age-related reference range. Replacement doses of hydrocortisone, testosterone and DDAVP, for those suffering from other pituitary hormone deficiencies were not adjusted during the study period. Tests were repeated after 3-6 months on a stable dose of GH.

6.3 Results

Baseline clinical and hormonal characteristics, similar to previous studies, are outlined in Table 6.1. Seventeen (85%) had multiple pituitary hormone deficiencies (MPHD) defined as two or more pituitary hormone deficiencies; three patients had isolated GH deficiency.

Serum testosterone levels did not change during the course of the study (14.20±11.95nmol/L vs. 14.95±10.40nmol/L, p=0.45). Three patients had cranial diabetes insipidus. Serum electrolyte levels were recorded at each visit; there were no abnormalities suggestive of over- or under-replacement with ddAVP. All patients had serum freeT4 and free T3 levels within the normal reference range prior to commencing GH replacement (Table 6.2).

Mean daily dose of growth hormone was 0.34±0.11 mg (range 0.15 - 0.5mg) administered subcutaneously. Following GH replacement, serum IGF-1 levels rose significantly, as expected, during the course of the study (+114.4±12.3µg/L, p<0.0001). FreeT4 levels declined (-1.09±1.99 pmol/L, p=0.02). Reverse T3 levels also fell (-3.44±1.42 ng/dL, p=0.03) and freeT3 levels increased (+0.34±0.15 pmol/l, p=0.03) (Table 6.2).

Table 6.1

Baseline characteristics of the full study cohort. BMI body mass index, GHD growth hormone deficiency, MPHD Multiple pituitary hormone deficiencies, NFPA Non-functioning pituitary adenoma . Data for age and BMI displayed as median (range).

Male/Female	20/0	
Aetiology of GHD n(%)		
NFPA	10(50)	
Prolactinoma	4(20)	
Other	6(30)	
MPHD n(%)	17(85)	
On Thyroxine n(%)	13(65)	
Age (median)	53.1 years	range 22.3-69.3
BMI (median)	31.3 kg/m^2	range 19.2-49.7

Table 6.2

Alteration in circulating thyroid hormones following replacement of growth hormone. Data is reported as mean±SEM where variable is normally distributed (TT4, fT3, rT3) and median±SD where variable is not normally distributed (fT4 & TT3). Only those with a detectable pre-treatment TSH level (n=13) were included in this analysis – reported as median (range). * p<0.05. GH Growth Hormone.

	Pre GH	Post GH	Δ	Ref. range	P value	95% CI
FreeT4 (pmol/L)	12.9±4.0	12.1±3.2	-1.09±1.99*	9-20	0.02	-2.24 to -0.32
Total T4 (nmol/L)	109±4.9	99.5±4.5	-9.6±4.25*	69-141	0.04	-18.5 to -0.72
Free T3 (pmol/L)	5.4±0.2	5.7±0.2	+0.34±0.15*	3.0-7.5	0.03	0.03 to 0.65
Total T3 (nmol/L)	1.72±0.4	1.62±0.4	-0.02±0.32	1-3	0.76	-0.1 to 0.19
Reverse T3 (ng/dL)	17.6±1.4	14.2±0.8	-3.44±1.42*	8-25	0.03	-6.44 to -0.45
TSH (mU/L)	1.1 (0.4-5.1)	1.1 (0.2-3.3)	-0.1±1.09	0.4-4.0	0.31	-0.87 to 0.17

Quality of life questionnaires

Assessment of GH deficiency in adulthood (AGHDA)

The baseline median AGHDA score was 16 (out of a possible total of 25) (range 0-24). Higher scores indicate a poorer QOL in the context of GHD. AGHDA score (median±SD) declined as expected during the study 16±8.7 vs 12±7.9; p=0.003. Subgroup analysis of those in whom serum free T4 declined during the study (n=12) demonstrated a blunted and non-significant rise in AGHDA score.

Nottingham Health Profile (NHP)

The self-reported scores in the six domains of the NHP are displayed in Table 6.3 and Figure 6.1. Lower scores indicate better health-related QOL. There was a decline in the score for social isolation during the study; however, this was only of borderline statistical significance. Raw score for social isolation (SI) score were – Pre GH 29.4±8.8 versus Post GH 17.3±6.2; p=0.05. There was no change in the other QOL domains of NHP during the study. Subgroup analysis of those with TSH deficiency (n=13) did not reveal any change in health-related QOL measured by NHP. Similarly, there was no change in NHP scores in subjects who experienced a decline in serum free T4 during the study (n=12)

Table 6.3

Quality of life scores before and after GH replacement for the full cohort (n=20). Raw scores are reported for Nottingham Health Profile (NHP) and transformed scores (raw scores converted to percentage) are displayed for Short Form 36 (SF36). Data expressed as mean (SEM), *p<0.1 **p<0.05 compared to baseline. High scores indicate better quality of life for SF36 and low scores indicate better quality of life for NHP.

NHP	Pre GH replacement	Post GH replacement
Energy level	47.4 (9.4)	44.9 (10.2)
Pain	13.9 (4.9)	12.2 (4.1)
Emotional reaction	25 (6.3)	21.9 (6.1)
Sleep	26.2 (7.9)	20.9 (6.7)
Social isolation	29.4 (8.8)	17 (6.2)*
Physical ability	14.1 (4.6)	16.6 (20)

Table 6.3 (contd)

SF36	Pre GH replacement	Post GH replacement
Physical functioning	63.8 (6.7)	74 (5.3)**
Role physical	43.8 (8.9)	56.3 (8.5)*
Bodily pain	72.8 (6.0)	74.6 (6.3)
General health	58.4 (3.7)	60.2 (6.0)
Vitality	41.3 (5.3)	50.8 (6.0)**
Social functioning	73.1 (5.3)	70 (5.3)
Role emotional	50.0 (10.1)	78.3 (8.1)**
Mental health	69.2 (4.4)	73.0 (3.7)

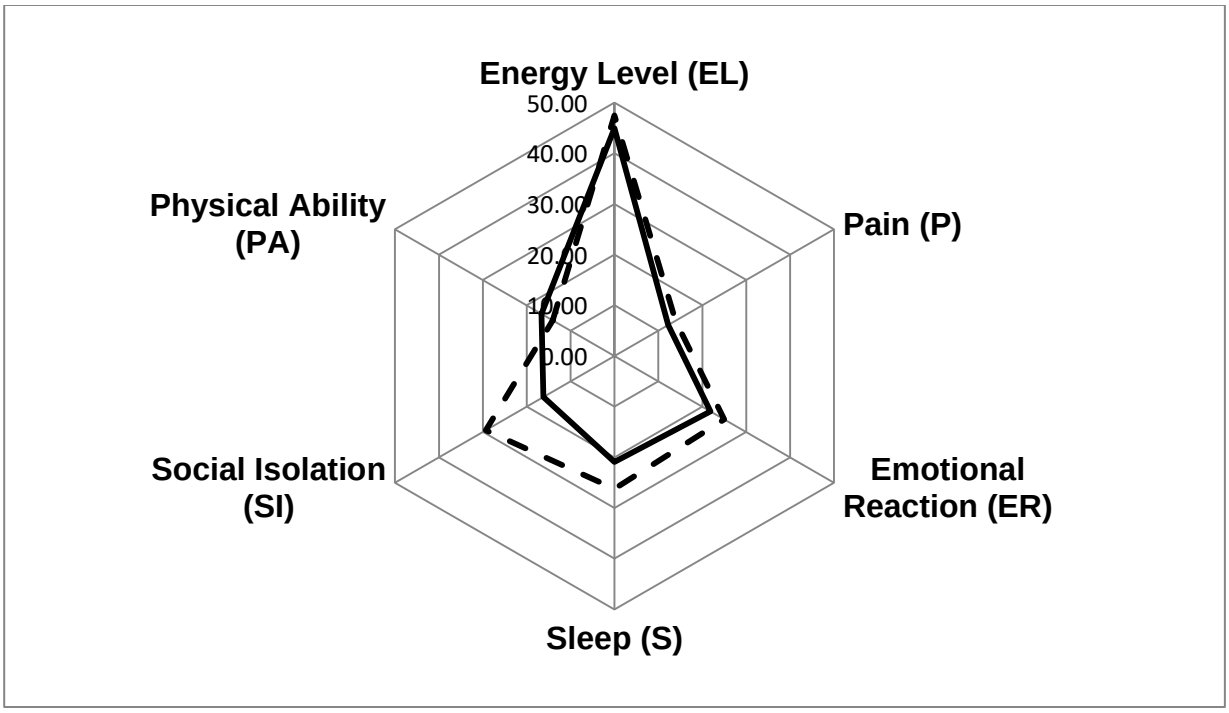

Figure 6.1

Radar plot showing the changes in the six domains of the Nottingham Health Profile (NHP), a generic health status questionnaire, during the study period. Lower scores indicate a better sense of well-being. Dotted line = pre GH at entry into study. Black line = 3 to 6 months after GH replacement.

Short Form 36 (SF 36)

Transformed scores (raw scores converted to a percentage) for SF 36 are displayed in Figure 6.2. Higher scores indicate a better state. There was a strong positive correlation between each component scale (all correlation coefficients >0.3) indicating good internal consistency. Overall, there was a significant increase in the scores for physical function, vitality and emotional role (Table 6.3). However, the rise in these scales did not correlate with either the rise in serum IGF-1 or the increase in freeT3/freeT4 ratio.

The improvements in health-related QOL observed in the full cohort were not apparent when subjects who experienced a decline in free T4 (n=12) were examined separately. Analysis of the subgroup with previously diagnosed central hypothyroidism (n=13) revealed less marked changes in QOL with a significant increase only in the physical function domain during the study.

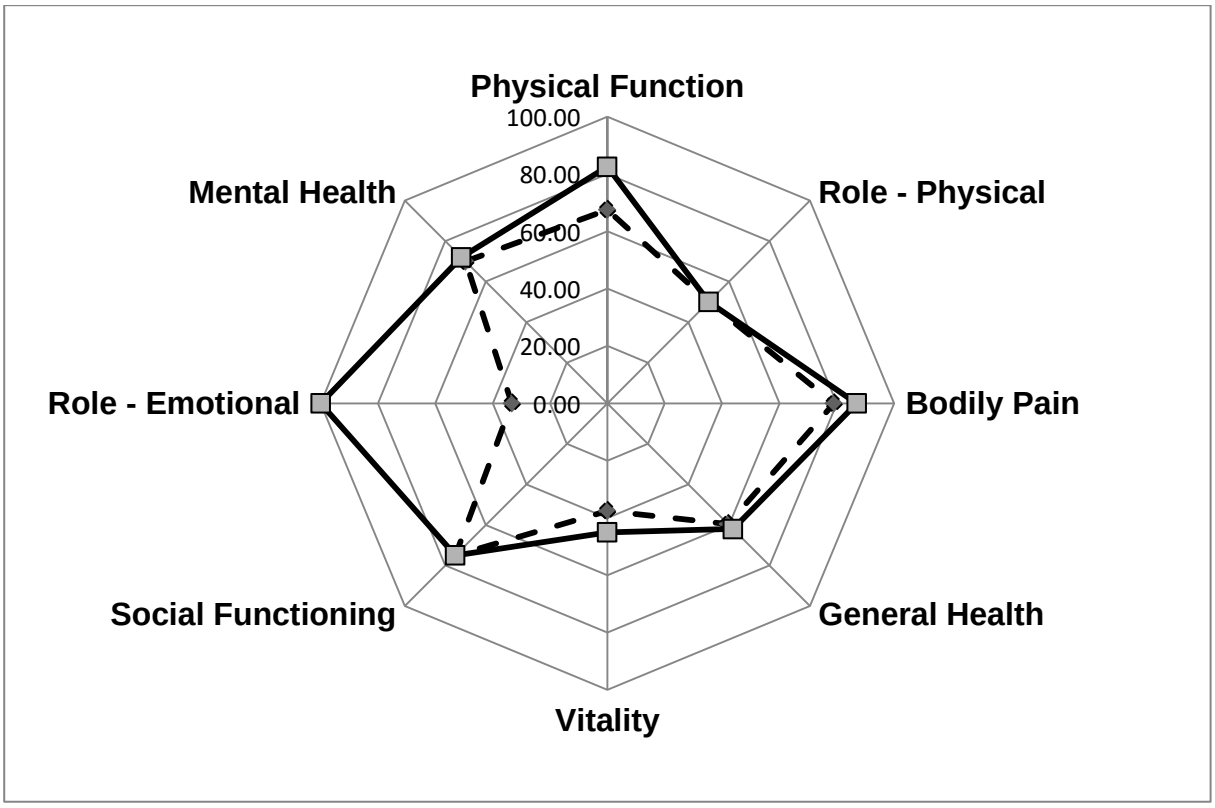

Figure 6.2

Radar plot showing the changes in the eight domains of the generic QOL assessment tool, Short Form 36 (SF 36), during the study period. Transformed scores are shown (i.e. raw score converted to percentage). Higher numerical scores indicate a better health status. Dotted line = pre GH at entry into study. Black line = 3 to 6 months after GH replacement.

6.3 Discussion

In Chapter 3, I discussed the changes in the HPT axis following growth hormone replacement in hypopituitary men. In essence, serum free and total T4 declined and the ratio of free T3/free T4 rose in proportion to the rise in serum IGF-1 concentration. In this chapter we have examined to impact of biological alterations in the GH and thyroid axis on health-related QOL.

Scores from the AGHDA questionnaire, a GHD disease-specific QOL self-assessment tool, were relatively high before commencing GH (16/25) indicating a poor QOL. This score declined, as expected, while receiving GH replacement sufficient to raise serum IGF-1 concentration into the upper half of the age adjusted reference range. Interestingly, a significant fall in AGHDA score was not observed in the group of subjects who experienced a decline in serum freeT4 during the study.

Subject responses to the NHP, a generic health status survey, suggested an overall improvement in QOL, particularly in the social isolation domain. However, the decline in NHP scores (suggesting an improved QOL) did not reach statistical significance. This remained true when those with previously diagnosed central hypothyroidism – which included the subjects with the most severe hypopituitarism - were analysed separately. The SF 36, which focuses on general well-being during activities of daily living and social interaction, revealed an improvement in a variety of domains after GH supplementation. Six months of GH replacement was associated with enhancement of QOL in the physical function, vitality and emotional role domains. It is noteworthy that improvements in QOL, measured by SF 36, were not apparent in the subjects in whom GH induced a fall in serum free T4. This finding, in keeping with the data from the disease-specific AGHDA questionnaire, suggests that the decline in serum free T4 is clinically important by attenuating the expected improvement in QOL following GH replacement.

Impaired QOL and sense of vitality are well recognised features of the syndrome of adult GHD. GH replacement is restricted to adults with impaired QOL in many jurisdictions. Therefore, improvement in QOL is an important clinical measure of the effectiveness of GH replacement. The use of generic QOL questionnaires is widespread in both placebo-controlled and open label studies of GH replacement. For example, using NHP, previous researchers have shown that the dimensions of energy level and emotional reaction responded best to treatment (49). However, improvements in QOL were sometimes only apparent after 12 months GH replacement (354). Therefore, lack of significant improvement in NHP scores in our study may relate to the relatively short time course. Alternatively, some studies have not demonstrated an improvement in well-being using generic QOL questionnaires and it may be that these instruments are insensitive to QOL deficits in GHD.

The disease specific adult GHD assessment tool (AGHDA) has been shown to be a reproducible method of monitoring psychosocial well-being while replacing GH (55, 305). AGHDA consists of 25 questions with YES/NO answers; higher scores indicate a poorer sense of well-being. Patients who experience an improvement in health-related QOL after receiving GH replacement show a decrease in AGHDA score after three months which is sustained at six and twelve months (303). However, the response to GH replacement, in terms of QOL, is variable and unpredictable. The reasons for are not clearly understood. It may, in part, relate to differences in the severity of GHD. However, previous research hints that the unmasking of central hypothyroidism may also be implicated in a poor response to GH replacement in respect of QOL. Agha et al showed that patients who became hypothyroid after GH replacement had a higher AGHDA score (indicating poorer QOL) after three months than those who remained euthyroid (62). Following T4 replacement, AGHDA score were equivalent in both groups. In our study, all changes in thyroid hormone concentration were within the normal range. Nonetheless, our data supports the findings of previous research with an attenuated

fall in AGHDA score seen in those who experienced a fall in serum free T4 after GH substitution.

There is a wealth of data linking primary hypothyroidism, both overt and subclinical, with impaired QOL. Patient reported outcomes using generic questionnaires such as SF 36 and NHP have highlighted deficits in multiple areas of QOL. These deficiencies typically improve after thyroxine replacement; however, the data in this field is inconsistent and patients patients who are biochemically euthyroid on thyroxine replacement may still exhibit subtle deficits in QOl compared to healthy controls. The data regarding QOL in central hypothyroidism is less extensive. Previous researcher in central hypothyroidism has shown that using a body weight adjusted dose of thyroxine (1.6µg/kg) in comparison to an empiric dose did not have a significant impact on well-being assessed by generic health status questionnaires including SF 36. The lack of differential response to QOL contrasts with the biochemical findings which suggest that the body weight adjusted dose of thyroxine resulted in a higher free T4 levels and a healthier metabolic phenotype.

Central hypothyroidism is usually part of a syndrome of multiple pituitary hormone deficits. Notwithstanding the selective effects of GH and thyroid hormone, the impact of other hormone deficiencies must be considered when using patient-reported QOL measurements in patients with pituitary disease. Over and under replacement of glucocorticoids can impact negatively in self-perceived health status (355-357). In addition, sex steroid deficiency and its replacement can influence health-related QOL. In our study subjects with ACTH deficiency the dose of hydrocortisone remained stable throughout the dose. However, the dose was not fixed and was prescribed according to the degree of ACTH deficiency judged by treating endocrinologist. All subjects were male - 70% were deficient in gonadotrophins and were prescribed testosterone replacement. While the dose testosterone replacement and route of administration were variable and decided by treating physician, the median serum testosterone concentration did not change during the study.

The study has limitations. Self-assessment patient reported outcomes are subject to considerable placebo effect. Our study was not placebo controlled. However, each subject acted as their own control in the longitudinal observational design. Generic QOL questionnaires provide a broad overview of a subject's health status but may be insensitive to disease specific deficits in well-being. We used the GHD disease specific AGHDA questionnaire but we did not use a hypothyroid specific symptom or quality of life scale. A specific QOL tool for thyroid disease may have yielded further insights into the clinical relevance of the changes in the HPT axis observed during the study. Previous research has shown that changes in QOL following GH may take many months or a year to become clinically detectable. The short time course, in a small cohort, may have underestimated the improvements in health-related QOL. Finally, the gender imbalance may have affected the results – all subjects were men. However, this imbalance occurred, by chance, during open, unbiased recruitment.

In conclusion, GH replacement is associated with improvements in health-related QOL. However, in patients who experience a decline in serum free T4 concentration following GH substitution, the improvement in QOL may have an attenuated.

Chapter 7

Summary Discussion and Recommendations

7.1 Summary Discussion

In this prospective human study, we have examined the impact of growth hormone (GH) replacement on the hypothalamic-pituitary-thyroid (HPT) axis. Recent research, in hypopituitary patients, has highlighted a variety of changes in thyroid hormone economy following exposure to GH. However, conflicting results were reported by different investigators (61). Variability in patient characteristics (adult versus children, GH deficient versus GH replete) may account for some of the differences in the results of studies in this field. The formulation (cadaveric versus recombinant) and dose of GH, in addition to the retrospective nature of many studies, may also have had a significant impact on the result.

Previous investigators have speculated about the mechanism responsible for GH-induced changes in the HPT axis. Several hypotheses have been suggested, the most convincing of which is a direct effect of GH on the peripheral activation and clearance of thyroid hormone. However, there are no human studies, to date, directly examining this theory. Furthermore, there is very little research examining the clinical relevance, in adult patients, of changes in the circulating concentration of thyroid hormone following GH replacement. Many of the purported benefits of GH in hypopituitary adults can also be influenced by changes in thyroid hormone exposure. Lipid abnormalities, bone turnover, energy expenditure, cardiac function and health-related quality of life are all sensitive to changes in both GH and thyroid hormone exposure. However, there is a paucity of research, in human subjects, about the selective effects of these hormones on clinically-important biological functions in hypopituitary adults.

In previous chapters, I have reported the results of prospective observational studies conducted in a cohort of hypopituitary adult men with severe GHD. All subjects received recombinant GH replacement as part of routine clinical care. Research study measurements and procedures were conducted immediately prior to commencing GH replacement. The dose of GH was titrated to achieve a serum IGF-1 in the upper half of the age-related reference range. All study measurements were then repeated after three to six months on a stable dose of GH.

7.1.1 Changes in the HPT axis following GH replacement

In Chapter 3, I described the changes in the HPT axis following recombinant GH replacement in a cohort of adult hypopituitary men. In summary, mean freeT4 levels declined (-1.09±1.99 pmol/L, p=0.02). Reverse T3 levels also fell (-3.44±1.42 ng/dL, p=0.03) and freeT3 levels increased significantly (+0.34±0.15 pmol/l, p=0.03). The concentration of TSH in the serum remained unchanged. All changes in thyroid hormone levels occurred within the normal reference range while the subjects received routine replacement dose of GH, maintaining serum IGF-1 in the upper half of the age-related reference range. The rise in serum IGF-1 correlated with the fall in freeT4 (r -0.46; 95% CI -0.755 to -0.006, p=0.048) while there was a trend towards a significant correlation with the rise in freeT3 and IGF-1 (r 0.442; 95% CI -0.0291 to 0.753, p=0.058). Subgroup analysis of those subjects with TSH deficiency (n=13, all taking thyroxine replacement) revealed a similar pattern of fluctuations in circulating thyroid hormone levels.

7.1.2 Mechanistic studies

The mechanism (or mechanisms) responsible for the observed changes in the thyroid axis is a matter of debate among endocrinologists. In Chapter 4, I report

the findings of studies aimed at shedding new light on the mechanisms for GH-induced changes in the HPT axis. Alteration of the serum binding proteins for thyroid hormone has been suggested by some investigators (71). However, in our study we observed a parallel decline in both total and free T4 following exposure to physiological doses of GH – this argues against an alteration in binding proteins. In addition, the serum concentration of thyroid binding globulin (TBG), to which the majority of T4 in the serum is bound, did not change during the course of the study.

Suppression of TSH or alteration of TSH dynamics has also been proposed as potential mechanism for changes in thyroid hormone seen following replacement of GH (67). We did not directly evaluate TSH dynamics; however, the mean serum concentration of early morning, fasting TSH did not change during the study. Many patients with central hypothyroidism have a suppressed TSH level (127). In our study, the observed changes in thyroid hormone levels were less marked in the subgroup with a detectable TSH level arguing against a crucial role for TSH in manipulating thyroid hormone levels following GH replacement. Similarly, a direct effect of GH on the thyroid gland itself, suppressing T4 production or enhancing the synthesis/secretion of T3, is unlikely. The serum concentration of thyroglobulin, the substrate for thyroid hormone, did not change during the course of the study.

T4, the main hormonal product of the thyroid gland, is a prohormone. It is activated, in peripheral tissue, to T3 by removal of an iodine moiety from the molecule – a process known as deiodination (see Figure 1.1). This reaction is catalysed by the type 2 isoenzyme of deiodinase (DIO 2). T4 can also be deactivated to reverse T3 (an inactive hormone by product) by removal of a different iodine atom in peripheral tissue – this reaction is catalysed by the type 3 isoenzyme of deiodinase (DIO 3). The peripheral metabolism of thyroid hormones, by a process of deiodination, controls the local tissue exposure to T3 and, in turn, alters the circulating concentration of thyroid hormones in the serum (73). Therefore, the rise in the ratio of free T3/free T4 and free T3/reverse T3 in the serum, following GH

replacement is suggestive of an effect of GH on deiodinase enzyme activity in peripheral tissue.

Deiodinase enzymes are widely expressed in tissues throughout the body (72). We chose to examine the enzyme activity in subcutaneous adipose tissue, before and after GH exposure, as this tissue is easily accessible with minimal risk and discomfort to the patient. Also, fat tissue has been previously shown to express all three isoenzymes of the deiodinase system – DIO 1, DIO 2 and DIO 3 (See figure 1.1). We speculated that a fall in T4, accompanied by a rise in T3, following GH supplementation was due to increased DIO2 activity. Unexpectedly, the opposite effect on DIO2 was observed. In subcutaneous fat, DIO2 isoenzyme activity declined (-11.08 fmol/mg per min; 95% CI -28.95 to 0.19, p=0.036). The decline in DIO2 activity correlated with the fall in serum freeT4 and totalT4 concentration but not the changes in T3 or reverse T3. There was no significant correlation between the fall in DIO 2 activity and the rise in serum IGF-1 level. DIO1 and DIO3 isoenzyme activity remained unchanged following GH substitution

The explanation for this unpredicted observation not clear. However, there are several potential explanations. GH is a potent stimulus of lipolysis and a reduction in DIO2 activity could be an attempt to protect the fat tissue from the additional lipolytic action of T3 (336). Alternatively, the increased circulating and/or locally generated T3 may have caused the decline in DIO2 activity in an attempt to maintain tissue T3 homeostasis. Previous research from animal studies has confirmed that T3 itself can suppress tissue DIO2 mRNA expression and activity in various tissues (334).

Despite the discordance between the serum and tissue findings in our study, modulation of deiodination of thyroid hormones in peripheral tissue remains a likely explanation for the changes in circulating thyroid hormone levels following GH supplementation. Thyroid hormone influences gene expression in virtually every organ in humans and deiodinase enzymes are differentially expressed in a wide

variety of tissues (78). Deiodinase isoenzyme activity in other organs, such as the liver, skeletal muscles or kidney, may make a greater contribution to the circulating pool of T3 in patients with hypopituitarism. Isoenzyme activity in other organs may be more sensitive to inhibition by GH.

7.1.3 Clinical implications

Notwithstanding the mechanism responsible for changes in the serum concentration of thyroid hormone following GH replacement, the clinical importance of a fall in serum free T4, particularly in the presence of a rising serum T3, is uncertain. Furthermore, when changes in thyroid hormone occur within the normal reference range, the clinical impact may be even more challenging to define. In patients with hypopituitarism, one cannot rely on serum TSH to reflect tissue exposure to thyroid hormone. In Chapter 5, I report the results of our studies aimed at defining the clinical importance of GH-induced changes in the thyroid axis. We measured a wide array of tissue biomarkers of thyroid hormone action before and after exposure to GH in adults.

Liver biomarkers, sensitive to thyroid hormone action, including serum ferritin and caeruloplasmin declined during the study. Fluctuations in these biomarkers showed strong associations with serum free T4 suggesting that GH-induced changes in the thyroid axis lead to a more hypothyroid hepatic phenotype. However, other biomarkers of thyroid hormone action in the liver, including SHBG, were unchanged during the study. Similarly, minor changes in lipoprotein particles did not appear to be directly influenced by changes in serum T4 or T3 concentration. Alterations in serum SHBG and lipoproteins can be provoked by both thyroid and growth hormone. In contrast, ferritin and caeruloplasmin are less likely to be affected by routine replacement doses of GH. Therefore, serum markers of copper and iron metabolism may be more useful markers of the hepatic action of thyroid hormone in patients with hypopituitarism.

We also evaluated the peripheral action of thyroid hormone on bone. Serum bone turnover markers (BTMs), which are sensitive to the circulating concentration of thyroid hormone, increased during the study. There was a parallel increase in markers of bone formation and resorption indicating an increase in bone turnover with preservation of the bone remodelling cycle. While GH can also increase bone turnover, the rise in BTMs was more strongly correlated with the increase in free T3/free T4 ratio than the rise in serum IGF-1. This suggests that BTMs may also serve as useful biomarkers of peripheral thyroid hormone action in patients with central hypothyroidism.

Resting energy expenditure (REE), which is sensitive to the action of both thyroid and growth hormone action did not change during the study. GH and thyroid hormone have opposing effects on REE with the former promoting an increase in the metabolic rate (266). We have speculated that the lack of an expected rise in REE may have been due to the decline in circulating free T4 and a tendency toward a hypothyroid metabolic phenotype. Cardiac time intervals have been shown in many previous studies to be highly sensitive to circulating concentration of thyroid hormone (290). Indeed, studies in both primary and central hypothyroidism have shown a good correlation between thyroid status and duration of systolic time intervals, particularly the isovolumetric contraction time (ICT) (289, 293). ICT is the time when the ventricles have begun to contract, but the volume of the chambers has not yet changed. It occurs immediately after the period of electromechanical delay, following electrical stimulation of the ventricles (see Figure 1.2). In our study, median ICT values did not change following GH substitution. However, there was a trend towards shortening of ICT in those with more severe hypopituitarism. Fluctuations in thyroid hormone levels may not have been large enough to provoke significant changes in cardiac time intervals. Also, the small study sample and lack of a matched control group may have impacted on our results.

The results of studies reported in Chapter 5 suggest that the fluctuations in serum thyroid hormone concentration, following GH replacement, may have

complex, tissue-specific, clinical effects. The decline in liver-derived markers of thyroid hormone action suggests that these alterations in the thyroid axis may induce a hypothyroid hepatic phenotype. In contrast, the concentration of BMTs increased during the study, in parallel with the rise in the free T3/fee T4 serum ratio. This implies that GH replacement may improve the biological action of thyroid hormone on bone tissue.

Patient reported outcomes are an increasingly important measure of quality in health care outcomes. GHD, in particular, has been associated with an impaired health-related quality of life (QOL) when measured with both generic and disease-specific questionnaires (304). Indeed, poor QOL is one of the primary indications for the replacement of GH in adulthood (303). However, thyroid hormone can also affect QOL. Not surprisingly, hypothyroidism has been shown to negatively impact on many domains of well-being (309). In chapter 6, I described the effect of GH replacement on health-related QOL in adult hypopituitary men. As expected, QOL improved following GH replacement; this was demonstrated using both generic (SF 36) and disease-specific (AGHDA) questionnaires. Interestingly, self-reported improvements in QOL were less apparent when the subgroup of patients, who experienced a fall in serum T4 after GH replacement, were analysed separately. This was seen using generic and disease-specific questionnaires. Overall, this implies that the decline in serum T4, seen after GH replacement, may reflect clinically significant hypothyroidism and attenuate the expected rise in health-related QOL.

In conclusion, fluctuations in the thyroid axis are common following GH replacement in adult hypopituitary men. DIO2 activity in subcutaneous adipose tissue is suppressed, while DIO1 and DIO3 activity are unchanged, following growth hormone replacement. This does not explain either the decrease in circulating concentration of freeT4 and reverseT3 or the rise in freeT3 induced by GH replacement. Alterations in the serum thyroid hormone levels following GH supplementation may have important clinical implications. Complex, often

contrasting, tissue-specific effects were observed in thyroid-sensitive clinical parameters. In particular, the decline in serum free T4 was associated with an attenuation of the expected improvement in QOL following GH replacement.

7.2 Recommendations for clinical practice

1. Clinicians caring for adults with GHD should anticipate the likely impact of GH replacement on the thyroid axis. Patients with pre-existing central hypothyroidism should have the dose of thyroxine optimized prior to commencing GH supplementation. Targeting the upper half of the serum free T4 reference range may prevent T4 levels declining outside the normal range upon commencing GH.

2. A decline of serum free T4 within the normal reference range, following GH replacement, should prompt careful evaluation for signs and symptoms of hypothyroidism particularly in patients with multiple pituitary hormone deficiencies.

3. Longitudinal analysis of biomarkers of thyroid hormone action, before and after GH replacement, may provide useful clinical information about the impact of fluctuations induced in the thyroid axis. In particular, the temporal trend in multiple thyroid biomarkers may help define the clinical importance of equivocal or borderline changes in thyroid hormone levels in selected cases.

4. Suboptimal improvement in health-related QOL following GH replacement in adulthood may reflect the unmasking of central hypothyroidism. A poor response in QOL should prompt assessment of the effect of GH replacement on circulating thyroid hormone levels and consideration of adjustment or commencement of thyroxine to optimise serum free T4 levels.

These recommendations are based on my research findings in a group of adult men with severe GHD and, therefore, may only be applicable for this group of patients.

7.3 Future areas of research

Further research in this field should focus on defining the mechanisms and the clinical importance of fluctuations in the thyroid axis induced by GH replacement. There is compelling indirect evidence to suggest that GH affects peripheral deiodination of thyroid hormone. However, our findings suggest that GH may have differential, tissue-specific effects on deiodinase isoenzymes.

We analysed the effect of GH on deiodinase enzymes in subcutaneous fat as this tissue is easily accessible. Future research should analyse the effect of GH on deiodinase isoenzyme activity in other tissues including muscle and liver. Muscle tissue can be attained from human subjects using a technique similar to the one I used to extract subcutaneous fat. Human liver tissue samples cannot realistically be obtained before and after GH therapy in subjects with severe GHD. However, a hypophysectomised animal model could be investigated in this respect. Indeed, the analysis of multiple tissue samples from the same animal, before and after GH replacement may yield interesting insights into the differential effect of GH on specific tissues. The liver is a major site of peripheral deiodination of T4. A cell model examining deiodinase activity in cultured human hepatocytes, incubated with GH, may advance our understanding of hepatic deiodinase activity in hypopituitary patients. Also, measurement of deiodinase mRNA expression in peripheral tissue may give further insights into the peripheral modulation of thyroid hormone metabolism by GH.

Investigation of other possible mechanism responsible for GH-induced changes in the thyroid axis should also continue. Limited evidence suggests that GH replacement alters TSH dynamics (67). Jørgensen et al showed that the nocturnal surge in TSH is suppressed by a high dose of GH substitution in a study of eight hypopituitary adults. Further studies of TSH secretion profiles in a larger cohort of hypopituitary adult patients receiving a routine, replacement dose of GH may yield further insights into the potential role of TSH dynamics in the altered

concentration of thyroid hormone. The concentration of TSH in the serum is often suppressed to an undetectable level in patients taking thyroxine for central hypothyroidism (127). Therefore, an alteration in TSH dynamics is likely to be of greater importance in patients not already taking thyroxine.

Further investigation of peripheral biomarkers will help to inform the clinical importance of alterations in the thyroid axis following GH replacement. Preliminary evidence, from the studies described in Chapters 5 & 6, suggests that alterations in the circulating concentration of thyroid hormones are reflected in biomarkers derived from a variety of target peripheral tissues. Future studies should seek to evaluate biomarkers which could be utilised in routine clinical practice. Many serum biomarkers of thyroid hormone action including ferritin, SHBG, caeruloplasmin, lipoproteins and BTMs, can also be influenced by GH or other factors such as inflammation or medication. Therefore, their utility in routine clinical practice may be limited. The measurement of REE can be cumbersome; it requires specialist equipment and expertise. In addition, both GH and thyroid hormone can influence metabolic rate which may confound the interpretation of REE measurements in hypopituitary patients. However, cardiac time intervals in echocardiography, including ICT, can easily be measured in the course of routine clinical practice.

Preliminary evidence suggest that cardiac systolic time intervals can be used in patients with central hypothyroidism to separate those on adequate thyroxine replacement from those on insufficient supplementation (289). In the study described in Chapter 5, there was a trend towards a shortening of ICT while the serum ratio freeT3:free T4 rose in patients with more severe hypopituitarism. This suggests that the fluctuations in thyroid hormone, induced by GH, may have clinically important and detectable effects on the heart. However, our results did not reach statistical significance. A larger cohort of patients, examined prospectively before and after GH replacement, and compared with a matched control group with no pituitary or thyroid disease, may better define the utility of cardiac time intervals

in this clinical setting. This easily accessible clinical measurement holds the greatest promise as a biomarker of thyroid hormone action during GH therapy.

Finally, the clinical relevance of a decline in serum free T4 within the normal reference range could be further investigated by a clinical trial of thyroxine supplementation. In patients with hypopituitarism, the diagnosis of central hypothyroidism is complicated by the fact that a serum free T4 concentration within the population reference range, similar to the situation of primary hypothyroidism, does not exclude central hypothyroidism. Longitudinal observation of a fall in serum free T4, within the normal reference range, following GH replacement, may warrant thyroxine replacement in selected patients. A placebo-controlled or cross-over trial design should be used to evaluate the response of a variety of tissue biomarkers of thyroid hormone action and health-related QOL to thyroxine replacement.

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Appendices

Appendix 1 – Nottingham Health Profile (NHP)

Nottingham Health Profile

Overview:

The Nottingham Health Profile is intended for primary health care, to provide a brief indication of a patient's perceived emotional, social and physical health problems.

Breakdown of questionaire

(1) Part I: 38 questions in 6 subareas, with each question assigned a weighted value; the sum of all weighted values in a given subarea adds up to 100

- energy level (EL): 3
- pain (P): 8
- emotional reaction (ER): 9
- sleep (S): 5
- social isolation (SI): 5
- physical abilities (PA): 8

(2) Part II: 7 life areas affected

Completing questionaire

- each question answered "Yes" or "No"
- important that all questions are answered
- if the patient is not sure whether to say "yes" or "no" to a problem, s/he are instructed to answer the one more true at that time.

Part I

Question	Yes	No	Section	Weight
I'm tired all the time.			EL	39.20
I have pain at night.			P	12.91
Things are getting me down.			ER	10.47
I have unbearable pain.			P	19.74
I take pills to help me sleep.			S	22.37
I've forgotten what it's like to enjoy myself.			ER	9.31
I'm feeling on edge.			ER	7.22
I find it painful to change position.			P	9.99
I feel lonely.			SI	22.01

I can walk about only indoors.			PA	11.54
I find it hard to bend.			PA	10.57
Everything is an effort.			EL	36.80
I'm waking up in the early hours of the morning.			S	12.57
I'm unable to walk at all.			PA	21.30
I'm finding it hard to make contact with people.			SI	19.36

Question	**Yes**	**No**	**Section**	**Weight**
The days seem to drag.			ER	7.08
I have trouble getting up and down stairs and steps.			PA	10.79
I find it hard to reach for things.			PA	9.30
I'm in pain when I walk.			P	11.22
I lose my temper easily these days.			ER	9.76
I feel there is nobody that I am close to.			SI	20.13
I lie awake for most of the night.			S	27.26
I feel as if I'm losing control.			ER	13.99
I'm in pain when I'm standing.			P	8.96
I find it hard to get dressed by myself.			PA	12.61
I soon run out of energy.			EL	24.00
I find it hard to stand for long (e.g., at the kitchen sink, waiting in a line).			PA	11.20
I'm in constant pain			P	20.86
It takes me a long time to get to sleep.			S	16.10
I feel I am a burden to people.			SI	22.53
Worry is keeping me awake at night.			ER	13.95
I feel that life is not worth living.			ER	16.21

I sleep badly at night.			S	21.70
I'm finding it hard to get along with people.			SI	15.97
I need help to walk about outside (e.g., a walking aid or someone to support me).			PA	12.69
I'm in pain when going up or down stairs.			P	5.83
I wake up feeling depressed.			ER	12.01
I'm in pain when I'm sitting.			P	10.49

Part II

Is your persent state of health causing problems with your:	**Yes**	**No**
Work? (that is, paid employment)		
Looking after the home? (cleaning & cooking, repairs, odd jobs around the home, etc.)		
Social life? (going out, seeing friends, going to the movies, etc.)		
Home life? (that is, relationships with other people in your home)		
Sex life?		
Interests and hobbies? (sports, arts and crafts, do-it-yourself, etc.)		
Vacations? (summer or winter vacations, weekends away, etc.)		

Interpretation

• number of questions in each section affected

• relative level affected, in which the sum of the relative weights are subtracted from 100%, giving values between 0 and 1, with 0 indicating poor and 1 good health

Appendix 2 – Short Form 36 (SF 36)

Subject Initials:…… Subject No:…… Visit No:……

The SF-36 questionnaire

This survey asks for your views about your health. This information will help you keep track of how you feel and how well you are able to do your usual activities.

Answer every question by selecting the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

1 In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
○	○	○	○	○

2 Compared to one year ago, how would you rate your health in general now?

Much better than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now that one year ago
○	○	○	○	○

3 The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

		Yes, limited a lot	Yes, limited a little	No, not limited at all
A	Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	○	○	○
B	Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	○	○	○
C	Lifting or carrying groceries	○	○	○
D	Climbing several flights of stairs	○	○	○
E	Climbing one flight of stairs	○	○	○
F	Bending, kneeling, or stooping	○	○	○
G	Walking more than a mile	○	○	○
H	Walking several blocks	○	○	○
I	Walking one block	○	○	○
J	Bathing or dressing yourself	○	○	○

4 During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

		Yes	No
A	Cut down on the amount of time you spent on work or other activities	○	○
B	Accomplished less that you would like	○	○
C	Were limited in the kind of work or other activities	○	○
D	Had difficulty performing the work or other activities (for example, it took extra effort)	○	○

5 During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

		Yes	No
A	Cut down on the amount of time you spent on work or other activities	○	○
B	Accomplished less than you would like	○	○
C	Did work or other activities less carefully than usual	○	○

Subject Initials:…… Subject No:…… Visit No:……

6 **During the past 4 weeks, to what extent have your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?**

Not at all	Slightly	Moderately	Quite a bit	Extremely
○	○	○	○	○

7 **How much bodily pain have you had during the past 4 weeks?**

None	Very mild	Mild	Moderate	Severe	Very severe
○	○	○	○	○	○

8 **During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?**

Not at all	Slightly	Moderately	Quite a bit	Extremely
○	○	○	○	○

9 **These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.**

How much of the time during the past 4 weeks…

		All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
A	Did you feel full of pep?	○	○	○	○	○	○
B	Have you been a very nervous person?	○	○	○	○	○	○
C	Have you felt so down in the dumps that nothing could cheer you up?	○	○	○	○	○	○
D	Have you felt calm and peaceful?	○	○	○	○	○	○
E	Did you have a lot of energy?	○	○	○	○	○	○
F	Have you felt downhearted and blue?	○	○	○	○	○	○
G	Did you feel worn out?	○	○	○	○	○	○
H	Have you been a happy person?	○	○	○	○	○	○
I	Did you feel tired?	○	○	○	○	○	○

10 **During the past 4 weeks, how much of the time have your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc)?**

All of the time	Most of the time	Some of the time	A little of the time	None of the time
○	○	○	○	○

11 **How TRUE or FALSE is each of the following statements for you?**

		Definitely true	Mostly true	Don't know	Mostly false	Definitely false
A	I seem to get sick a little easier than other people	○	○	○	○	○
B	I am as healthy as anybody I know	○	○	○	○	○
C	I expect my health to get worse	○	○	○	○	○
D	My health is excellent	○	○	○	○	○

Thank you for completing these questions!

Subject Name (Please Print) :……**Subject Signature**:……**Date**:……

Subject to complete details above

Appendix 3 – Assessment of Growth Hormone Deficiency in Adulthood (AGHDA)

LISTED BELOW ARE SOME STATEMENTS that people may make about themselves.

Read the list carefully and put a tick in the box marked YES if the statement applies to you.

Tick the box marked NO if it does not apply to you.

Please answer every item. If you are not sure whether to answer YES or NO, tick whichever answer you think is most true in general.

	YES	NO
I have to struggle to finish jobs	☐	☐
I feel a strong need to sleep during the day	☐	☐
I often feel lonely even when I am with other people	☐	☐
I have to read things several times before they sink in	☐	☐

	YES	NO
It is difficult for me to make friends	☐	☐
It takes a lot of effort for me to do simple tasks	☐	☐
I have difficulty controlling my emotions	☐	☐
I often lose track of what I want to say	☐	☐

	YES	NO
I lack confidence	☐	☐
I have to push myself to do things	☐	☐
I often feel very tense	☐	☐

	YES	NO
I feel as if I let people down	☐	☐
I find it hard to mix with people	☐	☐
I feel worn out even when I've not done anything	☐	☐

	YES	NO
There are times when I feel very low	☐	☐
I avoid responsibilities if possible	☐	☐
I avoid mixing with people I don´t know well	☐	☐

	YES	NO
I feel as if I m a burden to people	☐	☐
I often forget what people have said to me	☐	☐
I find it difficult to plan ahead	☐	☐
I am easily irritated by other people	☐	☐

	YES	NO
I often feel too tired to do the things I ought to do	☐	☐
I have to force myself to do all the things that need doing	☐	☐
I often have to force myself to stay awake	☐	☐
My memory lets me down	☐	☐

Now please go back to the first question and make sure that you have answered "YES" or "NO" to every question, on all two pages of the questionnaire. Thank you for your help.