Mechanisms and effects of growth hormone deficiency (due to Subarachnoid Haemorrhage and Traumatic Brain Injury) on Quality of Life and regional body composition, and the influence of hormone replacement.

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Medicine by

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Declaration

This thesis is the result of work performed whilst registered as a candidate for the degree of Doctor of Medicine at the University of Liverpool. I declare that no portion of the work in this thesis has been submitted elsewhere for another degree or qualification in any other university or higher institute of learning

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Abbreviations

GH – Growth Hormone

GHD – Growth Hormone Deficiency

GHR – Growth Hormone replacement

BMI – Body Mass Index

MRI – Magnetic resonance imaging

NFPA – Non-functioning pituitary adenoma

RCT – randomised controlled trial

QoL – Quality of Life

QoL-AGHDA – Quality of life assessment in growth hormone deficient adults

IGF-1 – Insulin like growth factor 1

hs-CRP – high sensitivity C-reactive protein

GST – glucagon stimulation test

SST – short synacthen test

GAT – Combined Growth Hormone Releasing Hormone and Arginine Test

ITT – Insulin Tolerance Test

IHCL – Intrahepatocellular lipid

IMCL – Intramyocellular lipid

SAT – subcutaneous adipose tissue

VAT – visceral adipose tissue

PTHP – post-traumatic hypopituitarism

Aims of Thesis

- Section 1 – To ascertain the incidence of non alcoholic fatty liver disease in patients with growth hormone deficiency and the impact of hormone replacement.
- Section 2 – Validation of the glucagon stimulation test in healthy subjects and hypopituitary patients.
- Section 3 – A prospective longitudinal study of pituitary function in survivors of Subarachnoid Haemorrhage and evaluation of changes in Quality of life following growth hormone replacement.
- Section 4 – An analysis of the Pfizer KIMS database to evaluate changes in Quality of Life and Body Composition following Growth Hormone Replacement in patients with Traumatic Brain Injury.

Chapter 1

Introduction and Review of Literature

Section I: Hypothalamo-Pituitary Axis

The pituitary gland is embryologically derived from 2 sources, giving 2 distinct lobes. The anterior lobe is derived from Rathkes pouch and is functionally and endocrinologically distinct from the posterior lobe, which is embryologically continuous with the hypothalamus.

The posterior lobe of the pituitary contains nerve fibres from the hypothalamus and secretes hormones in response to neuronal excitation, whereas the anterior lobe contains a number of different cell types which each secrete the various anterior pituitary hormones. These cells are under neuro-hormonal control from the hypothalamus via the long portal veins. Main cell types, hormones produced and control mechanisms for the anterior pituitary are shown in table 1.

Cell Type	*Hormone*	*%Pituitary population*	*Hypothalamic hormone*
Thyrotroph	TSH	3-5%	TRH (+) Somatostatin(-)
Corticotroph	ACTH	15 – 20%	CRH(+) Vasopressin augments
Gonadotroph	LH,FSH	10-15%	GnRH (+) Prolactin (-)
Somatotrophs	GH	40-50%	GHRH (+) Somatostatin (-)
Lactotrophs	Prolactin	10 – 25%	PRF (+) TRH (+) Dopamine (-)

Table 1.1 – *Cell types and functions within the anterior pituitary together with neuro-hormonal regulation. Adapted from Endocrinology - An Integrated approach(1)*

Blood Supply to the pituitary gland and hypothalamus

The pituitary gland is one of the most vascularised tissues in the in the body, making it vulnerable to ischaemia. Its blood supply is complex, with the hypothalamus being supplied from the circle of Willis, and the two lobes of the pituitary gland being supplied from the superior and inferior hypopyseal arteries. The superior hypophyseal artery forms a capillary bed around the nuclei within the median eminence of the hypothalamus. These capillaries then drain to the long portal veins which then form a second capillary bed around the cells of the anterior pituitary, allowing the hypothalamic hormones to exert their paracrine actions on the anterior pituitary. Ischaemia can therefore affect the pituitary directly in addition to the hypothalamus resulting in disturbances to the endocrine functions of the pituitary gland. Ischaemic damage is similar in its effects to other causes of pituitary damage, such as pituitary tumours or radiotherapy, in that somatotrophs are normally most commonly affected, followed by gonadotrophs, with the relatively smaller numbers of thyrotrophs being the most resilient. In the absence of an anatomical lesion, posterior pituitary function is normally well preserved(1).

Section II: Growth Hormone

a) Structure and Synthesis

The human GH (hGH-N) gene is located on chromosome 17q, and is one of 5 similar genes. Only genes GH1 and GH2 (producing GH V) produce growth hormone (GH) with any systemic relevance.(2)

Human growth hormone (GH) consists of a 191 amino acid, 22,129 kDa single chain protein with 2 di-sulfide bridges (figure 1.1). Whilst this is the prototype pituitary hormone another isoform does exist in the healthy state, constituting around 5% of pituitary GH, with a third isoform known to be produced under disease conditions (Genetic GHD T2) but not thought to be important in the healthy state (3) . Placental growth hormone (GH V), is similar in structure and is produced by the placenta in increasing amounts during pregnancy, being the principal type of GH in the maternal circulation by term. It has similar biological effects to pituitary GH, although has less lactogenic activity(4). Further isoforms of GH are also produced by the placenta from the remaining 3 genes (called Chorionic Somatomammotrophins), however these have little or no biological activity at the GH receptor and are readily distinguished by most assays.

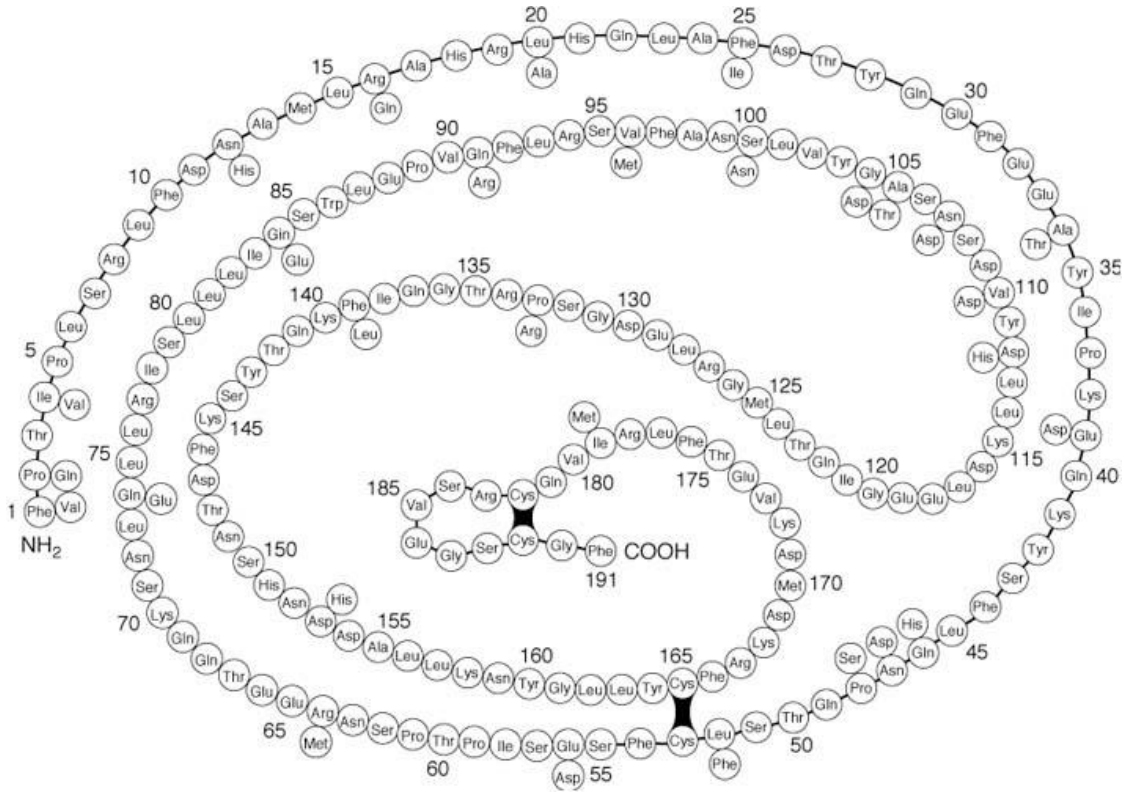


Figure 1.1- *Structure of human growth hormone*

(taken from Baumann et al(3))

b) Physiological control of GH secretion

i) Neuropeptide Hormones Growth Hormone is regulated by a number of endogenous and exogenous factors which may act directly or indirectly on the pituitary and hypothalamus.

Growth Hormone Releasing Hormone – This is the primary neuropeptide responsible for regulating the pulsatility of growth hormone release(5). It is a 44 Amino acid protein which is produced predominantly from the arcuate nucleus of the hypothalamus(6) It is derived from a 108 amino acid pre pro hormone, and has been found in the hypothalamus in 2 forms GHRH (1-40) and GHRH (1-44)(7, 8). Both forms are believed to have equal biological activity. GHRH acts by stimulating both gene transcription in pituitary somatotrophs, and release of stored GHRH from intracellular stores(9, 10). An intact GHRH system is required for the action of all other GH releasing stimuli.(5)

Somatostatin (also known as somatotrophin release inhibitory factor) - This neuropeptide hormone is produced in the paraventricular nuclei of the hypothalamus and is derived from a 116 amino acid pro hormone. It has 2 principal forms SS28 and SS14(11) and is responsible for inhibition of GH release(12, 13). It antagonises the actions of GHRH and ghrelin(14-16), and also acts directly on the pituitary to inhibit GH release, but not its production(17) which leads to a moderate rebound GH release on termination of SS infusion(18), particularly given its short half life of 3 minutes. It is the main negative regulator of GH secretion, however its main action seems to be to reduce the magnitude of GH pulses and interpulse basal GH levels rather than the frequency of GH pulses which are determined by GHRH and are unaffected by

SS infusion(19) In addition to GH inhibitory effects it also inhibits release of TRH stimulated TSH, glucagon, gastrin and insulin(20), as well as other gut hormones. It has a lesser inhibitory effect on ACTH and Prolactin(5).

Ghrelin. The most recently discovered neuroendocrine hormone, ghrelin is a 28 amino acid peptide which is formed from the 117 amino acid preproghrelin. It is produced in the endocrine cells of the stomach(21) however has also been isolated from the arcuate nucleus of the hypothalamus and the cells of the anterior pituitary(22), specifically somatotrophs, thyrotrophs and lactotrophs(23) where it is thought to exert a paracrine action. The actions of ghrelin are complex; it is known to stimulate GH release from somatotrophs in vitro(24) and in vivo however in this situation it requires an intact GHRH system(14) – it does not however act through this system as the amount of GH released by ghrelin is greater than that by GHRH alone(25). Ghrelin acts via the GH secretagogue receptor (GHS-R) which is strongly expressed in the hypothalamus and pituitary(22), as well as other peripheral tissues. In addition to its GH releasing actions, ghrelin is involved in appetite, gastric motility(26), gastric acid secretion, energy balance(27), insulin secretion, ACTH and prolactin release(25). It also has cardiovascular(28) and anti-proliferative effects(2).

Leptin – Leptin is produced by white adipose tissue, and is primarily involved in regulation of body fat via hypothalamic appetite sensors(29, 30). Whilst leptin and its receptors have been found in normal pituitary tissue and pituitary adenomas(31, 32), *in vivo* studies have failed to demonstrate an up-regulation of GH production in response to leptin(33).

Other neuro-peptides regulating GH release include GABA, Galanin, Neuropeptide Y(34) and endogenous opiates(35). Other physiological states will also affect GH release. GH levels are reduced in obesity and states of hyperglycaemia, Obesity has been clearly associated with impaired GH secretion: for each unit increase in BMI, there is a 6% reduction in the daily GH secretion rate(36). GH levels are however increased following exercise, physiological or psychological stress, hypoglycaemia and starvation(35). Sex steroids (oestrogen and testosterone) both amplify the magnitude of GHRH pulses and reduce the orderliness of pulse frequency leading to increased GH secretion(37-39), particularly during puberty.

Insulin Like Growth Factor 1: IGF-1 is produced in the liver and local tissues in response to GH stimulation; however it is hepatic IGF-1 which exerts the greatest feedback on the pituitary gland(6). IGF-1 levels in the blood declines rapidly if unbound, however because the majority of IGF-1 is bound by the IGF-1 binding proteins, measurable changes in IGF-1 may take 3 – 5 days to become evident after an intervention (e.g. prolonged fasting)(5). Whilst the precise site of IGF-1's feedback actions is unknown, it is suspected that it may work by feed back on both the hypothalamus and pituitary(40), although in humans the finding of a lower level of negative feedback by IGF-1 infusions in patients with hypothalamic hypopituitarism suggests that an intact hypothalamus is required for a full effect(41).

ii) Pharmacological Stimuli. Corticosteroids are known to have a biphasic effect on GH secretion, with an acute increase in GH measured over minutes to hours, followed by long term GH suppression(42, 43). GH secretagogues such as hexeralin and GHRP-6 have a potent effect on GH release by binding to the GHS-R1a receptor

on the somatotrophs of the pituitary(35) in a similar way to ghrelin. This forms the basis of several pharmacological stimulation tests(44-46). Alpha-adrenergic pathways stimulate GH release via increasing GHRH and reducing somatostatin, as do muscarinic pathways. This forms the basis for the use of clonidine (an alpha 2 receptor agonist), L Dopa (metabolised to adrenaline or noradrenaline) and pyridostigmine (an anticholinesterase) to stimulate GH secretion(47). Beta adrenergic pathways however inhibit GH secretion.

iii) Pulsatility of GH release. GHRH acts as the primary pulse generator, with somatostatin as the main inhibitor of GH release. GH release is governed by a complex interplay of these hormones, with SS inhibiting GHRH release, GHRH stimulating SS(48) and SS also inhibiting its own release(49). Whilst SS inhibits basal and peak levels of GH, it does not reduce pulse frequency. In addition to these 'short' feedback loops, GH also acutely inhibits hypothalamic GHRH secretion(50, 51) and stimulates SS(48, 52, 53). More long-term feedback is likely to be provided by IGF-1.

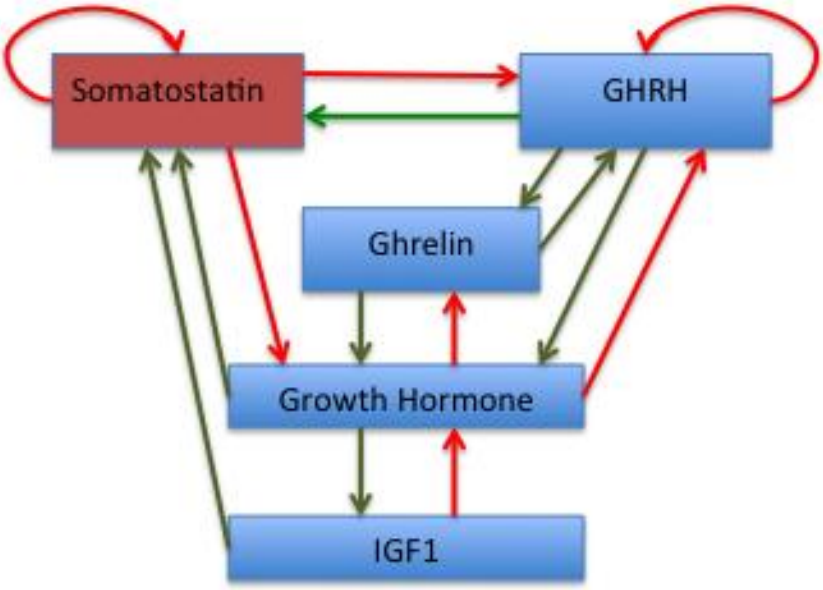


Figure 1.2 - *The physiological regulation of GH release. Adapted from Khoo B, Grossman A Normal and Abnormal Physiology Hypothalamus and Pituitary gland(54)*

iv) Other influences on GH secretion

Age: GH is secreted in reduced quantities in old age (the somatopause). This is a GHRH mediated response, since the pituitary response to GHRH remains intact despite aging(55, 56), and the pool of pituitary GH is relatively preserved into older age(57).

Gender: There is evidence of gender specificity in the pattern of GH release. Men demonstrate a predominantly nocturnal pattern of GH secretion with daytime quiescence, whereas women maintain both higher pulse frequency and basal GH levels during the day.(5)

Sleep: GH release is associated with the onset of slow wave sleep in humans(58), however the mechanism leading to the nocturnal pattern of GH secretion is uncertain. It has been demonstrated that cortisol release is not implicated in the nocturnal pattern of GH pulse generation(59), and neither is SS(60, 61), however GHRH is likely to play a role, given the finding that nocturnal GH release is reduced by 77% following infusion of a GHRH antagonist(62). Small peaks were however noted despite this which suggests that there maybe a further, as yet unknown factor regulating nocturnal GH release in humans(5).

Gonadal Steroids: It is known that the increase in gonadal steroids at the onset of puberty is accompanied by increased GH and IGF-1 levels(63, 64), however whether these events are coincidental or causal is unclear. Oestrogens are seen to increase GH secretion, an effect that is mirrored only by aromatizable androgens such as testosterone. It is however also known that oestrogens reduce hepatic IGF-1

synthesis which may simply remove the negative feedback resulting in increased GH levels.(5)

Nutrition: Total body fat and visceral fat are negatively correlated with growth hormone secretion(65), and hence important factors to consider when assessing patients with possible GHD. Obesity leads to markedly reduced levels of GH. This may be by 3 mechanisms. Firstly free fatty acids are powerful inhibitors of GH secretion, most probably via SS activation, since the GH response to GHRH is attenuated in healthy volunteers maintained on lipid infusions(66) and FFA's are raised in obesity(67) . Secondly, free IGF-1 levels may be elevated in obesity as a result in reduced IGFBP1 – this may lead to increased negative feedback on the pituitary(68). Thirdly decreased frequency of GH pulses is seen in obesity(69).

The role of Ghrelin in modulating the GH suppression in obesity is unclear. Ghrelin levels have been shown to be reduced in obesity(70-72), Reduced nocturnal secretion of ghrelin has also been noted in obese subjects(73). Lindeman *et al* however, despite confirming an exponential negative correlation between ghrelin and BMI, found a positive correlation between ghrelin and visceral fat in obese subjects, with a negative correlation between visceral fat and 24 hour GH secretion, and no change in ghrelin levels in patients who lost weight, despite an increase in GH secretion, suggesting that ghrelin is not involved in GH regulation in obesity(74).

Hyperglycaemia is known to suppress GH secretion acutely(75, 76), and this is associated with attenuated responses to GHRH and GH secretagogues suggesting increased SS tone(77, 78), however this is then followed by a rebound increase in

GH levels as the SS inhibition ceases and stored pituitary GH is released. Hypoglycaemia however causes a rapid increase in GH levels via GHRH activation(79) as part of the physiological counter regulatory response.

In conclusion GH release is predominantly under the control of the hypothalamus primarily influenced by the interplay of GHRH and Somatostatin. GH can be stimulated by a number of pharmacological agents, several of which are used in pharmacological testing. A number of physiological factors including age, gender, sleep status, gonadal steroids, nutrition and glycaemic state also influence GH production and release.

GH release is markedly reduced in states of hyperglycaemia and obesity and a number of possible mechanisms have been postulated. Whilst Ghrelin is a powerful GH secretagogue, it does not appear that ghrelin is responsible for changes in GH secretion in states of obesity.

c)Functions of GH

The primary function of GH is, as its name would suggest, the promotion of longitudinal growth in childhood. It does however have wide ranging action on muscle, bone and adipose tissue as well as a number of other organs of the body.

GH may act both directly on organs and tissues, and via the insulin like growth factors; Whilst GH is required for differentiation of precursor cells, IGF-1 is responsible for clonal expansion of the differentiated cells- the dual effector theory.(80)

GH has a diabetogenic effect by antagonising the effects of insulin. This has been seen in studies in GH replete volunteers given GH where hyperinsulinaemia has been noted during acute and chronic GH administration, leading to hyperglycaemia when given over longer periods(81). A lipolytic effect has also been demonstrated(82). GH increases lean body mass, primarily by an increase in skeletal muscle mass. GH has been shown to stimulate skeletal muscle protein synthesis(83). A summary of the effects of GH in health is shown in fig 1.3 and table 1.2.

The effects of GH are most potently seen by the effects of GHD. This will be discussed more fully in the following section.

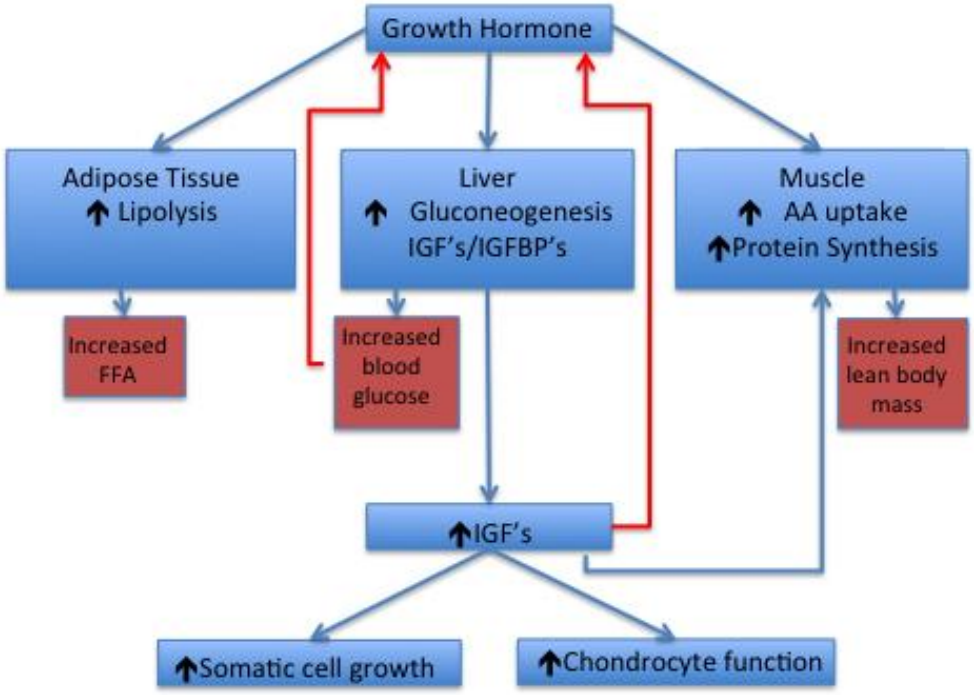


Figure 1.3 *Systemic effects of GH in health. Adapted from endocrinology an integrated approach(1)*

Promotion of somatic growth	Growth plate elongation
IGF-I generation	IGF-BP3 and ALS generation
Nitrogen retention	Amino acid transport into muscle
Lipolysis	Insulin antagonism
Beta cell hyperplasia	Early insulin-like effect
Sodium retention	Phosphorus retention
Lactogenesis	Stimulation of immune function

Table 1.2 - *Principal biological activities of GH. Adapted from Baumann et al(3)*

Section III: Growth Hormone Deficiency (GHD)

a)Causes

i) Childhood onset GHD: GH deficiency may arise in childhood or be adult onset in nature. Childhood causes may be subdivided into those which are congenital or acquired. Congenital causes include genetic mutations causing multiple pituitary hormone deficiencies such as PROP1, HESX1, PIT-1 and LHX3/4, or isolated deficiencies such as PITX2 mutations(84). Structural lesions such as empty sella, or midline defects such as septo-optic dysplasia many also be implicated.

As in the adult population, acquired GHD may be secondary to pituitary tumours, the most common being craniopharyngioma, or the treatment of such, namely pituitary surgery and hypothalmo-pituitary irradiation; post irradiation the chance of developing GHD is over 50% if the biological effective dose is greater than 40Gy(85). Infiltrative diseases such as Langerhans cell histiocytosis, sarcoidosis or tuberculosis are rare causes. Whilst traumatic brain injury has been described as a cause of childhood onset GHD(86) the precise incidence is unclear. By far the most common cause of childhood onset GHD is idiopathic. In this situation the majority of children will have normal GH secretion when retested on completion of growth(87, 88), whereas in those children with a defined genetic or structural cause, GHD is likely to persist into adulthood(89).

ii) Adult Onset GHD: In contrast to childhood, adult onset GHD is normally secondary to a structural defect. Adult onset isolated GHD is very rare, and in this situation consideration must be given to the accuracy of the diagnostic test, particularly where obesity is present, and in the presence of a normal IGF-1(84). The

commonest structural lesion in adulthood is a pituitary adenoma. Whist micro-adenomas are almost never associated with GHD, macro-adenomas are associated with pituitary hormone deficiencies in 30 – 60% of patients(84). This may be due to compression of long portal vessels or due to raised intrasellar pressure(90). Parapituitary space occupying lesions such as craniopharyngioma, arachnoid cysts, Rathkes cleft cysts, meningiomas, gliomas and astrocytomas may also affect pituitary function. As in children, pituitary surgery frequently causes hypopituitarism, although surgery may be followed by recovery of previously deficient pituitary hormones(91-93), however GHD is least likely to recover(91). Hypothalamo-pituitary irradiation causes hypopituitarism in over 50% of patients(94, 95).

Subarachnoid haemorrhage (SAH) and traumatic brain injury (TBI) have previously been thought to be rare causes of GHD, however interest in recent years has led to a number of studies challenging this view. A meta analysis in 2007 demonstrated an incidence in of hypopituitarism of 27% for TBI and 47% for SAH(96). This will be discussed further in later sections. The causes of GHD are summarised in Table 1.3.

Congenital	Acquired
Genetic:	Trauma (perinatal or postnatal)
Transcription factor defects	CNS infection
GHRH receptor gene defects	Tumours of hypothalamus or pituitary
GHS receptor gene defects	
GH receptor gene defects	Infiltrative/granulomatous disease
Post receptor gene defects	Cranial Irradiation
Structural:	Surgery
Agenesis of corpus callosum	SAH
Septo optic dysplasia	TBI
Empty sella syndrome	Idiopathic
Holoprosencephaly	
Encephalocele	
Hydrocephalus	
Arachnoid cyst	
Associated with midline defects	

Table 1.3 – *Causes of GHD (Adapted from Molitch et al(84))*

b)Diagnosis

The accurate diagnosis of GHD can be difficult. Whereas in children GHD is accompanied by growth failure, in adults no such firm auxiological end point exists. Diagnostic testing includes many confounding factors such as age and BMI, both of which may cause a physiological reduction in GH levels which cannot be differentiated biochemically from pathologically low GH levels. In patients with a structural lesion and multiple pituitary hormone deficiencies the diagnosis is somewhat easier, as in patients with greater than 3 pituitary hormone defects, the chance of GHD accompanying these is over 97%(97).

The much more common situation of patients with single or dual deficiencies does however need much more careful consideration. As GH is secreted in a pulsatile nature, basal hormone measurements are of little use and diagnosis is normally made by stimulation testing. Multiple stimulation tests are in use in clinical practice including the insulin tolerance test (ITT), the glucagon stimulation test (GST) and the GHRH/Arginine test (GAT). Other tests using GHRH and one of a number of GH secretagogues are also in use; however their use is less widespread. It is important that each test is used with an appropriate cut off value specific to the assay and the test used.

The ITT, GST, GAT, and GHRH/GHRP tests are all validated for the diagnosis of adult GHD. Only one test is required for a diagnosis of GHD, however in patients with possible hypothalamic disease, the combined tests may be inappropriately normal as these tests stimulate both the hypothalamus and the pituitary. Therefore in

patients with a history of cranial irradiation, an ITT should be performed if a combined test is normal(98).

Insulin Tolerance test: The ITT is considered to be the gold standard diagnostic test for GHD. This tests both GH and cortisol reserve by inducing hypoglycaemia (blood glucose <2.2mmol/l) and evaluating the response of GH and cortisol. This test works by physiological mechanisms and tests the complete GHRH-GH axis. It has however been shown to have poor intra subject reproducibility(99, 100), has a considerable number of unpleasant side effects, and is associated with a high potential for mortality and morbidity. Furthermore it can only be conducted in the inpatient setting under medical supervision, and is contraindicated in seizure disorders and cardiovascular disease

Glucagon Stimulation Test: The GST is widely used due to its ease, low cost and generally well tolerated protocol. It has been shown to be a safer and effective test of GH reserve(101-105). Side effects are few, but include nausea, tiredness, flushing and vomiting in around 20% of patients. It has few contraindications (hypothyroidism and insulinoma). It is however the most time consuming of all tests, and can produce differing results depending on whether given subcutaneously or intramuscularly, The IV route of administration has been demonstrated to have a poor efficacy for GH stimulation. There have also been concerns about the ability of glucagon to stimulate cortisol release which will be discussed further in chapter 4.

Combined Growth Hormone Releasing hormone Arginine Test: The GAT has been widely used in Europe and the United States. This test uses the hypothalamic stimulus of arginine, which is thought to act by inhibition of somatostatin, paired

with the direct effect of GHRH in releasing stored pituitary GH. Both agents are weak stimuli in isolation in adults; however their combination is synergistic and has been shown to have good reproducibility and a higher level of tolerability than both the ITT and GST. Like all combined tests, falsely normal results can be seen in patients with disease suspected to be of hypothalamic origin e.g. post irradiation, because GHRH directly stimulates the pituitary.

Effect of age on GH stimulation tests

Age has been shown to be a significant influence on GH release, and studies of spontaneous GH secretion have shown that this declines with increasing years, by around 14% per decade(106).

Biller *et al* however found no significant effect of age in any of 6 stimulation tests (ITT, arg, arg/ghrh, l dopa, arg/l dopa)(44), although when the GST was conducted in healthy older men age 50-69 years 20% were found to have a peak GH <3micrograms/l, which was not validated by ITT(107). Other factors such as obesity were not however taken into consideration in this study. Toogood *et al* investigated patients over the age of 60 years with and without hypothalamo-pituitary disease by arginine stimulation tests, 24 hour GH profiles and IGF-1 levels and found that there was a significant difference in all 3 indices between healthy older volunteers and patients with hypothalamo-pituitary disease suggesting that these patients may still benefit from GHR(106).

Effect of Gender on peak GH levels

Biller *et al* found a significant effect of gender in control subjects undergoing the GAT (p=0.03), and the arginine test alone (p=0.01) however the groups in this study were not equal (4 women, 20 men). There was no significant difference in the ITT, l dopa or L dopa/arg tests. Likewise there were no significant differences in the patients with MPHD. In females, phase of the menstrual cycle may affect response to GH stimulation testing, with higher levels found in the pre-ovulatory phase in women with normal menses.

Effect of BMI on GH stimulation tests

GH secretion declines in overweight subjects, with 24 hour GH secretion reduced by 75% in obese men compared with age matched controls(69). Abdominal fat mass is a better predictor of 24 hour GH release when compared to total body fat(65). GH secretion is suppressed to a varying degree even in mildly overweight subjects undergoing testing. This is a confounding factor, since most patients with GHD are likely to be obese(108). All tests examining GH secretion are affected by BMI, Colao *et al* also postulated a link between waist circumference and GH secretion(109). This reduction in GH secretion in obesity is mirrored by increasing insulin resistance and other adverse features of the metabolic syndrome as demonstrated by Di Somma *et al*(110). This has led to suggestions from Cordido *et al* who studied healthy volunteers with obesity with and without a suboptimal GH response to GHRH arginine, that reduction in GH secretion is the pituitary manifestation of the metabolic syndrome(111).

The GST has been shown to be unaffected by BMI in hypopituitary patients with GHD following structural lesions(103), however a correlation with BMI in healthy

controls has been demonstrated even though the mean population BMI of the controls in this study was only 25.8 kg/m^2 . A review of the KIMS database by Toogood demonstrated that the GST was affected by BMI, particularly between 20-30kg/m^2.(112). Yuen *et al* compared a weight based versus fixed dose GST protocol and demonstrated a link with age and BMI, finding that the weight based regime led to higher peak GH and cortisol levels. They concluded that because of the link with BMI weight based dosing was likely to be more accurate, however this could not be compared in this retrospective study(113). The link between BMI and peak GH has also been replicated in the ITT(114-116) and GHRH/Arg(117). This reduction in GH levels has also been seen in patients with normal IGF-1 levels(118) which are less affected by obesity(119).

The GAT has subsequently been studied in obesity, and validated cut offs by BMI established(98) although it has been suggested that waist circumference may be a more accurate measure than BMI in obese patients(120).

In conclusion a number of physiological tests are available for the diagnosis of GHD. Whilst the ITT is considered the gold standard, it has potential complications, has poor intra subject reproducibility and, like other tests is affected by BMI, age and gender. The GST is safe and effective however likewise the test can be affected by BMI, age and gender. The GAT has been validated with different BMI cut offs, however as it stimulates the pituitary directly it may fail to correctly diagnose patients with GHD following hypothalamic damage. Although only one test is required for the diagnosis of GHD according to consensus guidelines, it may be that multiple tests taking into account confounding factors in diagnosis may lead to the most accurate classification of GHD.

c) Assays

One obstacle to successful standardisation of GHD diagnosis has been the lack of standardisation within the GH assay. There has never been a standard assay, and cut offs depend upon the assay used. In 1997 polyclonal radio-immunoassays were replaced with 2 site monoclonal assays which normally report values <50% that of the polyclonal assays(119).

Furthermore prior to 2007 there was no universal calibration standard, however from this time the universal calibration standard IS 98/574 in which 1mg corresponds to 3IU somatotropin has been recommended by the Growth Hormone Research Society(121). Until this point GH was also reported in two different units, namely mU/L and μg/L. μg/L are now universally used for reporting of GH concentrations and all values in this thesis will follow this convention.

d) Markers of GH action.

The primary marker of GH action is IGF-1. IGF-1 is a single chain polypeptide hormone which is structurally homologous to pro-insulin(122). IGF-1 is produced both by the liver and in numerous peripheral sites, and circulates bound to the IGF binding proteins, of which IGFBP3 is the most functionally significant. IGF-1 is almost completely bound in the circulation which acts to prevent potential insulin like activity becoming manifest given the concentration in the circulation is around 1000 times that of insulin(123). The binding proteins also dramatically expand the half-life of IGF-1 in the circulation, and regulate the paracrine effects of IGF's. The main site of IGFBP3 is the liver and, like IGF-1 is under control of GH. In childhood, IGF-1 is a reliable marker of GH action, however in adulthood IGF-1

levels are dependant on a milieu of other influences, such as age, gender, oestrogen and prolactin levels, liver disease and fasting state(124).

As such compared to childhood onset GHD, IGF-1 levels have historically been felt to be of much less use in the diagnosis of GHD. Hoffman *et al*(125) found that even after correction for age, 70% of GHD patients had an IGF-1 level in the normal range, compared to Deboer *et al* who demonstrated 93% of young adults with childhood onset GHD had an IGF-1 level below the normal range(126). An analysis of the KIMS database by Lisset *et al* found that whilst 83% of patients with child hood onset GHD had an IGF-1 level less that 2 SD, in adult onset GHD this was only 52%(127). Age of onset of the GHD was the most significant factor predicting accuracy of the IGF-1 level in diagnosis, however gender, BMI and associated other hormonal deficiencies also contributed significantly to the variability. The age related reduction in IGF-1 levels means that IGF-1 is particularly unhelpful in this group, with only 21% of elderly patients with GHD having an IGF-1 level below the normal range(128).

Whilst IGF-1 levels are not found to be sensitive, they are specific. In the absence of poor nutrition or liver disease, Biller *et al* found that whilst 44% of patients with MPHD had normal IGF-1, 100% of these patients with IGF-1<2SD had GHD(44). Given the difficulties in interpretation of GH stimulation testing the role of IGF-1 which is similarly influenced by factors such as age and BMI is currently unclear.

e) effects of GHD

i) Glucose Insulin Metabolism. GHD has been shown to be associated with a state of insulin resistance(129, 130), and hypopituitary adults have an increased prevalence of the metabolic syndrome(131, 132). This is paradoxical as in early physiological studies, infusions of GH were also shown to antagonise insulin(133), and in conditions of GH excess such as acromegaly, the diabetogenic effects of GH are clear to see.

Whist the insulin resistance of GH excess may be due to the inhibitory effect of the GH induced lipolysis on glucose uptake (the Randle hypothesis)(82), the insulin resistance of GHD may be due to inhibition of the glucose storage pathway and reduced activity of glycogen synthase in peripheral tissues(130).

High dose GH treatment is known to reversibly increase insulin resistance in children taking supra-physiological doses of GHR. In adults, short term GHR has been shown to increase insulin resistance (134-136). It has been shown by Segerlantz *et al* that this is due to the lipolytic effect of GH as hypothesised, since inhibition of lipolysis during acute GH administration does not result in insulin resistance (137). Regardless of causation however, it is thought that the long term improvements in body composition and visceral fat may ultimately result in improvement in insulin resistance, particularly when given at low dose with the aim to maintain an IGF-1 level within the normal range(138, 139).

ii) Lipid Profiles. GHD is known to be associated with an adverse lipid profile with worsening of total and LDL cholesterol, and in some cases reduced HDL with

elevated triglycerides(129, 140, 141). Abdu *et al* demonstrated that this adverse lipid profile was directly associated with the increased cardiovascular risk seen in these patients(142). GHR has been shown to reduce LDL, however Mcallum *et al* did not demonstrate any change in triglyceride levels. Deepak *et al* demonstrated a reduction in LDL with an increase in HDL cholesterol after 6 months GHR, which was associated with a reduction in hs-CRP implying a reduction in overall cardiovascular risk(140).

Colao *et al* demonstrated an improvement in lipid profiles and cardiac performance in 15 patients with GHD given GHR, however in 15 untreated patients with GHD a further deterioration in both indices was seen when compared with controls(143). A recent analysis of the German KIMS database did not however show any benefit of GH therapy on long term (4-10 years) lipid profiles(144). This was postulated to be due to the lower GH doses, and the long term effects of aging which lead to increasingly adverse lipid profiles in the normal population. Studies implying benefit as above have tended to be over the shorter term. The reason for concentration on lipid profiles is the implied benefits on cardiovascular mortality and morbidity.

Whilst the effects of GHR would be expected to reduce cardiovascular mortality and morbidity, evidence is currently lacking of long term cardiovascular benefit(145).

iii) Body Composition.. GH is an anabolic hormone, however also has both lipolytic effects and anti-natriuretic effects(146). Patients with GHD therefore suffer with an overall gain in fat mass and reduction in lean body mass. A variety of studies have looked at the effect of GHR on body composition and despite a number of different

methodologies and the possible confounding effect of the GH induced restoration of circulating volume, all have demonstrated the same conclusions. Table 1.4 shows these studies, methodologies and their conclusions.

Author	Year	n (onset)	Duration (months)	LBM	FM	TBW	ECW
Salomon	1989	24(MO)	6	↑	↓		
Jorgensen	1989	21(CO)	4	↑	↓		
Orme	1992	8(AO)	2	↑	↓		
Binnerts	1992	8(AO)	6	↑	↓		
Whitehead	1992	14(MO)	6/6	↑	↓		
Amato	1993	7(CO)	6	↑	↓		
Bengtsson	1993	10(AO)	6/6	↑	↓	↑	
Chong	1994	7(AO)	3	↑	↓		
Herlitz	1994	16(MO)	6			↑	
Jorgensen	1994	10(CO)	36	↑	↓		
Hansen	1995	29(AO)	12	↑	↓		
Beshyah	1995	38(MO)	6	↑	↓	↑	
De Boer	1995	46(CO)	12				↑
Snel	1995	30(MO)	6	↑	↓		
Lonn	1996	9(AO)	6	↑	↓		
			24		↑	↓	
Moller	1996	7(AO)	1				↑
Johannsson	1996	34(AO)	24	↑	↓		↑
Johannsson	1996	68(AO)	6	↑	↓		
			12/18	↑	↓		
Baum	1996	32(AO)	18	↑	↓		
Hoffman	1996	7(AO)	7 days				↑
Christ	1997	13(MO)	3			↑	
Attanasio	1997	74(CO)	6	↑	↓	↑	
		99(AO)	12/18	↑/↑	↓/↓	↑/↑	

MO, mixed onset; AO, adult onset; CO, childhood onset; LBM lean body mass; FM, fat mass; TBW, total body water; ECW, extracellular water.

Table 1.4 *Studies examining the effect of GH replacement on body composition (Carrol et al 1998)(146)*

Lean Body mass: GH has been shown to be involved with regulation of lean body mass. Healthy volunteers given GH demonstrated increased whole body protein synthesis and mitochondrial oxidative capacity(147). Compared with healthy controls patients with GHD have been shown to have reduction in lean body mass of 7-8%. Salomon *et al* demonstrated that in 24 patients given GH for 6 months, lean body mass increased by a mean of 5.5kg whereas in a control group treated with placebo, no significant difference was seen(148). This has been replicated in subsequent studies. This has been shown to be due to increases in skeletal muscle, with increases in thigh muscle cross sectional area having been shown(149, 150).

Fat mass: As previously discussed, GH has been demonstrated to have lipolytic actions. Compared with predicted values of fat mass matched for gender and BMI, Salomon *et al* demonstrated that patients with GHD had a mean of 7% greater fat mass(148). Recently data from the KIMS database has demonstrated that whilst patients with hypopituitarism have an increased fat mass at a younger age, they do not demonstrate the age related increase in fat mass seen in normal controls(108). The distribution of fat mass in untreated GHD is mainly central and in particular in the visceral fat compartment(151). Snel and others have demonstrated that these changes are reversed by GHR with a reduction of 4-6kg in GHD adults treated with GHR(146, 152).

iv) Extracellular fluid. Studies have demonstrated that total body water is reduced in GHD, mainly due to reduction in extracellular fluid(153-155), although reduced plasma volume and total blood volume contribute to this(155, 156). Treatment with

GH results in increases in extracellular fluid after 3-5 days of treatment(157) with increases in blood volume after 3 weeks(156).

v) Bone mineral density. In a study of 36 adults with GHD, 19% were found to have osteopaenia(158). Furthermore in a study of 89 GHD adults and 360 controls, GHD patients had a 2.5 fold higher fracture rate(159). Patients with GHD have been shown in many studies to have reduced bone mineral density(146). GHR does not result in short term improvements in bone mineral density, however longer term studies demonstrate improvements in BMD at 2 years(160) and at 6 years (161). Data on whether GHR reduces fracture risk in the long term are currently not available(162).

vi) Muscle strength and exercise performance: Patients with GHD have reduced strength and exercise capacity with easy fatigability and markedly reduced energy levels(163). These patients have been shown to have reduced muscle mass and isokinetic muscle strength compared to normal controls, although intrinsic muscle strength remains the same. These features have been shown to improve with GHR 5 years after replacement commenced(164) and to normalise after 10 years of GHR(165).

vii) Quality of Life: QoL response to GH therapy has been reported on in detail over a number of years. Psychological well being has been demonstrated to be reduced in hypopituitary adults fully replaced with the exception of GH when compared with controls(166). Stabler found increased prevalence of anxiety and social phobia in adults who suffered with GHD as children who subsequently discontinued GH,

despite achievement of normal adult height(167). In a study of 86 GHD patients and 86 age matched controls, Rosen *et al* reported reduced energy, difficulties in sexual relationships increased emotional labiality and social isolation(168). Wallymahmed *et al* compared patients with diabetes, GHD with controls and demonstrated increased levels of depression, mental fatigue and reduced self esteem and life fulfilment in patients with GHD compared with the other 2 groups, suggesting an effect of GHD rather than simply chronic disease(169). Likewise Mukerjee *et al* demonstrated similar impairments in QoL in patients with GHD secondary to cancer or pituitary adenomas, with a similar response to treatment, implying an independent GH effect on quality of life(170).

Increased levels of atypical depression have been reported in GHD patients, again improving with treatment(171). Deijen found that QoL was worse in patients with multiple pituitary hormone deficiencies compared to isolated GHD(172), however a retrospective review of the Pfizer KIMS database did not corroborate this finding(173).

Double blind placebo controlled trials examining QoL changes after GHR have shown mixed and sometimes confusing results; Macauley *et al* demonstrated increased mood and energy in GHD patients after 6 months of replacement when assessed by the Nottingham health profile, Psychological general well being schedule and the general health questionnaire(174). Likewise Bengtsson *et al* showed improvements in the comprehensive psychological rating scale in 26 patients given GH, although no improvements were seen in a second questionnaire, the symptom checklist 90(157). Whitehead *et al* did not demonstrate any changes in QoL in 14

patients after 6 months of GHR, however IGF-1 failed to rise in the treatment group which may have reflected poor compliance(150). Beshyah *et al* conducted a randomised controlled trial in 40 adults with GHD(175); In the blinded portion of the study (first 6 months) improvements were seen in the general health questionnaire (GHQ) in the placebo group but not in the treated group, however the placebo group had significantly worse GHQ scores prior to treatment which may simply reflect a return to baseline in this group. After 6 months the study continued as an open label study, and improvements in QoL were seen at 12 months but not 18 months following commencement of treatment.

Burman *et al* measured psychological well being and sense of well being in 36 adults and 36 controls(176). They confirmed reduced QoL in GHD patients prior to treatment; Following treatment in this blinded cross over study, improvements were seen on placebo, however further improvements were seen once GHR was given. Arwert *et al* produced a meta-analysis of patient reported outcomes in 2005 and concluded that there was no evidence that GHR improves patient reported outcomes in GHD patients(177). A further meta-analysis however by Deijen *et al* looked at improvements broken down into effects on QoL, well being and health status. Whilst QoL showed only a small effect size (d=0.18) well being demonstrated a medium effect size (d=0.47). Deijen concluded that many studies claiming to demonstrate an improvement in QoL were in fact demonstrating an improvement in well being(178).

One confounding factor in all studies is the range of measures of change seen in different studies. Attempts have since been made to produce a single validated QoL

questionnaire with the publication of the QOL AGHDA score by Mckenna *et al* (179), and the Questions on Life Satisfaction Hypopituitarism Module(QLS-1H) by Blum et al(180). The QoL AGHDA score has been validated in a number of different populations(181-185), and the score has also been divided into dimensions, namely memory, tiredness, tenseness, socialisation and self confidence(186).

Normative data using a number of different populations has also been collated and published(187-189). A recent review of the KIMS data base looking at patients treated for between 4 and 10 years did demonstrate improvements in QOL AGHDA score(144), which were equal in patients with isolated GHD or multiple hormone defects in patients with structural pituitary lesions(190).

In conclusion insulin resistance is seen both in GHD and in GH excess, however the long-term benefits of GHR are thought to outweigh the effects of insulin resistance seen with GHR. Whilst lipid profiles have been shown to improve in short term studies there is not currently long term evidence of benefit or changes in morbidity and mortality. There is however overwhelming evidence of benefit on body composition with increases in lean body mass and reduction in fat mass, which would be expected to have beneficial effects to health. There is however insufficient long term evidence to be able to demonstrate reductions in cardiovascular mortality or morbidity due to GHR.

GHR also results in increases in extracellular fluid. Such body composition abnormalities in patients with GHD mean that any body composition analysis

depending on algorithms from normative data must be validated in this group. QoL does improve in patients with GHD given GHR, however in some patients the effect may be short lived. Improvements depend on the tools used and domains measured. There is not currently evidence of long term changes in QoL in patients with GHD and other long-term comorbidities as is the case in conditions such as TBI and SAH.

Section IV: Other pituitary hormone deficiencies

Cortisol deficiency:

Whilst primary adrenal failure is commonly isolated, secondary corticotrophin insufficiency is usually associated with hypopituitarism, with the main causes being as described in the above discussions. Isolated corticotrophin deficiency is rare, unless associated with suppression of the hypothalamo-pituitary –adrenal axis by exogenous corticosteroids, however may occur in chronic alcoholism and lymphocytic hypophysitis(191). Unlike primary hypoadrenalism corticotrophin deficiency is not associated with deficiencies of mineralocorticoids.

The symptoms of hypocortisolaemia can be vague and difficult to separate from other pituitary defects. Whist acute deficiencies may result in weakness, dizziness, nausea and vomiting, hypotension and hypoglycaemia, chronic cortisol deficiency produces tiredness, pallor, anorexia and weight loss(192). Replacement is easily given with oral hydrocortisone, however the aim of treatment – to mirror the normal diurnal variation in cortisol with highest concentrations on waking can be difficult to achieve, and modified release preparations are not yet available.

Cortisol is normally highly bound to albumin and other plasma proteins. Diagnosis of cortisol insufficiency based on total cortisol measurements should therefore be made with caution in otherwise sick patients in whom hypoalbuminaemia may also be a concern. (193).Measurement of free cortisol is more helpful, and is becoming more widely available by measurement of salivary cortisol.

The gold standard for diagnosis of cortisol insufficiency has always been the insulin tolerance test(194). Originally a cut off value of 580 nmol/L was proposed based on a comparison of the ITT against 20 patients undergoing major surgery(195). Hurel *et al* examined normal responses to ITT and synacthen in 57 volunteers (30 SST, 27 ITT)(196). They demonstrated a mean (SD) response to synacthen of 628(392-864) nmol/L and to ITT of 693(519 – 866) nmol/L with good correlation between cortisol responses to ITT and SST.

They went on to test these values in patients with pituitary disease, however and found that in patients who failed the ITT, 60% would have passed the SST at the lower end of the normal cut off found in healthy volunteers (390nmol/l). They found however that this figure reduced to 10% if a cut off of 600nmol/l were to be used in the SST, and therefore proposed this higher threshold for assessment of patients with hypothalamo-pituitary disease by SST, with a normal cut off for the ITT of 520nmol/l.

A lower cut off of 500nmol/ has since been recommended with the acknowledgement that normal individuals may peak lower than this(193), however the finding of a lower sensitivity for diagnosis of secondary AI by synacthen testing in a meta-analysis by Dorin *et al* led the authors to suggest that either the ITT or the metyrapone test should be used in patients with a high probability of secondary AI(197). Pavord *et al* looked at cortisol responses to ITT and concluded that a basal cortisol response >400nmol/l was predictive of satisfactory cortisol reserve thus making dynamic testing unnecessary(198). Karacca et al(199) demonstrated 100% of 30 volunteers passed SDSST at a cut off of 550nmol/l however they defined an

acceptable response as attaining this level up to 2 hours post stimulus. Had the 30 min level been taken as is the case in other studies, 20% of healthy volunteers would have failed this test. The SST has far less risk than the ITT, however as this tests the adrenal glands directly, this mechanism relies on the presumed atrophy of adrenal glands as a result of lack of stimulation by ACTH. Therefore the SST can not be used in patients less than 1 month after the acute insult(193).

The synacthen test has historically been performed using a dose of 250μg, however this supra-physiological dose led to concerns about the sensitivity of this test in predicting adrenal insufficiency, and so the low dose test was introduced with the aim of improving the sensitivity of this method(200). Since this time however both standard and low dose tests have been demonstrated to have similar operating characteristics(194, 197). Despite these findings the Standard dose test remains the test of choice for secondary adrenal insufficiency in the united kingdom(201).

The GST has also been used to assess cortisol reserve in patients with hypothalamo-pituitary disease(104), however the precise cut off remains unclear. Littley *et al* compared cortisol responses to ITT and GST in 6 healthy men, finding a mean peak of 481nmol/L (range 289 – 717) in the GST compared to 602nmol/L (range 493-792) in the ITT(202). Whilst there was no significant difference between the means of the two tests, the range in the GST was considerably wider, with 4 subjects of the 6 failing to attain a level of >500nmol/L. The lack of a demonstrable difference in this study is likely to be due to insufficient statistical power. Of note the peak cortisol in two of the six subjects was greater than 3 hours post glucagon, demonstrating the

requirement for longer testing protocols when testing cortisol reserve. The GST has previously been shown to produce great variability in normal subjects(203-205).

Tanriverdi *et al* looked at cortisol responses to glucagon in 22 healthy volunteers, reporting a median value of 450nmol/L with the lowest normal value being 295nmol/L – this was recommended as the normal cut off value for subsequent studies, however a low dose SST was used to confirm deficiencies(206). The largest study of cortisol responses to glucagon in health was undertaken in 55 healthy volunteers by Karaca *et al* who compared the GST with standard and low dose synacthen tests(199). They reported a mean cortisol response to glucagon of 592nmol/l with the lowest response of 251nmol/L. When compared to synacthen tests, the LDSST returned similar results with a mean peak cortisol of 697nmol/l and lowest value of 345nmol/l. If the conventional cut off of 550nmol/l was applied to the GST, it demonstrated only 43.6% specificity, with 56% of healthy volunteers demonstrating a suboptimal response. Of these suboptimal responses 25% also failed the LDSST whereas all volunteers passed the SDSST however as previously mentioned, an acceptable response to SDSST in this study was any response up to 2 hours post stimulus.

In conclusion subtle corticotrophin deficiency, whilst less common than GHD, can be difficult to spot, with vague symptoms which overlap with other conditions. The ITT and metyrapone tests are acceptable methods of testing cortisol reserve, however their potential morbidity and side effect profile make them impractical. Both standard and low dose synacthen tests show good correlation with the ITT however up to 20% of normal volunteers will fail these tests at a cut off of 500nmol/l;

furthermore they do not test the complete HPA axis. The GST has been shown to act via an ACTH dependant mechanism(202) however produces wide variability in results leading to a much lower threshold for cortisol response of between 250 and 300nmol/l. It is likely that in the absence of an optimal response to a single test, multiple tests are more efficacious in reducing the false positivity in this field.

Hypothyroidism:

In common with corticotrophin deficiency, thyrotrophin deficiency is normally found in association with other pituitary hormone defects. Once again symptoms can be non specific, however generally mirror those found in primary hypothyroidism, namely fatigue, weakness, failure to lose weight appropriately and cold intolerance(192).

Levels of thyroxine are generally higher than in primary hypothyroidism as a result of some residual TSH secretion. Thyrotropin deficiency can be diagnosed by the finding of a free T4 level below the normal range, in the face of an inappropriately normal or low TSH level(207). The TRH test has historically been used to differentiate secondary (pituitary) hypothyroidism from tertiary (hypothalamic) hypothyroidism however Pavord *et al* audited 232 TRH tests and found that the TRH stimulation frequently gave results at odds with the baseline samples, and therefore recommended that routine use was not advisable(198).

Hypogonadism:

Male hypogonadism:

Pituitary disease in men is associated with hypogonadotrophic hypogonadism. Hypogonadism has been defined as a low serum testosterone in the presence of symptoms and signs of testosterone deficiency including reduction in testicular size, reduced facial hair, decreased libido, depression, lethargy and defects of cognition(208, 209). This becomes more complicated in older and more obese individuals, as testosterone declines with age (see figure 1.4), and with increasing BMI, with 19% of otherwise healthy men satisfying the criteria for hypogonadism(210).

Testosterone in adult men is required for maintaining libido, secondary sexual characteristics and spermatogenesis, however is also involved in accrual and maintenance of muscle and bone mass and erythropoeisis. Testosterone exhibits a normal diurnal rhythm and circulates in 3 forms - free testosterone, bound to albumin and bound to sex hormone binding globulin – only the first 2 forms are biologically active(209), thus it is the free testosterone level which is a better marker of testosterone status than total testosterone.

One major confounder in men with hypogonadism is the effect of obesity. It has been show that the age related decline in testosterone is worsened in obesity, and affects both total and free testosterone levels(211). This reduction in androgen levels may be a marker for disorders of insulin-glucose metabolism, and may in fact contribute towards their pathogenesis (212). Weight loss will normalise testosterone levels in

around 70% of hypogonadal obese men(213), however the benefits of testosterone replacement in obesity are yet to be determined(212).

Testosterone replacement results in improvements in body composition with reduction in fat mass and visceral fat(214, 215) however a meta-analysis by Haddad *et al* failed to demonstrate any benefit of testosterone replacement on cardiovascular morbidity(216). Replacement does however result in improvements in libido, QoL, bone mineral density, muscle mass, mood, erythropoesis and cognition(209).

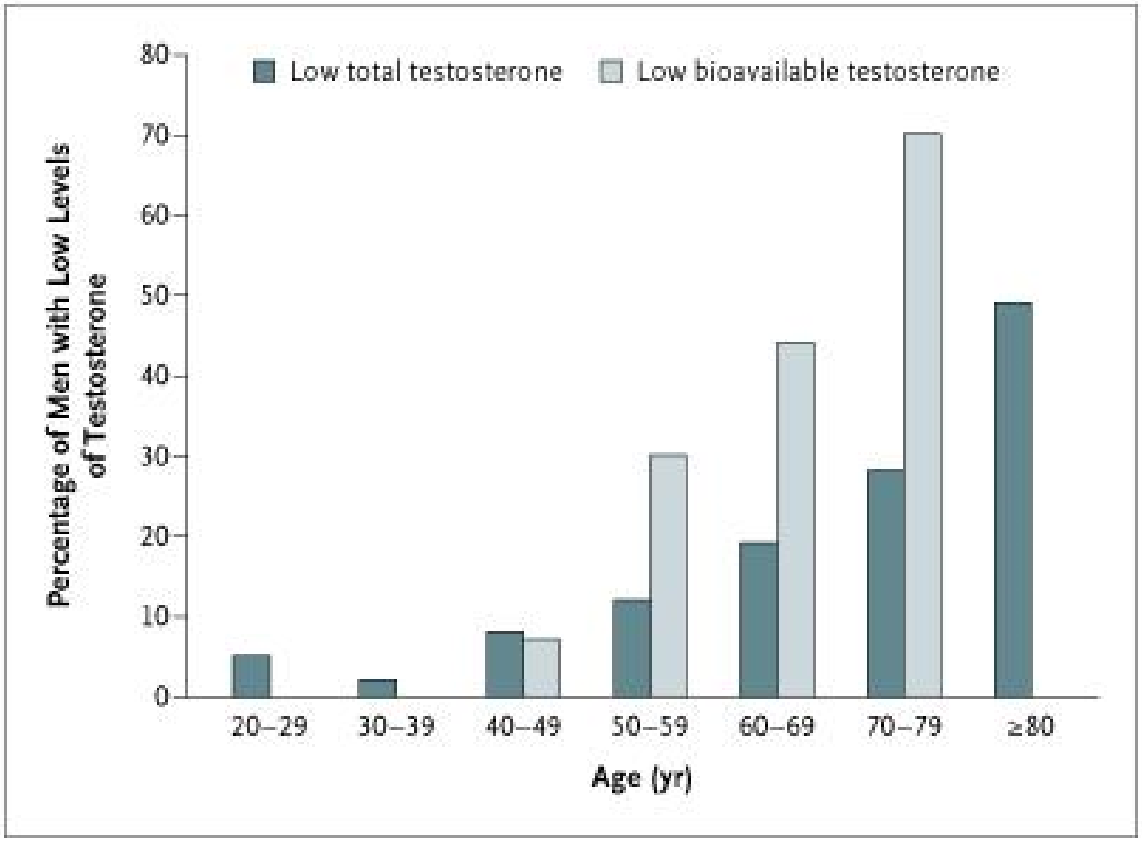


Figure 1.4: *Reducing levels of total and bioavailable testosterone with increasing age(208).*

Female Hypogonadism:

Female hypogonadism is found in patients with menstrual disturbance or amenorrhoea in whom a low oestrodiol level is found in the presence of normal or low gonadotrophins. In postmenopausal women where LH and FSH should be elevated, normal or low levels indicate hypogonadism(192, 207). Since the oestrodiol and gonadotrophins levels vary, it is important to use reference ranges appropriate to the menstrual stage.

Symptoms of hypogonadism are similar to in the male with the addition of breast atrophy, dyspareunia and infertility(207). Replacement therapy results in improvements to symptoms and re-establishment of menses, however infertility remains as only end hormones and not gonadotrophins are replaced.

In conclusion, diagnosis of male and female hypogonadism requires careful attention to symptoms and interpretation of biochemistry. False diagnosis, particularly in men can occur from failure to take account of age related norms, and the effects of obesity on binding proteins. Where deficiency is confirmed to be present, sex steroid replacement in males and females is effective in reversing symptoms.

Section V: Subarachnoid Haemorrhage

a) Aetiology:

SAH is caused by rupture of intracranial aneurysms in 85% of cases, the remainder being attributable in 5% of cases to other malformations or bleeding disorders(217). In 10% of cases, no cause is found(218-220). Most aneurysms are found on the circle of Willis with 15% of aneurysms occurring in the vertebro-basilar circulation. Aneurysms commonly arise at bifurcations with the commonest site being the junction of the internal carotid and the posterior communicating artery (41%) followed by the junction of the anterior communicating artery and anterior cerebral artery (34%) and middle cerebral artery (20%)(221). 20% of patients have multiple aneurysms(222). Aneurysms may be associated with polycystic kidney disease and connective tissue disease(217). Risk factors for aneurysm rupture include haemodynamic stress, atherosclerosis and hypertension(223, 224)

b) Epidemiology:

SAH affects in the region of 10/100,000 individuals in the UK per year(225), with peak incidence between the ages of 50 and 60 years(226, 227). Haemorrhage is more common in women than men in a ratio of 3:2, however this ratio is reduced in patients less than 40years(217). A seasonal variability has been noted in SAH incidence with a biannual peak in spring and autumn(228).

In aneurysmal SAH risk is increased if there is a history of SAH in first-degree relatives, and is tripled if 3 or more relatives are affected by SAH (227, 229). Other risk factors include hypertension, smoking(230, 231) and heavy alcohol

consumption(232, 233). Intracranial aneurysms are detected in 2-5% of autopsies(234) and a prospective study of over 8000 asymptomatic individuals by MRI scanning demonstrated an aneurysm incidence of 6.8% rising to 10.5% in participants with a family history of SAH(235).

c)Grading:

SAH may be graded clinically by the Hunt and Hess Scale or the World Federation of neurosurgeons scale(236, 237). Grading of intracranial blood is calculated by the fisher scale(238, 239).

d) Management:

Aims of management are to manage symptoms, identify and treat the underlying cause, and prevent complications. Analgesia, control of hypertension, adequate hydration and seizure control are all mainstays of acute management(217). Nimodipine, a calcium channel blocker is given prophylactically to reduce the incidence of vasospasm. Following confirmation of the clinical diagnosis by CT scan or lumbar puncture, further imaging by CT angiography and/or Angiography is performed to identify aneurysms which are now managed most commonly by endovascular coiling, although craniotomy with aneurysm clipping may still be used. Whilst 1 year outcome has been shown to be better with coiling(240, 241), there remains concern about the increased likelihood of coiled patients requiring retreatment due to re-bleed or recurrence with a 6.9 times likelihood of this in one long term follow up study(242).

e) Complications:

i) Re-bleeding: This is commonest in the first 24 hours with a 4-6% risk(243, 244) declining thereafter. 50% of patients who re-bleed will die.

ii) Hydrocephalus: Early hydrocephalus occurs in 20-30% of patients, being more frequent in patients with a higher grade SAH. External ventricular drainage is effective and VP shunting may be required. One quarter of patients who survive the acute phase may develop delayed hydrocephalus(217).

iii) Hyponatraemia: Hyponatraemia occurs in around one third of SAH patients(217). This may be due to a combination of cerebral salt wasting and the syndrome of inappropriate antidiuretic hormone secretion (SIADH). Management of SIADH normally consists of restriction of free water with isotonic fluid replacement.

iv) Vasospasm: Vasospasm is the main risk factor for delayed ischaemic neurological deficits, and occurs in 28.5% of cases(245). It is caused by pathological changes leading to wall thickening, luminal narrowing and impairment of vessel relaxation in addition to vasoconstriction(246) reduced blood flow may lead to ischaemia and infarction in the affected territory. The amount of blood in the subarachnoid space is a good predictor of vasospasm(238, 247, 248). Management aims to prevent onset with prophylactic nimodipine, and treat aggressively when it occurs. Hypervolaemia, hypertension and haemodilution (the triple H therapy) has been used to prevent ischaemic damage (249-251), and measures to directly reduce the amount of subarachnoid blood such as intra cisternal thrombolysis have been trialled with some success(252). Using current management techniques 25% of

patients with vasospasm will die, whereas 54% of patients will have a good outcome, the intervening patients having varying levels of neurological compromise.

f) Outcome:

Up to one quarter of patients will die before receiving medical attention(226) and a further 25% of patients developing vasospasm will subsequently die. In survivors, 4-12% have physical impairment such to impair their ability to perform activities of daily living(253-256). A much larger number of patients 44-93% suffer impairments in their instrumental activities of daily living, including skills such as managing finances, shopping and housekeeping(257-259).

SAH also results in major psychosocial problems(259, 260). Only one third of survivors regain functional independence(261) and 92% of patients assessed for their health related quality of life reported moderate to severe problems 12 months post event, although scores had improved by 12-14% since 3months post event(262).

Problems persist for many years; a study of 610 SAH survivors examined a mean of 8.9 years post SAH showed that 26% had stopped work, and a further 24% were working reduced hours or were in positions of reduced responsibility. 7% were divorced due to SAH related issues. 59% reported a change in personality, with increased irritability and emotionality being the most common findings. There was an increase in depressive symptoms, and 10% of patients had scores on the hospital anxiety and depression score suggesting significant depressive or anxiety symptoms. Only 25% of respondents reported complete recovery(261).

In conclusion SAH is an important condition with high mortality and morbidity. Complications such as re-bleeding and vasospasm are common. Whilst complete physical recovery may occur, patients are frequently left with complex psychological and psychiatric issues which are difficult to treat. Many of these complex difficulties appear similar to the problems faced by patients with untreated GHD as discussed in more detail in the following section.

Section VI: Hypopituitarism following Subarachnoid Haemorrhage

a) Aetiology:

Compared with other causes of hypopituitarism where there is an obvious and logical aetiology, the cause of hypopituitarism in SAH is more occult. Whist radiological studies may not document any abnormality, a post-mortem study of 102 patients dying as a result of SAH demonstrated hypothalamic lesions in 68%(263) consisting of ischaemic necrosis, macro-haemorrhages and micro-haemorrhages with lesions localised to paraventricular and supraoptic nuclei.

In the case of aneurysms closes to the midline, bilateral lesions were seen. Possible reasons postulated by Crompton *et al* included increased pressure in the chiasmatic cistern, direct damage of the perforating arteries by subarachnoid blood as they passed through the subarachnoid space, vasoconstriction, and subarachnoid blood passing up the sheaths of the perforating vessels then rupturing into the cerebral parenchyma. Other authors have suggested vasospasm(264) and damage done during craniotomy(265) as other possible explanations. In a case series of 10 patients with non aneurysmal SAH, necrotising pituitary adenoma was highlighted as a possible explanation for hypopituitarism(266).

b) Incidence:

Over the last decade SAH has increasingly been recognised as a cause of hypopituitarism, with an overall incidence in the chronic phase (over 5 months post injury) of between 37.5% and 55%(267-269). Schneider *et al* conducted a systematic review in 2007 concluding that the incidence of post SAH hypopituitarism was 47%, however studies have used different tests, cut offs and populations making comparisons between studies difficult. Recently a registry has been set up by German endocrinologists in order to evaluate the epidemiology of hypopituitarism after subarachnoid haemorrhage and traumatic brain injury.

The first case report of hypopituitarism post SAH was described in 1972, however this did relate to an intrasellar aneurysm(270). Osterman *et al* described 50 patients over 3.5 months post SAH investigated by baseline pituitary function tests and analysis of the circadian rhythm of 11-hydroxycorticosteroids and the metyrapone test. Whilst abnormalities of the HPA axis were found, it was concluded that frank hypopituitarism was rare(271).

Kelly *et al* recognised the commonality of symptoms between patients with hypopituitarism and patients with TBI or SAH, demonstrating an incidence of hypopituitarism of 40% in 24 patients (2 SAH) over 3 months post insult when compared to 18 closely matched controls. Investigation was done by ITT, GnRH test and TRH test. Five patients (four men) were found to be hypogonadal on GnRH test however all had normal testosterone levels. The GnRH test has however been shown to be unreliable in this context(198).

Kreitschmann – Andermahr *et al* investigated 40 patients between 1 and 6 years post SAH by ITT, Arginine, TRH and GnRH testing (265). They found deficits in 43% of patients from the cohort whose mean BMI was 26 kg/m^2. Defects are shown in Fig 1.5. Four patients had corticotrophin deficiency on ITT, whilst eight patients had GHD. Three of these patients did however pass the Arginine test having failed the ITT; two of these patients had an elevated BMI. Only one of the eight patients had an IGF-1level <-2 SD. Patients with GHD post SAH had gained significantly more weight since their SAH and also had a significantly higher BMI than those patients without GHD. The very high incidence of isolated corticotrophin deficiency has not been replicated, and may be accounted for by poor test- retest reproducibility, or borderline attainment of hypoglycaemia(272).

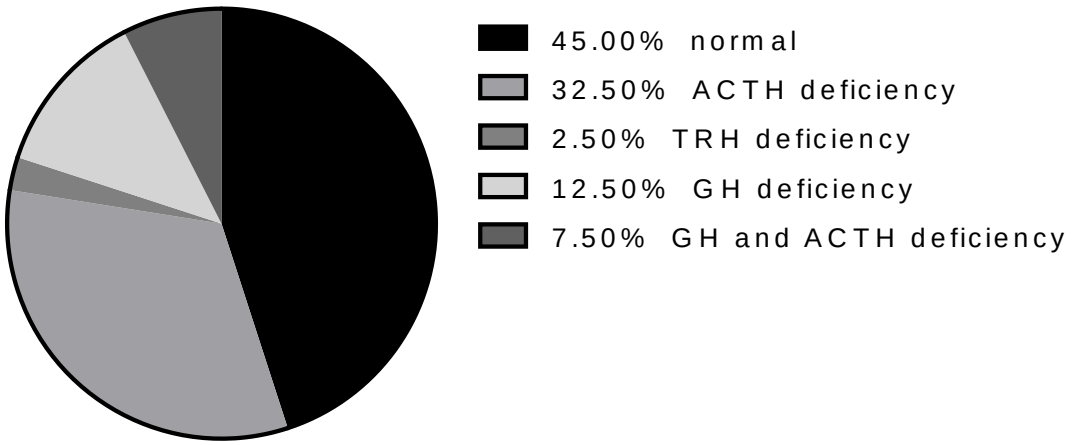


Figure 1.5 – *Adapted from Kreitschmann – Andermahr et al: Incidence of pituitary dysfunction in 40 patients with SAH(265)*

Dimopoulou *et al* investigated endocrine function 12-24 months post SAH by basal hormone testing and low dose synacthen testing(268). They found an overall incidence of 14/30 patients with endocrine abnormalities however no dynamic test for GH secretion was undertaken; the 37% incidence of GHD reported was based on IGF-1 levels alone.

Aimaretti *et al* conducted the first and only longitudinal study employing dynamic function testing at two time points in the first 12 months(267). 32 patients were included. This involved testing with the GHRH/Arginine test, and single early morning measurements of other pituitary hormones. They demonstrated an incidence of hypopituitarism at 3 months post SAH of 46.8% falling to 37.5% at 12 months. GHD (21.8% at 12 months) was most frequently observed followed by hypogonadism (6.25%) hypocortisolaemia (6.25%) and hypothyrotropinaemia (9.3%). Whilst mean BMI was 24.7kg/m^2 , the cut of off 9µg/L used in this test did not reflect the large changes seen in the GAT with increasing BMI, therefore the incidence of severe GHD is likely to be over estimated. Furthermore single early morning cortisol measurements are less sensitive than dynamic tests in diagnosing corticotrophin deficiency. Finally, when diagnosing hypogonadism, no account was taken of free testosterone or SHBG concentrations. Significantly this longitudinal study did not find any new cases of hypopituitarism at 12 months in patients with normal pituitary function at 3 months, in contrast to the findings in patients with traumatic brain injury in this study.

Further studies have broadly replicated the findings of these initial studies with minor variations in the subtypes of hormonal deficits described(273-275), deficits

have also been noted in the acute phase after SAH(275, 276) although differentiation from adaptive hormonal responses is difficult, with physiological responses to critical illness known to mimic central hypogonadism and hypothyroidism, and some drugs used in the intensive care setting known to affect the binding of hormones to their binding proteins(272).

Conflicting with previously published data Klose *et al*. reported only 1 case of GHD in a series of 62 patients following SAH, which was not verified when the test was repeated.(272) This study used robust BMI appropriate cut offs, and confirmatory testing of abnormal results. Whilst 58% of patients were found to have hormonal alterations correlated with lower GCS and presence of hydrocephalus in the acute phase, none was confirmed on testing at 12 months. Such rigorous testing may underestimate the prevalence of hypopituitarism and peak GH was significantly lower in the patient group compared with the controls (confounded by the fact that controls were significantly younger and fitter than the patients).

Despite the focus on this complication there is still a paucity of data in this area with only 122 SAH patients included in the systematic review performed by Schneider *et al*(96), and considerable differences between studies exist, confounded by issues such as differences in tests, different cut offs, different treatment modalities and different levels of morbidity in the patient population. Other questions remain about whether cardio-metabolic and psychological outcomes are influenced by the state of pituitary function.

In patients with traumatic brain injury, it has been shown that patients with hypopituitarism have an adverse lipid profile and unfavourable body composition compared with those with normal pituitary function(277). No such studies have however been performed in patients following SAH. Likewise both TBI patients and SAH patients have been shown to have reductions in QOL, energy, physical heath, emotion, well-being and increased rates of depression(278, 279). There have however been no prospective studies to demonstrate whether hormone replacement improves these factors as it is known to do in other causes of adult hypopituitarism(143, 170, 171, 176, 280-285).

In conclusion there is considerable overlap between symptoms seen post SAH and symptoms seen in GHD. Unlike other causes of GHD there is no overt pituitary damage although hypothalamic damages has been seen at post-mortem in SAH patients. Estimates of post SAH hypopituitarism vary greatly and are highly dependent on the test used, population studied, including age and adiposity, cut off for GHD employed, and treatment given for the original SAH. Compared with other patient groups only very small numbers of patients have been studied in this field. It is not yet known whether replacement of hormone deficiencies in this population improves symptoms or not.

Section VII- Hormonal influences on liver function

Non-alcoholic fatty liver disease (NAFLD) is an increasingly recognised cause of chronic liver disease affecting, in some series, up to 30% of the population(286). NAFLD is the commonest cause of abnormal liver function tests(287). NAFLD spans a spectrum of histo-pathological abnormalities ranging from simple hepatic steatosis, steatosis with necro-inflamation (NASH) and NASH with varying degrees of fibrosis which may progress to cirrhosis and its complications including liver failure and hepatocellular carcinoma (HCC)(288).

The hallmark of NAFLD is an echo-bright liver on abdominal ultrasonography, often accompanied by elevated liver enzymes, however liver enzymes may be normal(289), and most patients are asymptomatic, with liver disease identified incidentally. Both hepatic and cardiovascular mortality are increased in these patients. Regarded as the hepatic manifestation of the metabolic syndrome(290), NAFLD is associated with obesity, dyslipidaemia and abnormal glucose tolerance, and as such is a multifaceted disorder encountered in disparate clinical settings including primary care, gastroenterology, cardiology and gynaecology. Whilst obesity is the commonest metabolic cause, a number of other endocrine disorders may be associated with NAFLD including thyroid dysfunction, GHD, adrenal insufficiency and polycystic ovary syndrome(291).

The evolution of NAFLD to NASH is thought to involve a two hit hypothesis. In the first hit, hepatocytes develop an increased triglyceride content, followed by a second

hit involving increased oxidative stress(292). A number of endocrine mechanisms including hypothyroidism, PCOS and adrenal failure contribute to the development of NASH(291). Thyroid replacement has been shown to improve NAFLD after 6 weeks treatment(293). With adverse lipid profiles and elevated triglycerides being a feature of patients with hypopituitarism, and the reduction in lipolysis seen in GHD, an association between hypopituitarism and in particular GHD and NAFLD has been examined as discussed below.

Growth Hormone Effects in NAFLD:

Whilst the liver is known to have receptors for GH, and indeed is the site of production of IGF-1, the effect of GH in regulating liver health is thought to be indirect. As previously discussed, the metabolic phenotype of patients with GHD is one of hyperlipidaemia, hypertriglyceridaemia, obesity and insulin resistance and along with these features, NAFLD has been found to be common in this population. There is evidence to suggest that there is an independent association of GHD with NAFLD. Brobeck *et al* induced hypothalamic damage in animal models of pituitary disease, and found that this inevitably led to liver damage(294).

Lonardo *et al* looked at 61 patients with GHD and 104 controls, finding that GH levels were an independent predictor of fatty liver disease in men(295), and Bredella *et al* demonstrated that GH peak to GHRH/Arginine testing was correlated with hepatic triglyceride content in healthy obese patients(296). Furthermore individuals with cirrhosis have been shown to have reduced synthesis of IGF-1(291). Adams *et al* looked at 21 patients with GHD and NAFLD(297); they noted development of risk

factors for the metabolic syndrome, including development of central obesity and insulin resistance, with development of NAFLD a mean of 6.4 years after the development of hypothalamo-pituitary dysfunction. Biopsies on ten of the patients revealed cirrhosis in 6, NASH in 2 and simple steatosis in a further two patients. He concluded that GHD patients were at high risk for development of NAFLD.

In patients with hypopituitarism the increased risk of liver pathology would appear to be related to GHD; Ichikawa *et al* looked at 18 patients with hypopituitarism, with and with and without GHD, diagnosing NAFLD by the finding of an abnormal liver spleen ratio(298). They found an increased incidence of NAFLD only in patients with GHD (7/13 GHD vs. 0/5 no GHD). This was despite the two groups of patients demonstrating no significant difference in BMI, cholesterol or triglyceride levels. In a single case report Takano *et al* demonstrated improvement in NAFLD after GHR but not after prior replacement with hydrocortisone or thyroxine(299).

There is further evidence for the amelioration of liver disease by GHR. A case report in 2003 demonstrated reduction in liver fat as part of a constellation of improvements including improved lipid profiles, reduced insulin resistance and beneficial body composition in a 15 year old boy receiving GH for Alstrom syndrome(300). A further case in 2007 documented dramatic improvements in biopsy proven NASH after 6 months GHR as shown by normalisation of liver enzymes and improvements on biopsy(301).

Evidence for the molecular basis of these improvements comes from animal studies. Such studies have demonstrated reduced GH receptors in rats with cirrhosis with further reductions as cirrhosis progresses (302). GHR in this scenario has been shown to improve liver transaminases, increase levels of mRNA expression for the GH receptor, regress fibrosis and reduce portal hypertension. The mechanism for this is thought to involve activation of STAT 5A and 5B, transcription factors which induce transcription of genes critical for liver metabolism(303).

Whilst these clinical case reports suggest an increased prevalence of NAFLD in hypopituitarism and in particular patients with GHD, and the animal studies support a molecular mechanism, only one case control study matching for BMI (which is known to be independently associated with NAFLD(304)) has been performed. Nyenwe *et al* looked at 141 patients with hypopituitarism, retrospectively diagnosing the presence of NAFLD based on a variety of criteria including liver biopsy and abdominal imaging or biochemistry. They matched these patients with a similarly obese population of control subjects without hypopituitarism(305). They found an increased incidence of abnormal transaminases in this population (24% vs. 11%) however this study was limited by its retrospective nature and imprecise definition of NAFLD. No detailed observational studies looking at the effect of GHR on hepatic triglyceride content have been conducted.

Intramyocellular lipids

IMCL act as a source of rapid energy for exercising muscle, therefore IMCL are depleted by acute exercise. Likewise however athletes undergoing endurance training, have elevated levels of IMCL. There is however a paradox in that it has also

been noted that overweight sedentary adults have elevated levels of IMCL and that in this situation this abnormal build up of lipid can be associated with insulin resistance(306).

Bredella *et al* who looked at the relationship between GH levels, obesity IHCL and IMCL in premenopausal women, found increased levels of IMCL in obesity, together with an inverse correlation between IMCL and peak GH levels following GHRH/Arg stimulation(296).

Tripp *et al* went further looking at IMCL in detail in patients with GHD compared with matched controls(307), followed by re-examining the patients after 4 months of GHR. They looked at IMCL in states of both lipid accumulation and depletion. It was noted that patients with GHD exhibited greater variation between depletion and repletion, with volunteers retested after 4 months GHR returning towards the control group. Given that insulin sensitivity did not change between groups or following GHR it was concluded that changes in insulin sensitivity were not responsible for the difference.

Krag *et al* however using a higher dose of GHR in healthy volunteers did note an increase in IMCL in association with increasing insulin resistance(308), however they did not study depletion repletion cycles. It therefore remains unclear the effects or mechanism of effect of GH on IMCL in skeletal muscle.

In conclusion there is a demonstrable link between GH and accumulation of IHCL and IMCL. GHD patients are at increased risk of development of NAFLD post

diagnosis and this would appear to be an independent GH effect. Animal studies provide a possible molecular mechanism. There is however an increase in both obesity and insulin resistance in patients with GHD and both of these factors are risk factors for increases in both IHCL and IMCL. There are currently insufficient studies looking at IHCL and IMCL in a quantifiable way using closely matched controls to be able to isolate the individual risk factors and to measure the effect of GHR on IMCL and IHCL.

Chapter 2

Patients and Methods

1. Patient Recruitment for studies

GHD and NAFLD study. All patients with biochemically severe GHD (Peak GH <3nmol/l) and QOL AGHDA score >11/25 ("symptomatic patients") who were about to commence treatment were approached for inclusion in this study provided they did not have any of the exclusion criteria asset out in chapter 3.

Patients with severe GHD who did not have a QOL AGHDA score >11/25 ("asymptomatic patients") and who therefore did not warrant treatment were also approached for inclusion in the cross sectional arm of this study. Other asymptomatic patients were recruited during routine follow up visits to the endocrine clinic. A number of known asymptomatic patients were also invited to take part in the study by letter after authorisation from the local ethics committee.

Healthy controls were recruited from university and hospital staff; in addition a number of our patients' relatives also volunteered to act as healthy controls in the study.

TANITA validation study. Patients and volunteers undergoing the studies above underwent estimations of body composition by TANITA analysis. Data from all groups were used to validate the TANITA methodology and to highlight differences between groups.

Glucagon Validation study. Healthy volunteers were recruited from hospital and university staff by local advertisement on the relevant intranets. Relatives of patients undergoing the GST were also invited to take part.

SAH study. All patients referred prospectively for assessment to the neuro-dysfunction clinic were included in the prospective longitudinal study of pituitary function following SAH provided that they had undergone dynamic function testing at more than one point. The neuro-dysfunction clinic was set up by myself to screen patients following TBI and SAH and ran from may 2009 until December 2010. Referrals were accepted from any source including other tertiary specialties and general practitioners, however the majority of patients were referred on discharge from the Walton centre by the neurovascular nurse specialist. In addition a number of patients who had suffered an SAH some years earlier were referred from the neurosurgical clinic or general practitioners. The progress of these 'retrospective' patients, including response to treatment was also tracked.

TBI study. Data were obtained from the Pfizer KIMS database. The database was interrogated for all patients with adult onset GHD due to traumatic brain injury or NFPA. Patients who had previously received growth hormone replacement were excluded, as were those NFPA patients who had received cranial irradiation.

2. Ethical Approval

Ethical approval was not required for the prospective SAH study, as this was an NHS clinical service, and the data collection constituted clinical audit. The GH and non-alcoholic fatty liver disease study was approved by Liverpool East Regional Ethics committee, as was the glucagon study.

3. Hormonal and Lipid measurements

Total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG), glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltranspeptidase (GGT), were measured using standardised assays in the hospital laboratory at Aintree University Hospital.

Serum GH and IGF-1 levels were analysed in the hospital laboratory using chemiluminescent immunometric assays using IMMULITE 2000 (Siemens Medical Solutions Diagnostics) according to the manufacturer's instructions. Details of all assays are shown in table 2.1.

4. Diagnosis of GHD

All patients deemed to be at risk of GHD underwent dynamic function testing for measurement of GH reserve. This was initially performed by GST. Glucagon testing was carried out in the outpatient clinic or on the day case ward. Following an overnight fast an indwelling venous cannula was inserted, and blood was drawn for growth hormone, cortisol and glucose measurement. Glucagon (Novonordisk) 1mg (1.5mg for patients weighing >90kg) was administered subcutaneously following which blood for cortisol, GH and glucose was drawn at 90,120,150,180 and 210 minutes. By convention in our unit fixed dose glucagon stimulation testing was used.

Weight based dosing has been evaluated, being associated with higher side effects and a later GH peak, however may be of benefit in older and more obese patients.(113)

Analyte	Analyser	Assay	Analytical Sensitivity	Total CV %	Reference Range
GH	Siemens Immulite 2000 from Siemens Healthcare Diagnostics	Solid phase 2-site chemiluminescent immunometric assay	0.01 μ/L	4.2 – 6.6	Dependent on stimulation test
IGF-1	Siemens Immulite 2000, as above	Solid phase, enzyme labelled chemiluminescent immunometric assay	2.6 nmol/l	3.7 – 8.1	11-31 nmol/L
Cortisol	Siemens Centaur, as above	Competitive immunoassay using direct chemiluminescent technology	5.5 nmol/l	4.22 – 6.58	07.00-09.00 119- 618 nmol/L
TSH	Siemens Centaur, as above	2 site sandwich using direct chemiluminescent technology	0.01 mIU/L	3.17 – 5.87	0.4- 4.5 mIU/L
FT4	Siemens Centaur, as above	Competitive immunoassay using direct chemiluminescent technology	3.9 pmol/l	1.89 – 3.4	8- 21.0 pmol/L
Prolactin	Siemens Centaur, as above	2 site sandwich using direct chemiluminescent technology	6.4 mIU/L	2.9 – 6.6	<500 mIU/l female <350mIU/L male
Testosterone	Siemens Centaur, as above	Competitive immunoassay using direct chemiluminescent technology	0.35 nmol/l	2.7 -7.6	8.5 – 29 nmol/L
Oestrodiol	Siemens Centaur, as above	Competitive immunoassay using direct chemiluminescent technology	25.7 pmol/l	7 – 12.3	Females ND-587 pmol/L Males ND-150pmol/L
LH	Siemens Centaur, as above	2 site sandwich using direct chemiluminescent technology	0.07IU/L	2.7 – 3.8	0.8- 7.6 iu/L
FSH	Siemens Centaur, as above	2 site sandwich using direct chemiluminescent technology	0.3IU/L	2.2 – 3.9	Male 0.7- 11.1 IU/L, Female mid cycle 5.8- 21.0 IU/L
Cholesterol	Beckman Coulter AU 2700 from Beckman Coulter UK Ltd	Enzymatic method producing red quinoneimine dye measured at 540/600nm	0.07 mmol/l	1.06 – 1.45	<5mmol/L
HDL-Cholesterol	Beckman Coulter AU 2700, as above	Enzymatic method producing blue dye measured at 600/700nm	0.002 mmol/l	1.32 – 1.92	>1 mmol/L
LDL	Calculated not assayed	Calculation: [-(trig)/2.2 – (HDL) + (chol)] = LDL			N/A

Table 2.1 – *Endocrine Assays and methods used in this thesis*

Following the test patients were given refreshments and advice on frequent meals for the rest of the day. Patients found to have isolated GHD underwent a second test of GH reserve by combined growth hormone releasing hormone (GHRH) and arginine testing. Following an overnight fast an intravenous cannula was inserted and blood was drawn for GH measurement. GHRH (1 microgram/kg to a maximum of 200 micrograms) was given intravenously following which the patient received an infusion of Arginine 10% solution (0.5g/kg to a maximum of 30g). Samples for GH were drawn 30, 60, 90 and 120 minutes after the start of the test. Following the test the patient was allowed to eat and drink. GHD was defined as a peak GH level <3 micrograms/l for the GST; the response to arginine was stratified by BMI (cut off for severe GHD: GH<11µg/l for BMI <25kg/m^2, <8µg/l for BMI 25 – 30kg/m^2 and <4µg/l for BMI >30kg/m^2).

5. Diagnosis of other hormone deficiencies

All patients underwent dynamic function testing for cortisol reserve by GST as previously described followed by standard dose (250µg) short synacthen testing (SDSST) if inadequate reserve was demonstrated on GST. Synacthen testing involved insertion of an intravenous cannula. A blood sample was then drawn for estimation of baseline cortisol. Following this tetracosactrin, a synthetic ACTH preparation was instilled into the cannula over 5 minutes. A blood sample was then drawn 30 minutes following the injection for peak cortisol. A peak cortisol less that 500nmol/l to GST and SDSST would confirm suboptimal cortisol reserve.

Gonadotrophin levels were measured together with the relevant sex hormone (either testosterone or oestrodiol) to screen for gonadotrophin deficiency. In males samples were measured between 9am and 10am. An early morning testosterone level below the normal range of 8.5nmol/l in the presence of normal or low gonadotrophin levels would indicate gonadotrophin deficiency. Due to the effects of obesity on free testosterone, overweight patients underwent measurement of sex hormone binding globulin and free testosterone to confirm gonadotrophin deficiency. In females, premenopausal women were considered gonadotrophin deficient if they had a low oestrodiol level in the face of normal or low gonadotrophins. Postmenopausal women would be considered gonadotrophin deficient if gonadotrophins were within the premenopausal range.

Secondary hypothyroidism was defined as a free thyroxine level below the normal range, in the face of a normal or low thyroid stimulating hormone.

6. MRI Scan Protocols

MRI scanning was undertaken at the Magnetic Resonance and Image Analysis Research Centre (MARIARC) at the University of Liverpool. Patients attended on a mutually convenient day. Participants were fasted and asked to refrain for exercise for the preceding 24 hours, however were allowed to drink water freely on the morning of the test.

Following completion of a screening form to ensure there were no contraindications to scanning, patients underwent whole body MRI scanning followed by non-invasive quantification of intrahepatic and intramyocellular lipid content by proton magnetic

resonance spectroscopy (^{1}H-MRS) in a 1.5T Siemens Symphony scanner (Siemens Medical Solutions, Erlangen Germany).

Whole body MRI was conducted as follows: Whole body axial T1 weighted fast spin echo scans were taken coronally with a 10mm slice thickness followed by a 10mm gap using the body coil. The patient was scanned in the prone position. Image fat analysis was conducted by an external company (Vardis) with fat volume in litres reported.

Participants underwent MRI scanning in a 1.5T Siemens Symphony scanner (Siemens Medical Solutions, Erlangen, Germany). Coefficients of variation for all the following methodologies have been previously published (309-311), methodologies are summarised below:

Volumetric analysis of subcutaneous and visceral fat: Abdominal subcutaneous adipose tissue (SAT) and abdominal visceral adipose tissue (VAT) was calculated from whole body axial T1-weighted fast spin echo scans (axial scans, 10 mm slice thickness followed by a 10 mm gap using the integral body coil). The abdominal region was defined as the image slices from the slice containing the femoral heads, to the slice containing the top of the liver/base of the lungs. All scans were analysed centrally. Scans were anonymised prior to analysis thus ensuring the observer was blinded to all clinical details.

Proton magnetic resonance spectroscopy (^{1}H-MRS): In *liver*, NAFLD was defined as IHCL > 5.6%(312). Three voxels of interest were identified in the liver standard sites avoiding ducts and vasculature (figure 2.1). In skeletal muscle a single voxel was identified in each of the tibialis anterior (TA) and soleus (Sol) muscles, avoiding bone, fascia and the neurovascular bundle. Single voxel spectroscopy was conducted at each of these five sites. Voxel size was 20x20x20 mm, TE 135 ms, TR 1500 ms, with 64 acquisitions. Where the musculature was too small to allow placement of a 20mm voxel, a 15x15x20 mm voxel was placed and the number of acquisitions was increased to 200 to maintain signal-to-noise ratio. In both liver and muscle, voxel placement in post-treatment studies was guided by reference to the pre-treatment images. ^{1}H MR spectra were quantified using the AMARES algorithm in the software package jMRUI-3.0(313, 314). As previously described, IHCL is expressed as % of CH_2 lipid signal amplitude relative to water signal amplitude[31] after correcting for T_1 and T_2(311), and IMCL is expressed as CH_2 lipid amplitude relative to total creatine amplitude after correcting for T_1 and T_2 (315).

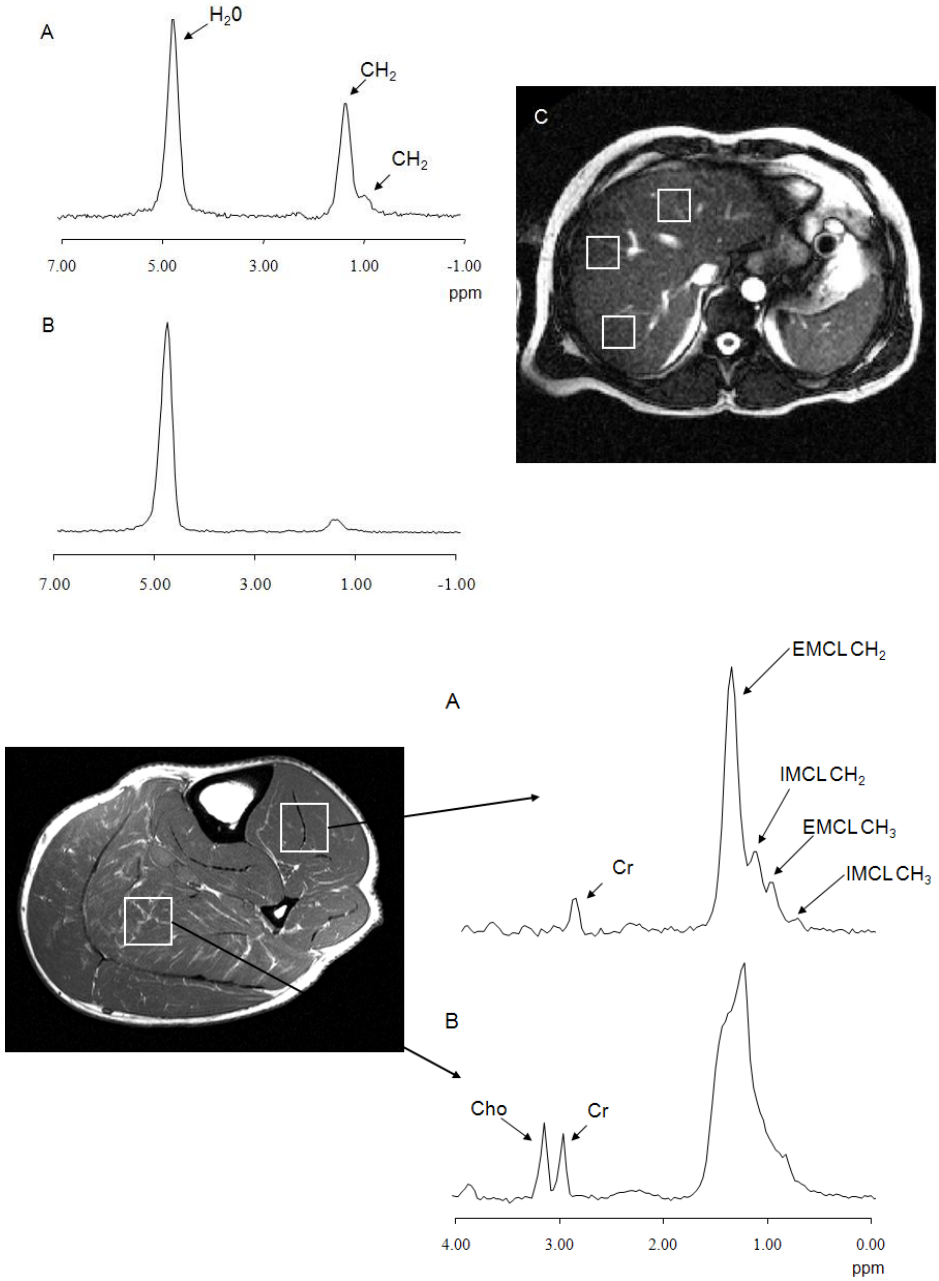


Figure 2.1: *Upper: Representative 1H MR spectra from the livers of subjects with (A) high liver fat content 18.1% and (B) low liver fat content 2.1%. The MR Image shown (C) shows typical placement of the PRESS voxel in different regions of the liver. Lower: Representative 1H MR spectra from the (A) tibialis anterior and (B) soleus muscles of calf. The image shows position from which each spectrum was acquired.*

7. Diagnosis of NAFLD

Non alcoholic fatty liver disease was defined as an average intrahepatic fat content by 1H MRS of >5.6% as defined by Szczepaniak et al (312).

8. Growth Hormone Replacement

Individuals who were biochemically GH deficient, and who had impaired quality of life defined as a QOL AGHDA score ≥ 11 points ('symptomatic' patients) were commenced on GH replacement in accordance with UK National Institute of Health and Clinical Excellence (NICE) guidance. The initial GH dose was 0.2 mg/day titrated at monthly intervals to achieve an IGF-1 level within the normal range. Patients were supervised by an experienced endocrine nurse and symptoms and signs carefully monitored. Patients with biochemical GHD but with a QOL AGHDA score <11 did not commence growth hormone.

9. Body Composition Analysis and Auxology

Weight, percentage body fat and visceral fat rating was measured fasted to the nearest 0.1 kg wearing light clothing on TANITA scales (Tanita BC420, Dolby Medical, Stirling, UK) and height measured to the nearest 0.5 cm using a stadiometer. Patients were advised to avoid vigorous exercise in the 24 hours prior to testing and to drink normally on the morning of the investigation to avoid errors in analysis caused by an abnormal state of hydration.

10. Quality of Life assessment

Quality of life was measured using the validated questionnaire Quality of Life Adult Growth Hormone Deficiency Assessment (QoL AGHDA). Participants were asked to fill this in making reference to their perceived quality of life over the previous month.

11. Protocol for Prospective screening for hypopituitarism following SAH

Patients referred for screening prospectively were seen 3 times over the course of 12 months following their SAH. The initial visit was 3 months after their SAH. At this point they underwent a full clinical history and examination. They then underwent a glucagon stimulation test of pituitary function provided that there was no contraindication in addition to fasting lipid profile, liver function and renal function (sodium, potassium, urea, creatinine, GFR). Patients were seen a second time 3 months later went changes were noted and basal hormone levels, fasting lipids, renal and liver function repeated. If patients demonstrated a suboptimal cortisol response to glucagon stimulation then they had a short synacthen test at this visit, unless their initial peak cortisol level was less than 300nmol/l in which case they would have had a synacthen test as soon as possible after the glucagon stimulation test results were received.

Patients were seen one further time 12 months after their subarachnoid haemorrhage at which point they had a repeat glucagon stimulation test to detect evolving hormone deficiencies, or to confirm persistence of deficiencies already found on earlier screening. At all 3 visits patients also filled in the quality of life questionnaire

QOL AGHDA. A summary of the screening protocol is included in the table 2.2 below.

12. Protocol for Retrospective screening for hypopituitarism following SAH

Patients referred over 12 months after their SAH or TBI underwent the same evaluation as in visit 1 above. Repeated testing was not undertaken in this group as it was assumed that all deficiencies would be static in this chronic phase of the recovery.

Visit number	Investigations
1. 3 months post insult	GST, fasting lipids, renal, liver, QOL
2. 12 months post insult	GST, fasting lipids, renal, liver, QOL

Table 2.2 - *Prospective screening programme for patients following SAH*

13. Grading of SAH

Note was made of the severity of the subarachnoid haemorrhage in all patients attending the neuro-dysfunction clinic. This was done both clinically using details from presentation at the neurosurgical centre (the world federation of neurosurgeons [WFNS] grade), and radiologically by measuring the blood load on the initial CT scan (the Fisher grade). Data from case notes was used to calculate the WFNS grade whilst Fisher Grade was calculated by a two observers, Dr Mani Purthuran and Dr Biswas, consultant neuro-radiologists at WCNN. Both grading systems are shown in the table 2.3.

Grade	WFNS		Fisher
	GCS	Motor Deficit	
I	15	-	No blood detected
II	14-13	-	Diffuse blood <1mm thick
III	14-13	+	Localised clot or thick layer >1mm thick
IV	12-7	+/-	Diffuse or no blood with intracerebral or intraventricular blood
V	6-3	+/-	-

Table 2.3 – *Description of fisher grades and WFNS grading system used in grading severity of SAH(316, 317)*

Chapter 3

Estimation of Liver Fat in patients with Growth hormone Deficiency and the effect of growth hormone replacement

Abstract

Non-alcoholic fatty liver disease (NAFLD) is reported to be more common in patients with GHD than the general population. We aimed to determine i) liver fat in patients with GHD compared to age and BMI-matched controls, and, ii) effect of 6 months growth hormone replacement (GHR) on liver fat. We also aimed to examine the relationship between changes in liver fat and changes in other fat depots of the body, namely abdominal visceral and abdominal subcutaneous fat. In doing so we also sought to validate bio impedance analysis as a method for quantifying different fat depots by comparing this to the gold standard of MRI scanning.

We concluded that NAFLD is equally common in patients with GHD and matched controls. GHR is associated with a hierarchical reduction in fat deposition (fat loss: visceral >subcutaneous>liver). Bio impedance was less well correlated to MRI measures of body composition in patients with GHD in comparison to controls, and hence this is not an accurate surrogate measure in this patient group. Further studies involving GHD patients with NAFLD are required to conclude the role of GHR in treating NAFLD.

Introduction

Untreated GHD is associated with multiple features of the metabolic syndrome including insulin resistance, obesity, dyslipidaemia and hypertension(297, 298, 318). Body composition analysis in GHD has shown increased central adiposity and visceral fat, and reductions in lean muscle mass, which have been linked to the metabolic syndrome(148, 319). Furthermore reductions in extracellular fluid volume have been seen in patients with GHD(155, 320), which is important when considering bio impedance as a method for calculating body composition. Treatment with recombinant growth hormone (rGH) has been shown to improve many of these features(321-324).

Non-alcoholic fatty liver disease (NAFLD), as the hepatic manifestation of the metabolic syndrome, has similarities with adult GHD, being associated with insulin resistance and increased cardiovascular mortality(290). Patients with GHD tend to gain weight more rapidly and are on average more obese than controls(108, 325), so it could be argued that the increased prevalence of the metabolic syndrome in GHD is simply related to obesity. Against this, GHD is an independent predictor of NAFLD in patients with hypopituitarism(295). Furthermore, patients with hypopituitarism including GHD have increased prevalence of NAFLD compared with body mass index (BMI) matched patients with hypopituitarism and normal GH secretion(298). These data suggest that GHD may be an independent risk factor for the development of NAFLD. Previous studies in this area have been limited by reliance on less sensitive methodologies to detect NAFLD such as abdominal

ultrasonography. Studies have also suffered from poor matching of clinical characteristics, such as age and BMI, between the cases and controls.

Whilst rGH has been shown to produce clinically significant improvements in body composition, lipid profiles and insulin sensitivity, reports of improvement in NAFLD have been limited to isolated case reports(299-301). Likewise, treatment with rGH has been shown to improve insulin sensitivity in obese patients without GHD(326-328) and in patients with type 2 diabetes(329), but the effect on liver and muscle fat has not been documented. With proton magnetic resonance spectroscopy (^{1}H-MRS) it is possible to non-invasively quantify triglyceride within liver(311) and skeletal muscle(315) (intrahepatocellular lipid, IHCL and intramyocellular lipid, IMCL). These measurements are metabolically and physiologically relevant as IHCL is a better predictor of metabolic risk than visceral fat in obese patients(330), and IMCL is also inversely associated with insulin sensitivity in sedentary patients(306). These measurements can easily be combined with whole body magnetic resonance imaging (MRI) for volumetric analysis of total and regional subcutaneous and visceral fat.

Whist MRI scanning provides accurate body composition data, its use is time consuming and expensive, limiting its use both in large scale research studies and in clinical practice. Furthermore many categories of patients are unable or unwilling to undergo MRI scanning due to a number of medical contraindications or due to inability to tolerate the scan itself. Finally the dimensions of the scanner can prove difficult when considering that by definition the population we intend to study is obese. Body composition analysis by bio impedance provides a comparatively inexpensive and portable method(331) with few contraindications hence provides a possible solution and has been developed and validated in particular sectors of the

population. Its use has been validated for estimation of skeletal fat(332), total body fat and abdominal adipose tissue(333-335) although inaccuracies have been seen in patients with greater abdominal adiposity(336), possibly due to the larger quantity of subcutaneous adipose tissue(335).

Bio impedance has not however been validated in patients with GHD in whom altered body composition has been well described, and in whom hydration of tissues differs to healthy participants. This has been demonstrated by changes in bio impedance when GH therapy is commenced(154). In preparation for further studies we aimed to ascertain whether the calculations inherent in the estimation of abdominal visceral fat were appropriate and accurate in a group of patients with GHD.

The aims of this study were therefore as follows:

1. Based on the previous reports of increased prevalence of NAFLD in patients with GHD, compared with controls matched for age, gender and BMI, we hypothesised that that the primary outcome measure of liver fat would be higher in GHD patients compared to controls. We anticipated that the altered phenotype might explain the clustering of cardio-metabolic risk factors observed in this patient group.
2. Consistent with this hypothesis, we further hypothesised that treatment with rGH for 6 months would be associated with reductions in IHCL and IMCL.

3. We wished to ascertain whether bio impedance analysis was suitably accurate in this population of obese patients with and without GHD to enable its use in future studies in whom MRI scanning would not be practically possible.

Methods

The Liverpool research ethics committee approved the study. Written informed consent was obtained from all subjects before the study.

Study design

This was a cross-sectional study comparing long standing (>1 year) untreated GHD patients who were fully GH naive with controls who were mean matched according to age and BMI; thus we used 2 independent but comparable groups. Within this study a nested cohort of 12 patients commencing rGH were studied longitudinally before and six months after commencement of rGH. The nested cohort was necessary, because only patients with a symptom score >11/25 using the validated questionnaire (Assessment of Growth Hormone Deficiency in Adults)(179) received treatment in accordance with UK National Institute for health and clinical excellence guidance.

Clinical and laboratory measurements

Twenty eight GHD patients attending the tertiary endocrine service at University Hospital Aintree, Liverpool were recruited between April 2009 and February 2011. The time from the pituitary insult to study recruitment was a median of 5 years (range 1 to 33 years). Baseline characteristics are shown in Table 3.1. Of the 2 patients with Cushing's disease, both were in long term remission (>5 years). Inclusion criteria included GH response less than 3 µg/l after glucagon

stimulation(104). Twenty-four controls were recruited by local advertisement. Exclusion criteria included alcohol intake > 21 units/week for men and >14 units per week for women(329), drugs associated with steatosis, a history of viral hepatitis, auto-immune or metabolic liver disease (haemachromatosis, alpha-1 antitrypsin deficiency or Wilson's disease). In addition controls were ineligible if they had a history of traumatic brain injury, subarachnoid haemorrhage or an intracranial intervention which may have hypopituitarism as a consequence. Controls were screened for GHD by clinical history and examination.

Patient No.	Diagnosis	No of hormone deficiencies in addition to GHD
1	Macroprolactinoma	2
2	Idiopathic Hypopituitarism	2
3	Meningioma, craniotomy, DXT	3
4	Mid brain tumour, DXT	0
5	Macroprolactinoma, DXT	3
6	SAH	0
7	Macroadenoma, TSS	0
8	Frontal Oligoastrocytoma, craniotomy, DXT	1
9	SAH	0
10	Cushing's TSS	0
11	Macroadenoma,	0
12	SAH	0
13	TBI	1
14	Macroadenoma	0
15	Pineal germinoma, DXT	1
16	Macroprolactinoma, DXT	3
17	Macroadenoma, TSS DXT	3
18	Macroprolactinoma	1
19	Macroadenoma, TSS	0
20	Empty sella	0
21	Pituitary apoplexy, TSS	1
22	SAH	0
23	Cushing's, TSS, DXT	2
24	Suprasellar arachnoid cyst	2
25	Pituitary apoplexy	2
26	Intrasellar meningioma, craniotomy	2
27	TSHoma, TSS, radioiodine	0
28	Macroprolactinoma	3

Table 3.1 *Original diagnoses, management and number of associated pituitary hormone deficits in 28 patients with growth hormone deficiency. SAH – subarachnoid haemorrhage, TBI traumatic brain injury, DXT radiotherapy, TSS transphenoidal surgery.*

Of the eight women in the growth hormone deficient group all except two were pre-menopausal, of which two women were taking hormone replacement therapy. In the control group three women were post menopausal. Seven patients and two controls were taking statins for hypercholesterolaemia, whilst seven patients in each group were on anti-hypertensive medications, none of which would be expected to affect liver fat. One patient in the growth hormone deficient group was taking metformin for type 2 diabetes and remained on the same dose. Two patients in the GHD group were taking anti-convulsants. Six patients with GHD were also taking hydrocortisone replacement. Following an overnight fast, patients had blood drawn for IGF-1, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG), glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltranspeptidase (GGT).

Weight, percentage body fat and visceral fat rating was measured to the nearest 0.1 kg wearing light clothing on TANITA scales (Tanita BC420, Dolby Medical, Stirling, UK) and height measured to the nearest 0.5 cm.

MRI methods

MRI methods were as previously described in chapter 2.

Statistical analysis

As this was an exploratory study, no power calculation was undertaken. By the same rationale, no correction for multiple comparisons has been undertaken and as such results should be considered as hypothesis generating rather than conclusive. Normally distributed variables are presented as mean and standard deviation and

non-normally distributed variables as median and inter-quartile range. Categorical variables are presented as counts. Where the relevant assumptions were met, the independent samples t-test was used to compare the healthy control and GHD groups on continuous demographic variables. Where natural log transformation was unsuccessful in achieving normality, the Mann-Whitney test was used. For categorical variables the chi-squared test was used to compare the healthy control and GHD groups. ANCOVA was used to compare IHCL between the healthy control and GHD groups in order correct for potentially confounding differences between groups, despite mean matching.

For those patients measured before and after GHR, paired t-tests were used to determine the size of the difference between time points, where the relevant assumptions were met. Otherwise the Wilcoxon matched-pairs test was used. For all parametric statistical tests undertaken an estimate of the difference between groups with corresponding confidence interval is provided. Where variables have been log transformed this 'difference' represents the ratio of geometric means. Correlation analyses were performed using Spearman's non-parametric rank correlation coefficient. Calculations were performed using Stata Statistical software (Stata Statistical Software: Release 12. College Station, TX: StataCorp LP.) and GraphPad Prism (version 5.0 for Windows; GraphPad Software, San Diego, CA). $P<0.05$ (two-tailed) was considered statistically significant.

Results:

When comparing GHD patients and controls, differences in age, BMI, gender and waist circumference were sufficiently small to be able to consider them comparable (see Table 3.2). As expected, IGF-1 was significantly lower in GHD; however no differences were noted in ALT, AST, GGT, or plasma lipid measurements. Alcohol intake was greater in the control group (median eleven units, controls vs. three units, patients, p=0.01), however there was no association between alcohol intake and liver fat in this group (R=0.24, p=0.27). There were no differences between symptomatic GHD patients (SGHD) and asymptomatic patients (AGHD) in any measurements, including IGF-1 (SGHD 12 (0-29) nmol/l vs. AGHD 11 (7-36) nmol/l; p=0.77) and peak GH(SGHD 1.53 (0-3.0) µg/l vs. AGHD 1.09 (0-2.33); p=0.82). All patients with GHD were therefore analysed as a single group. There was no correlation between severity of GHD (as measured by number of associated pituitary hormone defects) and the level of IHCL, IMCL or subcutaneous adipose tissue. There was a positive association between the number of associated pituitary hormone defects and quantity of visceral adipose tissue (r=0.4, p=0.03).

Measurement	Controls (n=24)	GHD (n=28)	Difference (95% CI)	p value
Age (years)	52.6 (12.0)	52.6 (14.0)	-0.1 (-7.5 to 7.4)	0.99
BMI (kg/m^2)	27.9 (25.1, 32.1)	27.8 (24.7, 34.7)	-	0.93
Male: female	16:8	20:8	-	0.71
Waist circumference (cm)†	101 (94, 112)	101 (93, 115)	0.99 (0.92 to 1.06)	0.70
Hip circumference (cm)†	105 (100, 114)	101 (97, 117)	1.0 (0.95 to 1.08)	0.61
AGHDA	-	14 (5.5, 19.5)	-	-
Peak GH (µg/l)	-	1.1 (0.2, 2.3)	-	-
IGF-1 (nmol/l)	18.0 (14.0, 23.0)	11.5 (9.0, 16.5)	-	0.0007
Random GH (µg/l)	0.11 (0.06, 0.26)	0.15 (0.06, 0.40)	-	0.68
Fasting glucose (mmol/l))	5.0 (0.6)	4.8 (0.7)	0.2 (-0.2 to 0.5)	0.41
ALT (u/l)	26.0 (24.0, 35.0)	24.5 (20.0, 31.5)	-	0.35
AST (u/l)†	27.5 (21.5, 31.5)	25.0 (21.0, 35.0)	1.06 (0.85 to 1.31)	0.61
GGT (u/l)†	32 (24, 45)	25 (18, 56)	1.05 (0.74 to 1.52)	0.75
Cholesterol (mmol/l)	5.6 (4.6, 6.0)	5.0 (4.7, 5.6)	-	0.38
HDL (mmol/l)	1.3 (1.2, 1.6)	1.2 (1.1, 1.4)	-	0.05
LDL (mmol/l)	3.4 (2.9, 4.0)	3.0 (2.7, 3.5)	-	0.19
Triglycerides (mmol/l)	1.2 (0.9, 1.5)	1.4 (1.1, 1.9)	-	0.11
IHCL (% CH_2/water)†	5.0 (1.5, 12.7)	2.8 (1.3, 8.6)	1.17 (0.49 to 2.78)	0.72
IMCL-Sol (CH_2/creatine)†	11.5 (6.6, 15.8)	12.3 (6.4, 18.4)	0.97 (0.70 to 1.36)	0.87
IMCL–TA (CH_2/creatine)†	6.9 (5.3, 15.0)	6.9 (2.0, 32.7)	0.78 (0.50 to 1.22)	0.27
Abdominal SAT (l)	5.8 (4.3, 7.4)	5.7 (4.6, 11.8)	-	0.78
Abdominal VAT (l)†	3.6 (3.0, 5.8)	5.8 (4.3, 7.4)	0.75 (0.57 to 0.99)	0.04

Table 3.2 - *Baseline characteristics of control subjects and GHD patients. Results presented as median and range. †Variables analysed after logarithmic transformation.*

Abdominal visceral and abdominal subcutaneous adipose tissue: VAT was significantly greater in GHD than in controls despite there being no significant difference in SAT. There was a correlation between VAT and IHCL and IMCL-TA but not IMCL-Sol in controls (table 3.3), whilst in GHD only IMCL-TA was correlated with VAT.

Intrahepatocellular lipid: IHCL was correlated with BMI in both the patient and control groups (Figure 3.2), ANCOVA was used to compare liver fat between the two groups whilst correcting for BMI in order to robustly correct for small differences between the groups. In this analysis the ratio of geometric means was 0.81 (95% CI: 0.38, 1.72), p=0.72 indicating that it was not possible to demonstrate a difference between the two groups even after correction for BMI. There was no significant difference in the proportion of patients with IHCL > 5.6% in each group (GHD 32%, controls 50%, difference 19% (95% CI: -45%, 7%) p=0.16), and no significant difference in IHCL after adjustment for the covariates of age, gender and BMI. (p=0.35). There was no correlation between IHCL and age or gender in either group. IHCL was negatively correlated with IGF-1 in the control group (r=-0.50, p=0.02) but not the patient group.

Intramyocellular lipid: There was no significant difference between the groups in IMCL in either TA or soleus muscles (p= 0.3 (TA); p=0.9(Sol)). IMCL increased with age with the exception of IMCL-TA in GHD. IMCL-TA was positively correlated with VAT in both groups, but not with BMI. There was no association between IMCL and IHCL in either group.

Hepatic enzymes: IHCL was associated with ALT and GGT in both controls and patients (Table 3.3). In addition ALT was positively correlated with VAT in patients but not controls, and GGT was inversely associated with IGF-1 in both groups (controls r=-0.65 p=0.001; GHD r=-0.65, p=0.001)

CONTROLS	IHCL			IMCL - TA			IMCL - Sol		
	n	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*
Clinical									
Age (years)	24	0.119	0.607	23	0.531	**0.009***	23	0.590	**0.003***
Gender	24	0.139	0.5	23	-0.05	0.829	23	-0.032	0.886
BMI (kg/m^2)	24	0.608	**0.003***	23	0.356	0.1	23	0.176	0.422
Waist circumference (cm)	24	0.464	**0.03***	23	0.445	**0.03***	23	0.241	0.268
Biochemical									
ALT (u/l)	22	0.455	**0.05***	21	0.026	0.911	21	0.006	0.980
AST (u/l)	24	-0.31	0.884	23	-0.099	0.652	23	-0.03	0.884
GGT (u/l)	22	0.533	**0.015***	21	-0.183	0.428	21	0.294	0.196
Fasting glucose (mmol/l)	24	0.237	0.302	23	0.101	0.647	24	0.002	0.991
Total Cholesterol (mmol/l	23	0.3	0.199	22	-0.118	0.6	22	-0.144	0.522
Fasting triglyceride (mmol/l)	23	0.304	0.2	22	-0.039	0.862	23	-0.025	0.912
HDL (mmol/l)	23	0.287	0.2	22	0.088	0.7	23	0.003	0.990
LDL (mmol/l)	23	0.122	0.6	21	-0.110	0.627	23	-0.172	0.444
IGF-1 (nmol/l)	23	-0.504	**0.02***	22	-0.259	0.244	22	-0.260	0.243
Body composition									
Abdominal SAT (l)	22	0.216	0.4	22	0.06	0.793	22	-0.04	0.859
Abdominal VAT (l)	22	0.677	**0.001***	22	0.580	**0.005***	22	0.300	0.175

Table 3.3 - *Association of clinical, biochemical and MRI parameters with IHCL, IMCL Soleus and IMCL Tibialis anterior in 24 controls. *p value <0.05*

GHD	**IHCL**			**IMCL - TA**			**IMCL - Sol**		
	n	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*
Clinical									
Age (years)	28	-0.12	0.52	27	0.119	0.555	27	0.680	**<0.001***
Gender	28	0.03	0.88	27	0.062	0.757	27	-0.187	0.349
BMI (kg/m^2)	28	0.48	**0.01***	27	0.216	0.279	27	-0.332	0.09
Waist circumference (cm)	27	0.46	**0.02***	26	0.305	0.130	26	-0.163	0.425
Biochemical									
ALT (u/l)	28	0.52	**0.004***	27	0.000	0.999	27	0.1	0.625
AST (u/l)	22	0.21	0.34	21	-0.104	0.655	21	0.073	0.753
GGT (u/l)	23	0.45	**0.03***	22	-0.018	0.936	22	-0.019	0.932
Fasting glucose (mmol/l)	27	0.5	**0.008***	26	0.453	**0.02***	26	0.108	0.599
Total Cholesterol (mmol/l	28	-0.22	0.24	27	0.399	0.169	27	0.119	0.555
Fasting triglyceride (mmol/l)	25	0.06	0.78	24	-0.150	0.485	24	-0.068	0.751
HDL (mmol/l)	28	0.05	0.78	27	-0.274	0.167	27	-0.096	0.633
LDL (mmol/l)	23	-0.08	0.97	22	0.140	0.536	22	-0.013	0.954
IGF-1 (nmol/l)	28	-0.26	0.18	27	-0.319	0.104	27	-0.171	0.393
Body composition									
Abdominal SAT (l)	28	0.370	**0.05***	25	0.301	0.127	27	-0.402	**0.04***
Abdominal VAT (l)	28	0.184	0.34	25	0.395	**0.041***	27	0.026	0.899

Table 3.4- *Association of clinical, biochemical and MRI parameters with IHCL, IMCL Soleus and IMCL Tibialis anterior in 28 patients with GHD. * p value <0.05*

Lipid profiles and fasting glucose: There was no significant difference between total cholesterol, HDL or LDL between the groups. No components of the lipid profile had any significant association with IHCL in either group; however fasting TG was negatively correlated with IGF-1 in GHD. There was no difference in fasting glucose between patients and controls, although there was a positive correlation with age in both groups (controls r=0.46, p=0.02; GHD r= 0.46, p=0.02). Whilst there was no association between IHCL and fasting glucose in controls, there was a positive correlation in GHD (see table 3.3).

IGF-1: In controls IGF-1 was associated with all markers of adiposity (waist circumference, BMI, VAT and SAT, the correlation being most striking for VAT (r =-0.615, p=0.035). IGF-1 was inversely correlated with IHCL in controls, even after correction for age and VAT (r= -0.504, p=0.028). In GHD there was no association between IHCL and IGF-1 levels, or with markers of adiposity (BMI, waist circumference, VAT or SAT).

Effect of rGH: Results are summarised in Table 3.4 and Figure 3.2. Twelve patients completed six months of rGH treatment, of whom four had NAFLD (liver fat >5.6%). Both VAT and SAT reduced in response to treatment, with the VAT: SAT ratio also reducing significantly (p=0.02), indicating a proportionately greater reduction in visceral fat. There was no overall change in IHCL (Pre GH 2.7% (0.2 – 44.8%) Post GH3.2% (0.1 – 20.6%)) (p=0.1). However, subdividing the patient group into those with high (n=4) and low (n=8) baseline liver fat (defined as > or <5.6% liver fat), reductions in liver fat were seen in the high liver fat group treated

with rGH (Figure 3.1b) however this was not statistically significant (p=0.07). The magnitude of change in IHCL was positively correlated with the baseline IHCL (r= 0.906, p=<0.001), thus patients with the highest higher initial liver fat demonstrated the greatest improvement (Figure 3.2c).

Parameter	Pre GH	Post GH	Difference(95% CI)	p value
Weight	75.4 (69.5, 96.4)	77.7 (69.4, 98.4)	0.4 (-1.9 to 1.1)	0.58
BMI (kg/m^2)	28.9 (24.6, 33.0)	30.0 (25.6, 33.2)	0.2 (-0.4 to 0.8)	0.54
IGF-1(nmol/l)	12 (9, 12)	25 (21, 29)	13 (9 to 17)	<0.00005
ALT (u/l)	21 (17, 34)	28 (14, 32)	1 (-13 to 15)	0.88
AST (u/l)	24 (22, 37)	23 (20, 26)	2 (-12 to 16)	0.75
GGT (u/l)	29 (20, 56)	33 (20, 45)	-	0.06
Cholesterol (mmol/l)	5.3 (4.7, 5.7)	5.0 (4.7, 5.6)	-0.2 (-0.6 to 0.2)	0.26
LDL (mmol/l)	3.3 (2.6, 4.4)	3.1 (2.7, 3.8)	-0.2 (-0.9 to 0.6)	0.65
HDL (mmol/l)	1.15 (1.0, 1.4)	1.3 (0.9, 1.4)	-	0.65
TG (mmol/l)	1.6 (1.4, 1.9)	1.6 (1.3, 2.5)	-	0.80
Glucose (mmol/l)	4.8 (4.4, 5.3)	5.0 (4.5, 5.4)	0.24 (-0.06 to 0.53)	0.10
IHCL (% CH_2/water)	2.8 (0.6, 14.7)	3.2 (0.8, 9.2)	-	0.10
IMCL TA (CH_2/creatinine)	7.0 (6.2, 9.5)	7.4 (6.9, 11.5)	1.0 (-2.4 to 4.6)	0.51
IMCL Sol (CH_2/creatinine)	10.2 (6.0, 16.6)	12.3 (8.9, 16.1)	0.9 (-3.2 to 5.1)	0.63
Abdominal VAT (l)	5.6 (4.2, 6.7)	4.4 (3.2, 5.1)	-1.5 (-2.3, -0.62)	0.0027
Abdominal SAT (l)	6.2 (4.3, 11.9)	6.8 (3.8, 11.2)	-0.6 (-0.9 to -0.2)	0.0026
VAT:SAT ratio	0.8 (0.6, 1.3)	0.71 (0.4, 1.0)	-	0.02

Table 3.5 - *Anthropometric, biochemical and MRI derived parameters pre and 6 months post GH replacement in 12 patients (5 female) with GHD.*

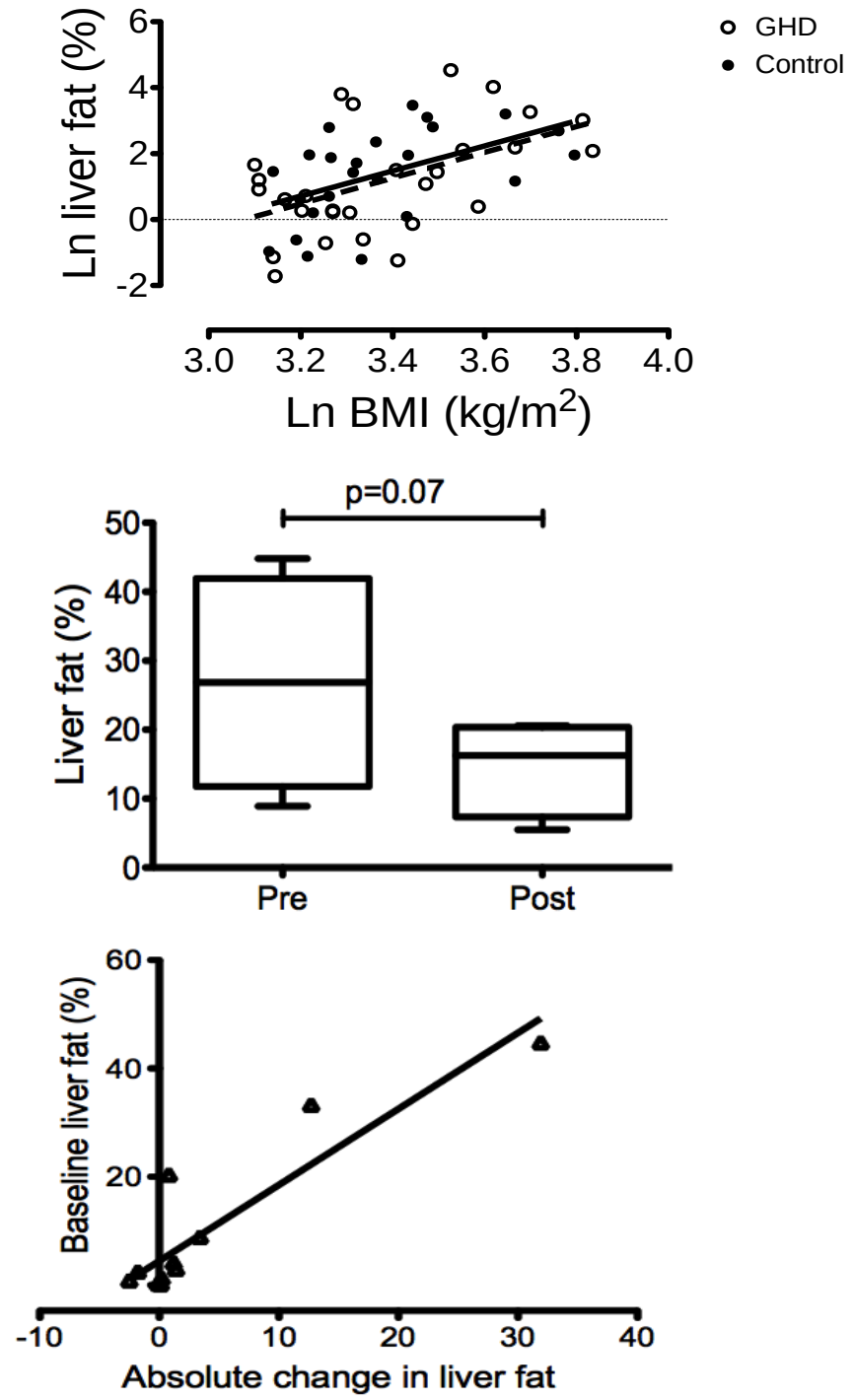


Figure 3.2 ***A.*** *Relationship between liver fat (logarithmically transformed) and body mass index in GHD patients and healthy controls demonstrating no difference in liver fat between patients and controls;* ***B****. Box and whisker plot demonstrating reduction in liver fat following 6 months rGH in 4 patients with elevated liver fat at baseline.* ***C****. Scatterplot demonstrating the correlation between baseline liver fat and absolute change in liver fat (r=0.906;p=<0.001).*

Correlation between Bio impedance and MRI derived measurements.

Clinical characteristics

Altogether 23 patients and 24 controls were entered into this comparison. Smaller numbers were due to unavailability of the bio impedance equipment at the time of some participant's attendance. Date for these subgroups are therefore presented separately. Groups were well matched for gender, age and BMI. There were 17 males in the patient group and 18 males in the control group. Median (range) age of patients was 53.08 (23,77) years and controls 54.5 (25,77) years. Median BMI was 27.5 (22.2,46.3) kg/m^2 and 27.9 (22.9,44.5) kg/m^2 for controls. Results of both Tanita analysis and MRI analysis are shown in table 3.6. Graphical illustrations of clinical correlations are shown in Figure 3.3.

Measurement	Patients (n=23)	Controls (n=24)	p value
Total Body Fat Tanita (%)	27.3(18.2,51.7)	28.1(16.7,52.6)	0.9
Visceral fat Tanita (units)	12(4,33)	12(3,35)	0.7
Abdominal Subcutaneous Fat MRI (litres)	6(3,23)	6(3,17)	0.8
Abdominal Visceral Fat MRI (litres)	5.2(2.6,10.7)	3.5(1.6,14.2)	0.07

Table 3.6 - *Body composition measurements on 23 patients and 24 controls demonstrating no significant difference between the 2 cohorts.*

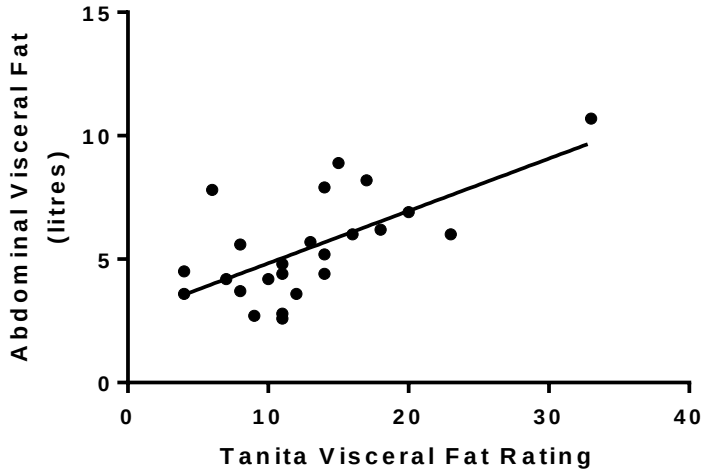
Correlations between MRI and TANITA data

Controls: MRI measured abdominal visceral fat correlated strongly with Tanita visceral fat rating (Figure 3.1B R=0.83 p=<0.0001). In regression analysis age, BMI and gender were not significant. R^2 for regression model 0.83.

GHD pre treatment: MRI measured abdominal visceral fat correlated significantly with Tanita visceral fat rating (Figure 3.3A R=0.67 p=0.001). In Regression analysis BMI was the most significant predictor of visceral fat. Age, gender and visceral fat rating were not significant after correction for BMI. R^2 for regression model 0.73.

GHD post treatment: MRI measured abdominal visceral fat correlated with Tanita visceral fat rating (R=0.64, p=0.034). In regression analysis Tanita visceral fat was the most significant predictor. Age, BMI and gender were non significant. R^2 for regression model 0.582.

A



B

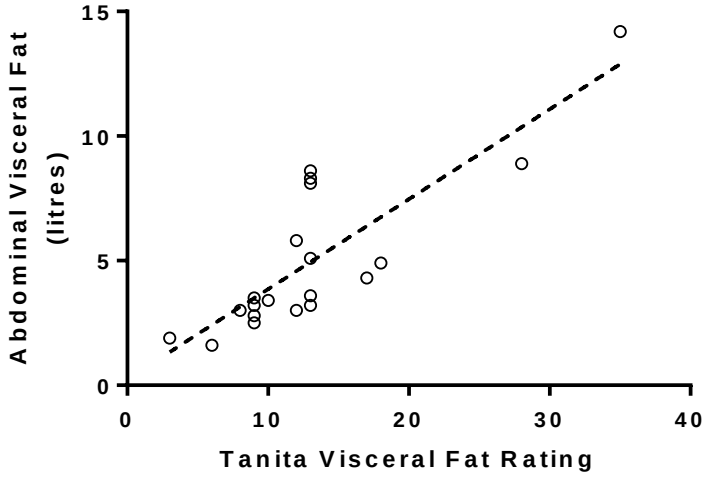


Figure 3.3 - *Clinical correlation of Visceral fat rating ascertained by Tanita scales vs. MRI estimation of abdominal visceral fat in patients with GHD (Figure A R=0.67) and normal controls (Figure B R=0.83).*

Discussion

This study reveals several interesting and novel observations. Firstly, despite higher VAT in GHD patients compared with age, gender and BMI matched controls; there was no difference in the liver or skeletal muscle fat content between the two groups, neither was there any difference in the proportion of people with hepatic steatosis in either group. This finding does not support previous observations of an increased prevalence of NAFLD in patients with GHD. Secondly, these data provide an important insight into regulation of fat deposition by GH with a differential effect of GH on the various fat depots. Clearly the most significant effect was the reduction by GH treatment on visceral (versus subcutaneous) fat. Whilst GHD patients with hepatic steatosis experienced a reduction in liver fat content following treatment with rGH, this did not attain statistical significance ($p=0.07$), most likely due to the small number of patients with elevated liver fat in this group. This differential effect has led to a poorer correlation of bio impedance with MRI measurements in patients with GHD.

We deliberately included a range of BMI and ages to make our study group representative of patients with GHD encountered in clinical practice. The BMI of our patients at 27.8kg/m^2 was similar to the range of BMI (28.5 – 29.9kg/m^2) seen in the GHD patients in a recent analysis of the KIMS database (Pfizer International Metabolic Database)(108). Furthermore our study group reflects a range of pituitary pathologies, which is a good representation of the average clinic population. We therefore believe we have obtained a representative sample of the GHD population. Despite the reductions seen in IGF-1 with obesity, our GHD patients had

significantly lower IGF-1 levels than our control group, reflecting the pituitary rather than hepatic origin of the IGF-1 deficiency in this group.

By using ^{1}H-MRS rather than modalities such as CT (measuring liver to spleen attenuation ratio) or ultrasonography which are able to assess steatosis semi-quantitatively(337), we were able to quantify steatosis very precisely. Many previous studies used liver transaminases, as a surrogate marker of NAFLD; these may be normal in up to 80% of cases(338). In contrast, we can be certain of identifying all cases of NAFLD within both of our study groups. The elevated VAT in this population is consistent with the previously reported phenotype of patients with GHD, having a higher ratio of visceral to subcutaneous fat compared with controls matched for BMI. Furthermore, in those patients given GHR, improvements in body composition were seen with significant reductions in both subcutaneous and visceral fat in keeping with recent data from Eggar *et al*(339) and the recent meta analysis by Hazem *et al(340)* .

Our primary outcome measure was IHCL. Our finding that hepatic steatosis is equally common in our cohort of patients with GHD and controls is in contrast to a number of previous studies, all of which have significant limitations. Ichikawa *et al* found an increased incidence of NAFLD (7/13 vs. 0/5) in 18 lean patients with hypopituitarism, but with and without GHD(298). The two groups had similar BMI, but neither group of patients was overweight. Furthermore diagnosis was made by liver to spleen attenuation ratio using CT, a much less sensitive technique than ^{1}H-MRS. Adams *et al* also examined the link between NAFLD and GHD(297), however the retrospective methodology and lack of a control group means that no comment

could be made on the relative prevalence of NAFLD. Longitudinal analysis of these patients documented rapid weight gain with deterioration of lipid profiles after diagnosis, with NAFLD subsequently being diagnosed a median of 3 years after the hypothalamo-pituitary insult. These authors speculated that the cause and severity of NAFLD in GHD might be related to the rapid weight gain after diagnosis, which has been shown to be a feature in these patients(108, 297) rather than the GHD itself. Nyenwe *et al* examined patients with hypopituitarism retrospectively for features of the metabolic syndrome and compared with controls matched for age, obesity, gender and race(305). They found greater elevations of serum transaminases in patients with hypopituitarism and untreated GHD, although the overall number of abnormalities was not significantly different in the two groups (elevated AST 37% GHD vs. 34% controls). Finally, Hong *et al* examined 40 men with hypopituitarism with abdominal ultrasonography and compared with 34 age matched controls, finding a higher prevalence of NAFLD in hypopituitary patients (32.5% controls vs. 70.6% patients.)(341).The hypopituitary patients however had a significantly higher BMI than controls.

We demonstrated an inverse association between IGF-1 and IHCL in our control group but not in GHD. A similar association was found by Arturi *et al(342),* Stavastano *et al(337),* and by Bredella *et al* in premenopausal women using methodologies similar to our own, although in that study the association was non-significant after correction for age and visceral adiposity(296), while in our group the association remained after correcting for these variables. They also demonstrated that peak GH in the combined growth hormone releasing hormone/arginine stimulation test was inversely associated with IHCL in premenopausal obese women without

GHD(296). In regression analysis GH was found to be a significant predictor of IHCL after correction for age and visceral adiposity; however the cross sectional nature of this study means that causality cannot be inferred. Volzke *et al* found an inverse association between IGF-1 and severity of hepatic steatosis using a 4 point scale in a large cohort of 3863 subjects(343). They likewise were unable to elucidate the cause and effect relationship of IGF-1 and liver disease. Ichikawa *et al* demonstrated an association between low IGF-1 levels and fibrosis in patients with NAFLD(344), and an association between low GH levels and steatosis in the same cohort. Lonardo *et al* demonstrated that low levels of GH were an independent predictor of NAFLD in male patients(295). Despite a significantly lower IGF-1 in our GHD patients, neither incidence of NAFLD nor median IHCL were significantly different. However it is possible that blunted GH release existed in our control population as a result of their obesity.

We did not find an overall reduction in IHCL following treatment with rGH. In statistical terms this may be due to the lack of a significant change in IHCL in the majority of patients who had normal IHCL, but this masks reductions in steatosis in those individuals with elevated IHCL. Given the responsiveness of liver fat to hormone replacement demonstrated by our group in a patient after only 6 weeks of treatment(293), it would be anticipated that 6 months would be an appropriate length of time for some changes to be seen. It would thus appear that there is a differential regulation of the different fat depots (visceral, subcutaneous and liver fat depots) by the GH/IGF-1 axis with more critical involvement in the regulation of VAT and SAT mass compared with liver fat, which may primarily be regulated by other physiological mechanisms. The reduction in VAT:SAT ratio also suggests a

preferential reduction in VAT rather than SAT, independent of BMI suggesting that GH makes patients more metabolically healthy. The important correlation between baseline liver fat and the reduction in liver fat is worthy of further study to ascertain whether GH may be beneficial in patients with elevated IHCL although to answer this question, larger and appropriately powered randomized-controlled studies involving NAFLD patients, both with and without GHD, will be required.

We demonstrated that Tanita analysis of visceral fat correlated significantly in all groups. It is clear however that this correlation is lower in patients with GHD , and that BMI was more closely correlated with the visceral fat in this group. Looking at the group of patients who had undergone 6 months of growth hormone replacement the correlation was improved, however it is clear that they had not achieved body composition similar to healthy controls.

The finding of poorer correlation of bio impedance data with MRI generated measurements is unsurprising given the effects of GHD on body composition with increased fat mass, lower lean body mass (345) and reductions in extracellular fluid volume all being documented(155, 320). The differential effect of GHR on different fat depots will also confound accurate bio impedance analysis. It has been shown that in untreated GHD whole body resistance is abnormally high, rapidly reducing on low doses of growth hormone replacement(154). Bio impedance relies on information from transmission through all of these tissues and alterations in extracellular and intracellular fluid volumes are known to affect readings, as has been seen previously in subjects suffering dehydration after vigorous exercise(346). Tanita devices not only measure bio impedance but also interpret this information using algorithms

based on BMI, gender and age. Normative data for such algorithms are likely to be based on normally hydrated participants.

Thomas *et al* investigated the link between intra abdominal adipose tissue on MRI and visceral fat as estimated by the newer Tanita technology, the Viscan. In addition to multi-frequency bio impedance this incorporates estimation of abdominal circumference together with bio impedance analysis particularly of the abdominal area. They demonstrated a strong correlation between MRI derived values and TANITA generated estimates. They did however notice poorer correlations in obese individuals, particularly those with increased abdominal subcutaneous fat. They concluded that whilst this equipment was a useful screening tool for population analysis of total abdominal fat, prediction of abdominal visceral fat might be limited.

This study has some weaknesses. The relatively small numbers of patients, particularly in the intervention arm of this pilot study, mean that conclusions cannot be drawn about the effects of rGH on liver fat in NAFLD patients, and that population estimates of prevalence cannot be performed. We did find a marginally higher alcohol intake in our control population, however the absence of any association between liver fat and alcohol intake means that this finding is unlikely to be of clinical significance. All participants drank within the safe recommended limits(329). There was, as would be expected in a general clinic population, a varying length of time between diagnosis of GHD and inclusion in the study. The minimum period was 12 months, however this short period of time between diagnosis and testing in many patients may result in lower levels of IHCL compared with others who have had untreated GHD for a longer period, or those controls who

have been obese for many years.

We did not perform liver biopsies in our patients meaning that it was not possible to differentiate between simple steatosis and the more severe steato-hepatitis. We were also therefore unable to demonstrate potential benefits in terms of incidence of or improvement in Non Alcoholic Steato-hepatitis which has been reported previously(300), or in hepatic fibrosis, which can paradoxically result in lower IHCL despite severe liver disease. Future studies will need to address this issue, possibly with the inclusion of transient elastography, clinical fibrosis scores or both(347). Finally we accept that whilst this study is aimed to be a proof of concept exploratory study techniques such as MRS are unlikely to be helpful in many healthcare settings , where this is not routinely available.

In conclusion, we have demonstrated that there is no significant difference in the proportion of people with NAFLD in our patients with GHD compared with controls, despite the increased visceral fat volume in patients with GHD. Six months of rGH appears to demonstrate encouraging results in reducing liver fat in NAFLD patients with significant steatosis however this reduction is less pronounced when compared with the marked reduction observed in visceral and subcutaneous fat. We have demonstrated good correlation of bio impedance data predicting abdominal visceral fat with MRI derived data in normal controls. Whilst we have confirmed changes in abdominal visceral fat on MRI scanning in patients with GHD, we have also demonstrated that this is not translated into changes in visceral fat on Tanita analysis. This is likely to be due to changes in bio impedance in patients with GHDcaused by reduced extracellular fluid volume and reduced lean muscle mass, which are not compensated for by the standard Tanita algorithm. As such Tanita bio impedance

analysis cannot be used for body composition analysis of abdominal visceral fat in patients with GHD.

Chapter 4

Glucagon Stimulation Testing in Health and Disease, Validation of methodology and correlations with Body Mass Index

Introduction

GHD and hypopituitarism are consequences of many pituitary conditions and have been postulated to be common following Subarachnoid Haemorrhage(96). It is important to recognise these complications due to clear benefits of replacement on health and quality of life. Diagnosis in adults however is challenging, particularly with regard to GHD. Compared with childhood GHD, where growth velocity is the ultimate evidence of adequate GH secretion, the lack of an auxiological end point means that GHD in adult is based entirely on biochemical findings. IGF-1 measurement, which has been shown to be a reliable marker of GH secretion in childhood is felt to be unreliable in adult patients, and so diagnosis is made exclusively on the basis of stimulation testing.

Diagnostic uncertainty arises most frequently in the obese patient or the older patient, as GH levels are known to vary with both of these factors. Despite this however current UK and international guidance gives a single cut off value(97).

In this study we aim to validate our methodology and assay and to ensure that the internationally recognised cut off of 3 µg/L is appropriate going forward by comparing patients with known GHD with healthy volunteers. We also aim to explore the relationship between peak GH and BMI to ascertain whether this can be a confounding factor in the diagnosis of GHD in obese patients.

Methodology

Healthy volunteers were recruited, with varying BMI including volunteers with BMI>30kg/m^2. Volunteers were screened for underlying medical conditions which may either result in GHD, or interfere with the test. As such patients with a history of subarachnoid haemorrhage, traumatic brain injury, pituitary disease, long term or on-going steroid use, use of opiate medications, tricyclic antidepressant use or history of irradiation would not be eligible. Due to uncertainties about the reliability of the glucagon stimulation test in subjects with diabetes, this was also an exclusion criterion.

Following an overnight fast, patient underwent a standard glucagon stimulation test, with 1mg glucagon (1.5mg for patients weighing >90kg) given subcutaneously. Following insertion of an indwelling venous cannula, baseline blood tests for cortisol and GH were taken. Blood was drawn at 90,120,150,180 and 210 minutes for GH and cortisol. Results from this cohort were compared with historical patients from the endocrine clinic who have been diagnosed with multiple pituitary hormone defects. Since these patients have a >97% chance of being growth hormone deficient(98), it will be assumed that this group represents true GHD. A ROC curve was constructed for the two groups to determine the cut off giving maximum sensitivity and specificity.

Results

Healthy Volunteers

15 healthy volunteers were recruited. One participant was excluded however after being found to be taking Fibrates which can interfere with GH secretion. The median

(range) age of the controls was 47.5 (34,62) years with a BMI of 27.4(20.8,39) kg/m^2.

Of the 14 eligible volunteers, Median IGF-1 was 17.5(10,35) nmol/l. Peak GH was 6.46(0.56,14.9) µg/L and peak cortisol was 540(271,998) nmol/l. Of the 14 healthy controls recruited, four participants would have been classed as growth hormone deficient (peak GH<3µg/L). Three of these participants had a BMI greater than $30kg/m^2$. There was a non-significant correlation between BMI and peak GH(Spearman correlation coefficient R=-0.44, p=0.1,figure 4.1). There was no correlation between IGF-1 level and peak growth hormone.

Median cortisol level was 540(271, 998) nmol/L. There was no correlation between peak cortisol and BMI (R^2 =-0.14, p=0.62 figure 4.1). Six controls would be classed as having an inadequate cortisol response (<500 nmol/l) on the basis of the GST, with the lowest cortisol peak being 271 nmol/l. All these controls subsequently had a normal synacthen test.

Patients with Multiple Pituitary Hormone Deficiencies

33 patients with multiple pituitary hormone deficiencies who had undergone GST were identified. Median age was 45 (14, 78) years. Median BMI was 31.7 (19, 44.7) kg/m^2. Median peak GH was 0.76 (0, 3.1) µg/L. IGF-1 data was not available in this cohort.

Healthy Volunteers vs. Confirmed GHD

Using both datasets a ROC curve was constructed to ascertain the optimal GHvalue to correctly diagnose GHD . Using healthy volunteers as confirmed GHsufficient,

and patients with multiple pituitary deficiencies as confirmed growth hormone deficient. The best fit was achieved with a GH value of 3.0μg/L indicating GH sufficiency (figure 4.2). At this value the GST demonstrates an overall sensitivity of 97% and specificity of 71%. There were insufficient numbers of patients and volunteers in each group to stratify the ROC by BMI.

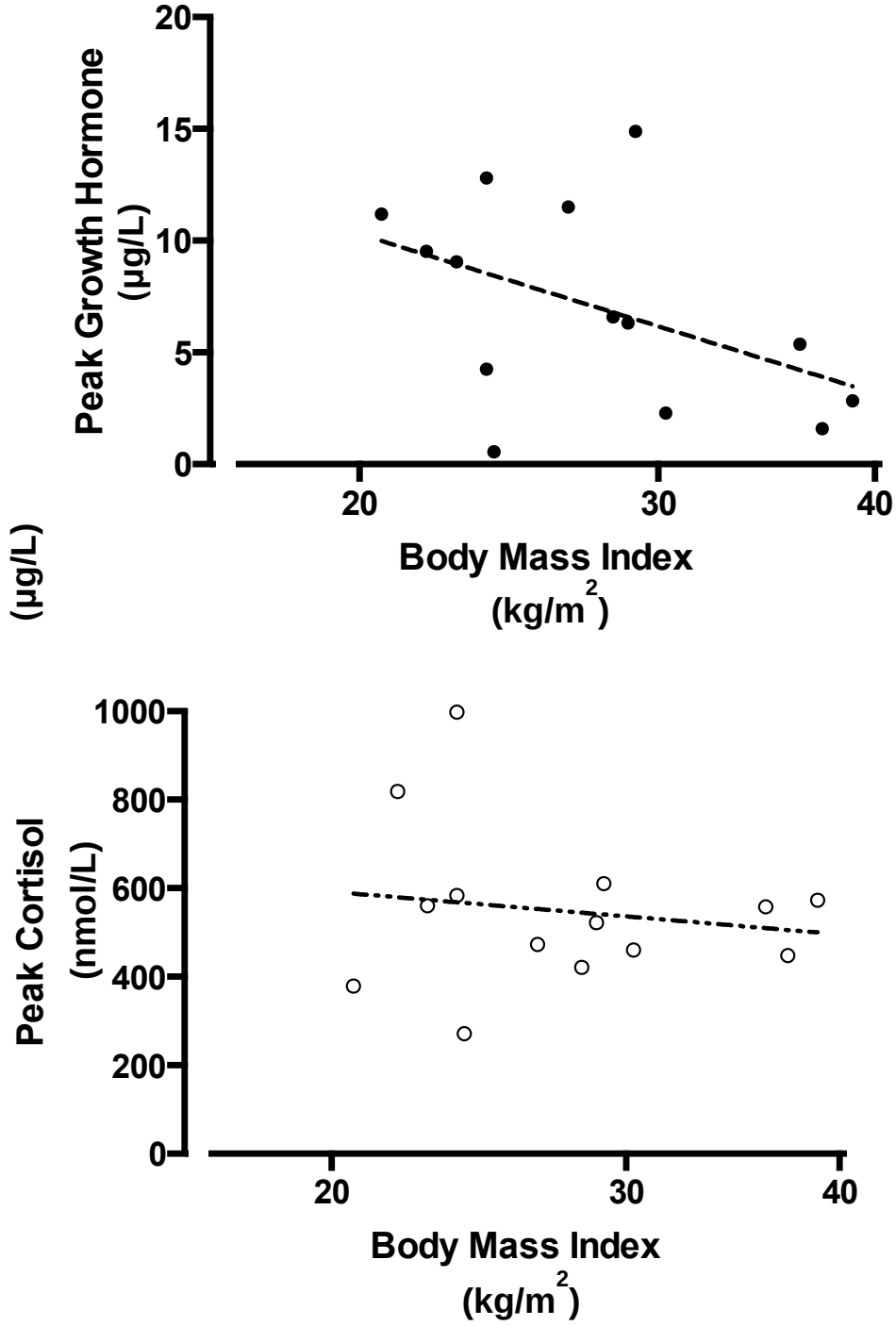


Figure 4.1 - *Peak GH (µg/L) and peak cortisol (nmol/L) plotted against BMI (logarithmically transformed). Demonstrating trend to reduction in peak GH with increasing BMI but no association between peak cortisol and BMI.*

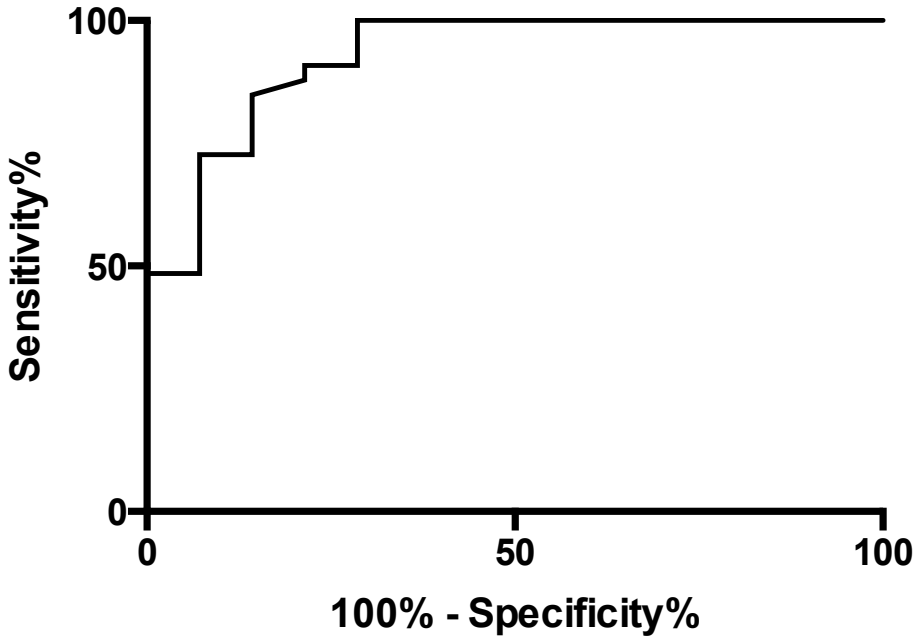


Figure 4.2 - *Receiver Operating Characteristics curve. Demonstrating sensitivity and specificity for peak GH level in patients with GHD and healthy controls undergoing the GST. Area under the curve 0.93, p<0.0001.*

Discussion

The aim of this study was firstly to ascertain whether a cut off of 3μg/L was reliable using our local methodology and assays, and secondly to explore the link between obesity and GH peak to stimulation to further understand the confounding effect of BMI on stimulation testing. We have firstly validated a cut off of 3μg/L, and secondly we have confirmed that there is an association between BMI and peak growth hormone, which is clinically significant when using this test as a mark of GH reserve.

The cut off of 3μg/L has been internationally agreed in consensus statements, however Ho *et al* have stated that local validation is required to ensure that false patients are not erroneously diagnosed with GHD(98). This was the primary reason for validating our methodology. The GST has been validated previously be other centres; Conciaco et al looking at 25 healthy volunteers vs. 33 patients with pituitary disease had validated cut off of 3 μ/L by ROC curve analysis (sensitivity 98%, specificity 88%)(102). Berg et al compared the GST to the ITT in 49 patients post pituitary surgery(101). Using a GH cut off of 3 μg/L, they noted that the GST was equally good at distinguishing GHD. ROC analysis however revealed an optimal cut-off of 2.5 μ/L with 95% sensitivity and 79% specificity for GHD using the ITT as the gold standard comparator – they did not however investigate healthy volunteers.

Furthermore Kokshoorn *et al*(348) noted that the frequency of GHD varies between studies of SAH and TBI in particular, depending on which test is used. They also documented a higher incidence of GHD in studies on TBI patients using the GST and GHRP – 6 test. They questioned whether the cut off of 3μg/L for diagnosis of GHD

is correct in the GST. Toogood *et al* however in a review of the KIMS database have reconfirmed similarity between the ITT and GST in diagnosis of GHD at a cut off of 3μg/L(349). In our study we demonstrated that the cut off of 3μg/L does provide the best discrimination between GHD and sufficiency, however whilst sensitive at 97%, it is only moderate specific 71%. This specificity is lower than Conciaco *et al*(102), however we deliberately selected volunteers with elevated BMI given that our local population has a high prevalence of obesity.

BMI is well know to have an effect on a number of stimulation tests, and has been shown previously(103, 113, 117, 120, 349-353). There has however been some disagreement as to whether, and when this is clinically important. Gomez *et al* showed that the peak GH response to glucagon is correlated with BMI in normal controls, even when the mean BMI is 25.8kg/m^2 (103), however did not find an association in patients with pituitary disease. Likewise Berg *et al* found no association between BMI and peak GH in patients following pituitary surgery(96). Tzanela *et al* however noted an association in both healthy controls and patients with pituitary disease, demonstrating that 14.3% of overweight controls and 32.1% of obese controls failed the ITT with a cut off of 3μg/L(354). Micmacher *et al* also found the GST to be less reliable in middle aged men(107).

We demonstrated an association between BMI and peak GH in our control patients, although not in our patients with pituitary disease. Indeed using a cut off of 3μg/L, 3 out of 4 of our healthy volunteers with a BMI over 30kg/m^2 would have been classified as GHD. Like Tzanela *et al*(354) we found that all these volunteers had a normal IGF-1.

With the advent of newer indications for GH stimulation testing namely SAH and TBI, new challenges are being presented in accurate diagnosis of GHD. These populations are distinct from previous groups of patients where a clear pituitary lesion is demonstrable on imaging, and patients have a high incidence of multiple deficiencies. Likewise unlike patients with primary pituitary disease, patient with TBI or SAH have a clear date of onset, and so it is possible to be clear about whether obesity was pre-existing or of new onset following the insult.

It is therefore important to consider what the best test is to be used for diagnosis of GHD in this patient group. The 2007 consensus statement on diagnosis of GHD in adults(98) stated that ITT, glucagon, GHRH Arginine, and GHRH/GHRP were all validated tests for the diagnosis of adult GHD, including TBI and SAH. They also stated that only one test was required for a diagnosis of GHD(98, 355). Given that there is evidence of post-mortem findings of hypothalamic damage in patients with SAH, it could be argued that caution should be applied in using newer combined tests, as by stimulating the pituitary directly these can be falsely normal in patients with hypothalamic damage(349). This would suggest that therefore in this patient group, either ITT or glucagon should be used. ITT however is contraindicated in patients with seizures, and cardiovascular disease. Seizures are common in this patient group and as the chance of occult cardiovascular disease increases with age, routine testing in this patient group, which tend to be aged 40 years and above, is problematic. This therefore leaves the GST as the test of choice(105).

Whilst in the GAT, normative values defined by BMI have been established (GH<11µg/l for BMI <25, <8µg/l for BMI 25 – 30 and <4µg/l for BMI >30)(356), this is not the case for the GST or the ITT. Given that the mean BMI in our clinic of

SAH patients is 28.2(Range 20.1 – 42.8) kg/m^2 this has to be a consideration. It has been shown that using weight based dosing regimens for the GST results in higher peak GH levels(113) compared to the fixed dosing regimen used in our study, however this requires a longer test as peak responses come later, and the incidence of side effects is higher. Given that age related IGF-1 SDS is becoming available this could be used, and has been shown to be reliable(107).

Ideally larger studies are required to further elucidate the GH response to glucagon in different ages and with varying BMI. Given that an AUC of 0.7 or greater signifies that the test is better than simple chance, and assuming a significance level of 0.05 and SEM 0.08, and assuming equal numbers of patients and healthy participants, a minimum of 22 healthy participants and 22 patients with multiple deficiencies would be required in each BMI stratified group. Until this point however it would seem reasonable to look to confirmatory testing in the absence of structural pituitary disease using a testing method which incorporates BMI specific cut offs.

We found a wide variability in peak cortisol in our healthy volunteers in common with other studies. Normal responses ranged from 271-998nmol/l, with 6 volunteers (40%) failing the GST with a cut off of 500nmol/L. These findings are similar to other authors, with Littley *et al(202)* finding responses 289-717, and Yuen *et al* (252-1104)(113). The breath of variation makes it difficult to find a level with both high sensitivity and specificity, and therefore patients with suboptimal cortisol responses to GST require an alternative test before being declared cortisol deficient.

In conclusion, we have confirmed that a level for GH peak of 3μg/L offers the best balance between sensitivity and specificity, however the relatively poor specificity

together with clear influence of BMI requires a confirmatory test, particularly in the absence of structural pituitary disease. The GST is a poor marker of cortisol reserve.

Chapter 5

Hypopituitarism following Subarachnoid Haemorrhage

Abstract

Objective: Hypopituitarism following subarachnoid haemorrhage (SAH) has been reported to be a frequent occurrence. However, there is considerable heterogeneity between studies with differing patient populations and treatment modalities, and most importantly employing differing endocrine protocols and (normal) reference ranges of growth hormone. We aimed to examine prospectively a cohort of SAH survivors for development of hypopituitarism post-SAH using rigorous endocrine testing and compare GH response to glucagon stimulation with a cohort of healthy controls of a similar body mass index (BMI).

Design and Methods: 64 patients were investigated for evidence of hypopituitarism three months post SAH with 50 patients tested again at 12 months. Glucagon stimulation testing (GST), with confirmation of deficiencies by GHRH/Arginine testing for GHD and short synacthen testing for ACTH deficiency, were used. Basal testing of other hormonal axes was undertaken.

Results: Mean age of patients was 53±11.7 years; mean body mass index (BMI) was 27.5±5.7 kg/m^2. After confirmatory testing the prevalence of hypopituitarism was 12% (GHD 10%, asymptomatic hypocortisolaemia 2%). There was no association between hypopituitarism and post-SAH vasospasm, presence of cerebral infarction, Fisher grade or clinical grading at presentation. There was a significant correlation between body mass index and peak GH to glucagon stimulation in both patients and controls.

Conclusions Identification of 'true' GHD after SAH requires confirmatory testing with an alternative stimulation test and application of BMI-specific cut- offs. Using such stringent criteria, we found a prevalence of hypopituitarism of 12% in our population.

Introduction

The true prevalence of anterior hypopituitarism after aneurysmal subarachnoid haemorrhage (SAH) remains debatable. A number of studies, often incorporating patients with both SAH and traumatic brain injury (TBI), have concluded that hypopituitarism is common in this patient group (264, 267, 268, 275, 357), with a systematic review by Schneider *et al* in 2007 concluding that hypopituitarism is present in 47% of patients in the chronic phase after SAH (96). In contrast Klose *et al*, evaluating 61 patients prospectively concluded that SAH was uncommon having failed to identify a single case (358). Lammert *et al* (359) have recently confirmed a prevalence of hypopituitarism lower than previously thought, although in this selected cohort not all patients underwent dynamic testing. Currently a number of endocrine societies have recommended that all patients should undergo evaluation of anterior pituitary function after SAH.(98, 360).

There are limitations to the current literature, which may explain the widely ranging prevalence figures of hypopituitarism post-SAH, with a variety of dynamic tests using differing cut-off values to determine GHD and a lack of confirmatory testing with a repeat or an alternative stimulation test to demonstrate GHD. Other confounding factors include assessments at varying time points post-injury and a failure to account for the effects of obesity on GH response to stimulation testing(118), where obese patients are more likely to receive a falsely positive diagnosis of GHD. Finally, treatment modalities have changed in recent years with most patients now treated by endovascular coiling compared with craniotomy and clipping(361), which may reduce the severity of the pituitary insult.

Accurate diagnosis is essential in this cohort as Subarachnoid haemorrhage (SAH) can be a devastating condition which can leave survivors with profound neurological disability. Even after a full neurological recovery, many survivors continue to suffer from impaired quality of life(279). Given the overlap in symptomatology between patients with SAH and hypopituitarism, interest has focused on whether these symptoms in SAH may be due to pituitary damage. If confirmed, the question then remains as to whether replacement of pituitary hormones, specifically GH may have a role to play in improvement in QOL as has been seen in patients with other pituitary diseases in which GHR has significantly improved QOL.

The aim of this study was to assess the prevalence and progression of anterior pituitary hormone deficiencies, and in particular GHD, with prospective evaluation at 3 and 12 months post-SAH, using confirmatory testing with an alternative dynamic test and accounting for the confounding effects of obesity on GH secretion. We hypothesised that by adopting such a rigorous approach, the prevalence of post-SAH hypopituitarism would be lower than previously reported.

We further report on a cohort of patients with documented severe, symptomatic GHD following SAH, and their response to GHR.

Methods

Patient recruitment

Between November 2008 and December 2009, we monitored the presentation and progress of 147 patients with a diagnosis of SAH admitted to the Walton Centre for Neurology and Neurosurgery, a tertiary neurosurgical referral centre in Liverpool, United Kingdom. The outcomes of these patients are shown in Figure 5.1. Eleven

patients (7.3%) died in the Neurological Intensive Care Unit and nine (6%) died following discharge from the hospital and prior to any endocrine follow up. The remaining 127 patients were invited to attend for endocrine follow up at University Hospital Aintree, of which 85 patients attended. 21 of these had incomplete evaluation for a variety of reasons (needle-phobia, declined consent for dynamic function testing or declined further follow up due to lack of symptoms). Thus 64 patients (50%) (prospective group) underwent complete evaluation of which three had a non-aneurysmal SAH. Fifty of these patients were evaluated both at three and 12 months post-SAH.

In order to ensure that there was no selection bias, baseline characteristics of non-participants were also recorded where available (table 5.1). The local research ethics committee approved the study.

In addition 15 patients (11 female) with a history of SAH over 12 months previously who were referred because of symptoms of chronic fatigue and poor QOL and were followed at baseline and 6 months. This included 6 patients found to have GHD who were evaluated post GHR. Clinical characteristics of these 'retrospective patients' are shown in table 5.2. Median age was 50(range 33,62) years. Median BMI was 28(range 20,45) $kg/m^{2.}$

Parameter		Participants (n=64)	Non Participants (n=63)
Age		53.7± 11.6	57.5±13.5
Body Mass Index		27.5± 5.7	-
Gender (M:F)		25:39	20:43
Fisher Grade	1	3(5%)	2(4%)
	2	12(21%)	2(4%)
	3	22(38%)	14(28%)
	4	21(36%)	32(64%)
	Unknown	6	13
World Federation of Neurological surgeons Score	1	49(78%)	27(62%)
	2	10(16%)	10(23%)
	3	0	2(5%)
	4	3(5%)	3(7.5%)
	5	1(1%)	1(2.5%)
	Unknown	1	20
Hydrocephalus		8 (12.5%)	-
Delayed Ischaemic Neurological Deficit		14(22%)	-
Aneurysm Size (n=55)	<=10mm	41	-
	11-25mm	14	-
Aneurysm Treatment (n= 64)	Endovascular Coil	60	
	Clipping	1	
	None	3 (all non-aneurysmal)	
Aneurysm Location	Middle Cerebral Artery	9	
	Basilar Artery	8	
	Posterior Communicating Artery	19	
	Anterior Communicating Artery	15	
	Pericallosal Artery	2	
	Anterior Cerebral Artery	3	
	Other site	5	

Table 5.1 - *Clinical Characteristics of 64* patients with subarachnoid haemorrhage*

*(*Fisher score unavailable in 6 patients due to transfer in from other hospitals).*

Numbers (and percentages) demonstrate the distribution of WFNS scores and Fisher scores.

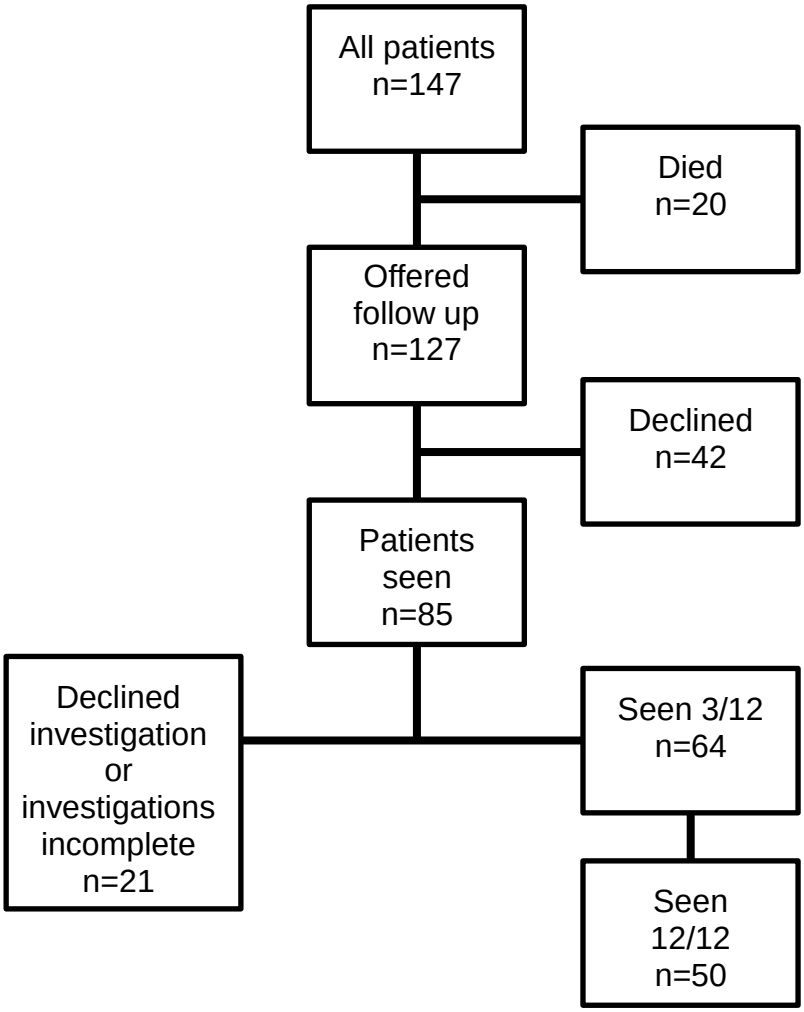


Figure 5.1 - *Patient flow diagram, demonstrating outcomes of 147 patients admitted with a diagnosis of SAH.*

Patient No.	Age	Sex	Condition	Time since insult	Treatment	WFNS grade (SAH)	Pituitary Deficiencies
1	39	F	ASAH	13 months	EVC	1	GHD
2	43	F	ASAH	14 months	EVC	1	
3	55	M	ASAH	12 months	EVC	1	
4	33	F	ASAH	30 months	EVC	1	GHD
5	47	F	ASAH	16 months	EVC	1	GHD
6	62	F	ASAH	24 months	EVC	1	GHD
7	51	M	ASAH	36 months	EVC	1	
8	35	F	ASAH	15 months	EVC	1	GHD
9	51	M	ASAH	12 months	EVC	1	
10	51	F	ASAH	11 years	Clip	1	
11	50	F	ASAH	12 months	EVC	2	
12	51	F	ASAH	7 years	EVC	1	
13	61	F	ASAH	4 years	Clip	3	
14	44	F	NASAH	4 years	None	1	
15	44	M	ASAH	12 months	EVC	1	GHD

Table 5.2 – *Clinical characteristics of 15 patients with SAH referred due to symptoms. ASAH, Aneurysmal SAH; NASAH Non aneurysmal SAH; EVC Endovascular coiling, GHD Growth Hormone Deficiency, WFNS – world federation of neurosurgeons*

Clinical protocols

Patients were assessed prospectively on two occasions, at 3 and 12 months post-SAH (or as close as possible to these time points as was practical). An investigation algorithm is shown in Figure 2. All patients underwent clinical evaluation, testing of basal pituitary hormones and dynamic function testing using the GST. All patients with biochemical evidence of cortisol insufficiency or severe GHD underwent confirmatory testing by an alternative method (short synacthen testing and GHRH + arginine test).

The 15 retrospective patients were assessed at just one time point, the time of referral to the clinic.

Anthropometric and Quality of Life (QOL) questionnaires

This was performed as described in the methodology chapter. In the retrospective cohort, treatment was offered to severely growth hormone deficient patients if their QoL-AGHDA score was ≥ 11 points, in accordance with UK guidance from the National Institute of Clinical Excellence (NICE). Growth hormone replacement was given at a dose of 0.2mg subcutaneously once daily, with monthly review and dose adjustment to achieve an IGF-1 level in the normal range.

Clinical and radiological markers of severity of SAH

Clinical severity was independently graded based on the clinical history and examination findings at presentation according to the World Federation of Neurosurgical Societies (WFNS) scoring system (362). Admission CT brain scans were reviewed by one of two radiologists who were blinded to the clinical condition of the patient and Fisher Scale recorded (316) (Table 5.1, 5.2). The number of aneurysms, size and location of aneurysm(s), treatment with inotropes and

medication use were also recorded. In addition, episodes of delayed ischaemic neurological deficit (DIND) (vasospasm) and presence of hydrocephalus were recorded. Follow up MRI scans at 6 months were reviewed for evidence of infarction.

Biochemical tests

Following a detailed history and physical examination all patients underwent basal pituitary hormone testing including IGF-I, TSH, FT4, FT3, LH, FSH, oestrodiol, testosterone and prolactin measurements and a GST for assessment of GHand cortisol reserve. The investigation protocol is shown in figure 5.2.

Glucagon Stimulation Testing

This was performed as previously described. All patients with an inadequate (<500nmol/l) cortisol response to glucagon underwent standard dose (250µg) short synacthen testing to confirm cortisol deficiency at 12 months, however patients with a peak cortisol <350nmol/l, or <500nmol/l with symptoms (such as lethargy) at 3 months underwent immediate SST – all were normal and no patient in the prospective group required hydrocortisone replacement between 3 and 12 month assessments.

GHRH/Arginine Tests

Patients with a GH response to glucagon stimulation <3µg/L underwent GAT as previously described.

Statistical analysis

Results are expressed as mean (standard deviation) unless otherwise stated. Smaller subgroup data are expressed as median(range). Data analysis was performed using SPSS 17.0 for Windows (SPSS, Chicago, IL). $p<0.05$ (two-tailed) was considered statistically significant.

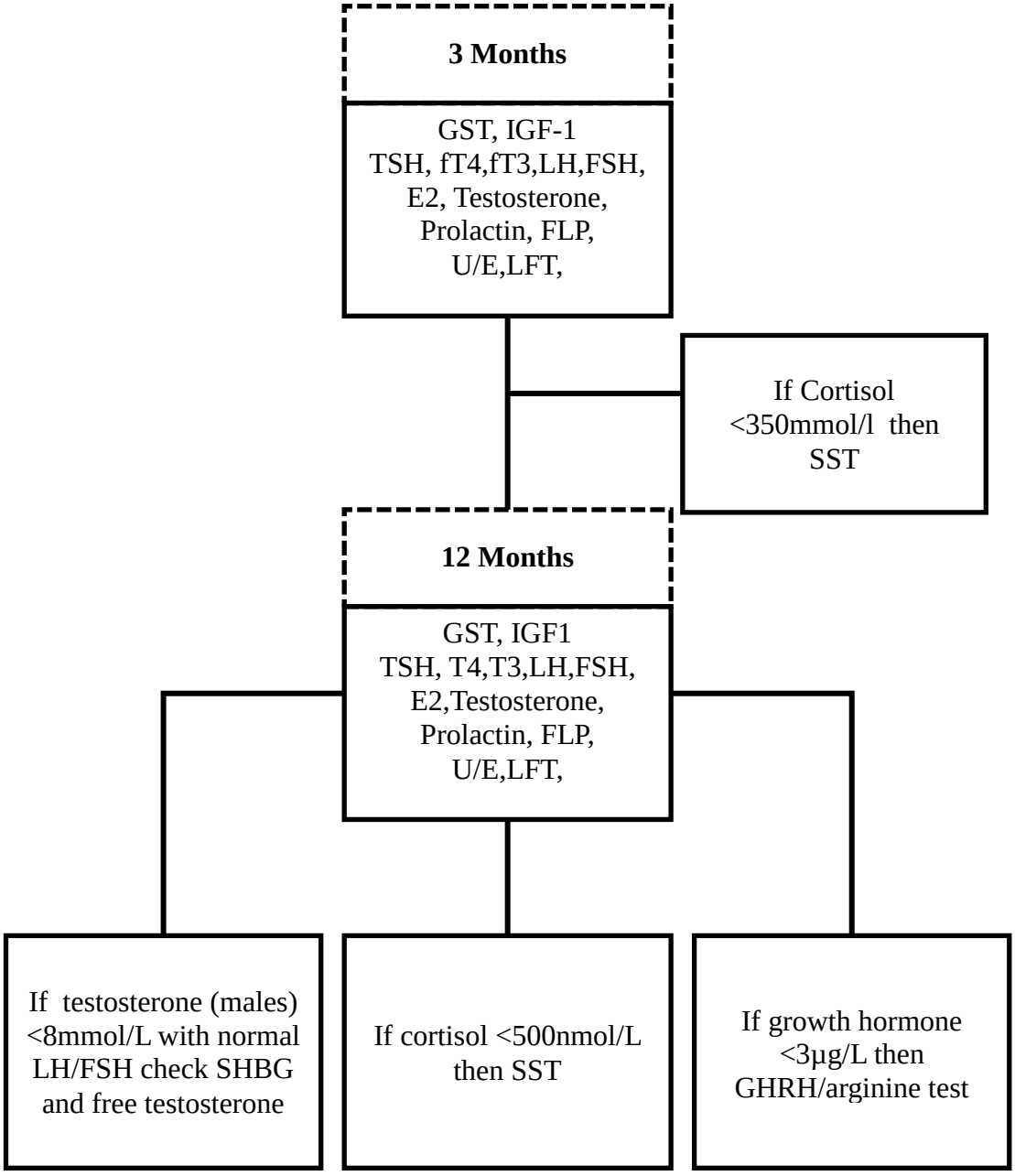


Figure 5.2 - *Investigation algorithm for hypopituitarism in 64 patients with SAH.*

GST glucagon stimulation test; TSH, thyroid stimulating hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone; E2, ethinyloestrodiol; FLP, fasting lipid profile; U/E, urea and electrolytes; LFT, liver function tests; SST short synacthen test; GHRH, growth hormone releasing hormone; SHBG, sex hormone binding globulin; IGF-1, insulin like growth factor 1, cortisol, peak cortisol on dynamic function testing.

Results – Prospective cohort

Clinical Characteristics

64 patients were clinically reviewed at three months, of which 50 completed follow up at 12 months. No patient had known neuro-endocrine disease before the pathological event. No patient was receiving glucocorticoid replacement during the course of his or her follow up. Clinical characteristics of these patients including treatment are summarised in table 5.1.

Of the 64 patients completing the initial testing, 25 (39%) were males and 39 (61%) females, mean age was 53.7±11.6 years and mean body mass index (BMI) was 27.5±5.7kg/m^2. 39 (61%) patients were either current or recent smokers. All patients underwent four-vessel angiography: in three patients however no aneurysm or arterio-venous malformation was detected. Of the 61 patients with aneurysms identified, 17 (27%) patients had more than one aneurysm. Additional surgery was required in nine cases (External Ventricular Drain insertion for hydrocephalus in eight (12.5%) patients, haematoma evacuation in one patient). 14 (22%) patients had evidence of DIND (radiological evidence only in 2 patients) during the acute recovery period. Eight patients suffered with hydrocephalus. Three patients had evidence of infarction follow up MRI scanning, frontal lobe in one patient and lacunar infarction in two other patients. Median duration of hospital stay was 12 days (range 4 to 130 days).

	Glucagon Stimulation Testing		GHRH/Arginine + synacthen testing
	3 months (n=64)	12 months (n=50)	12 months (n=50)
Hypopituitarism(any hormone)	29 (45%)	25(50%)	6(12%)
Growth Hormone Deficiency	13(20%)	11(22%)	5(10%)
Hypocortisolism	18(21%)	20(40%)	1(2%)
Hypogonadism	5(8%)	0	0
Hypothyroidism	0	0	0
Hyperprolactinaemia	0	0	0

Table 5.3 - *Prevalence of individual hormone deficiencies in 64 patients tested at 3 months and 50 patients tested at 12 months together with prevalence after confirmatory testing. Note 5 patients (8%) and 7 patients (14%) had multiple deficiencies at 3 and 12 months respectively.*

Anterior pituitary function

The results of pituitary function testing are shown in Table 5.3. Overall at 12 months the prevalence of hypopituitarism was 12% consisting of isolated GHD in 10% and asymptomatic hypocortisolaemia (not requiring treatment) in a single patient (2%). Likewise there was no change in BMI in the cohort as a whole during this time (3 months 27.4±5.7kg/m^2, 12 months 27.8±5.3kg/m^2 p=0.73).

Patients with hypopituitarism post-SAH were significantly younger, (45(27,50) vs. 54(20,77) p=0.02) however there was no difference in BMI either shortly after diagnosis or at the time of re-testing at 12 months (see table 5.4). Significantly however, hypopituitary patients had overall demonstrated weight loss, whilst other patients had marginal weight gain. No patients with hypopituitarism had hydrocephalus or required inotropes. Only one patient in the hypopituitary group had evidence of DIND. There was no association between DIND or requirement for surgery and subsequent development of hypopituitarism. The three patients with infarction all had normal pituitary function. There was no difference in the distribution of Fisher or WFNS grades between the groups.

Growth Hormone axis

There was no significant difference in the prevalence of GHD at 3 and 12 months based on glucagon stimulation testing. Five patients (8%) and seven patients (14%) had multiple deficiencies at 3 and 12 months respectively based on the GST. There was a significant reduction in the prevalence when a second confirmatory test using BMI specific cut offs was employed, and no multiple deficiencies were confirmed.

There was no difference in mean IGF-I between patients with or without hypopituitarism (table 5.4).

Thyroid axis

There was no significant difference in TSH between the groups at either 3 or 12 months. A single case of apparent secondary hypothyroidism was detected; however the patient was taking phenytoin for seizures related to the SAH. Thyroid function subsequently normalised. Two incidental cases of primary hypothyroidism were discovered, patients were treated and were biochemically and clinically euthyroid prior to pituitary function testing.

Adrenal axis

A single patient failed to mount a cortisol response greater than 500nmol/l at 12 months. Peak cortisol to synacthen was 473nmol/l, however the patient was asymptomatic without other pituitary deficits and therefore did not require treatment. Despite the low prevalence of confirmed adrenal insufficiency on stimulation testing, basal cortisol was significantly lower in the hypopituitary group at 3 months. Although this difference remained at 12 months, this was not statistically significant.

Gonadotrophin axis

No cases of female hypogonadism were detected. Five male patients were found to have low testosterone in the presence of normal gonadotrophins. Two of these patients had clinical symptoms compatible with androgen deficiency (impotence, loss of libido) and received androgen replacement between 6 and 12 months after repeat testing confirmed the results. In both of these patients however this resolved

spontaneously by 12 months and replacement was discontinued. The remaining three patients were asymptomatic and were found to have a low sex hormone binding globulin (SHBG) presumed secondary to obesity and a normal free testosterone on 12 month confirmatory testing.

Prolactin

There was no significant difference in prolactin levels between patients at 3 and 12 months or patients with and without hypopituitarism. No cases of hyperprolactinaemia were recorded.

Quality of life scores

Quality of life was impaired at both 3 and 12 months as demonstrated by the QOL-AGHDA score in which a score of >10/25 indicates significant impairment of quality of life(179). There was no significant change in mean QOL-AGHDA score over the period of follow up (3 months 11.1±7.6, 12 months 10.4±7.1, p=0.25). There was no significant difference in QOL-AGHDA scores between patients with or without hypopituitarism (table 5.3) although patients with hypopituitarism had a tendency towards higher scores. Quality of life scores at 3 and 12 months were not correlated with peak GH or basal cortisol at either time point.

Parameter	Hypopituitarism at 12 months (n=6)	Normal pituitary at 12 months (n=44)	p value
Age	45(27,50)	54.6(20,77)	0.02
Gender (M/F)	6/0	14/30	0.002
BMI 3 months (kg/m^2)	28.4(24.2,36.3)	26.6(18.3,43.9)	0.52
BMI 12 months (kg/m^2)	27.5(23.2,36.7)	27.7(18.7,47.1)	0.96
Change in BMI (kg/m^2)	-0.2(-1,0.4)	0.5(-3.4,3.5)	0.04
Basal Cortisol 3 months (nmol/l)	202(178,449)	458(146,760)	0.008
Basal Cortisol 12 months (nmol/l)	256(133,603)	348(192,556)	0.17
IGF-I 3 months (nmol/l)	18 (14,32)	16.5(3,34)	0.80
IGF-I 12 months (nmol/l)	22(10,29)	17(4,51)	0.90
Prolactin 3 months (mU/l)	105(71,163)	116(71,163)	0.94
Prolactin 12 months (mU/l)	106(95,117)	113(48,286)	0.64
QOL-AGHDA 3 months	17(6,23)	11(0,25)	0.10
QOL-AGHDA 12 months	16(3,25)	10(0,25)	0.10

Table 5.4 -*Comparison between patients diagnosed with hypopituitarism on confirmatory testing at 12 months and those patients with normal pituitary function*

Effect of BMI on glucagon stimulation testing

SAH cohort (n=64)

There was a significant correlation between peak GH to GST and BMI; R=-0.629, p<0.0001 (Figure 5.3). In stepwise linear regression in which the variables age, gender, need for inotropes, presence of hydrocephalus, WFNS score and fisher grade were entered, BMI and inotropes were deemed significant (R=0.581, BMI p=<0.001, Inotropes p=0.003). At 12 months the only significant variable affecting peak GH in linear regression analysis was BMI at 12 months (R=-0.43, p=0.003). There was no correlation between peak cortisol and BMI (R=-0.1, P=0.42).

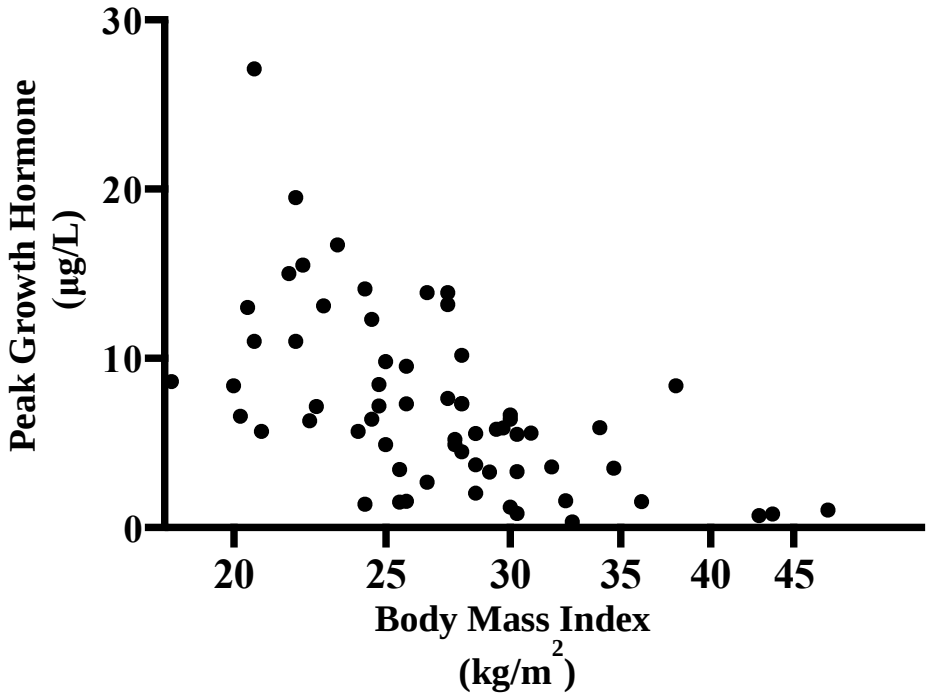


Figure 5.3 -*Peak GH response vs. BMI (logarithmically transformed) for patients 3 months following SAH.*

Results – Retrospective cohort

Clinical Characteristics

Fifteen patients (11 female) with a history of SAH were included in the study. The median time from insult to referral was 33 months with no patient being assessed less than 12 months after the SAH. The clinical grades at presentation according to the grading system of the world federation of neurological surgeons (WFNS) were: grade 1, n=13; grade 2 n=1, grade 3 n=1. Fourteen patients were found to have an aneurysm as the cause of their SAH, two patients required a ventriculo-peritoneal shunt for treatment of hydrocephalus, and one patient required evacuation of a haematoma causing raised intracranial pressure. Glasgow outcome score(GOS) at the time of assessment was GOS III n=1; GOS IV n=9; GOS V n=5.

For analysis, patients were divided into 2 groups, those with and without hypopituitarism. Results are summarised in table 5.5.

Patient anthropometry

There was no significant difference in age or gender in patients with or without hypopituitarism. BMI was significantly higher in the hypopituitary patients. Visceral fat and total body fat were also non-significantly higher in this group. In the patients treated with GHR there was no significant change in weight, BMI, total body fat or visceral fat during the 6 months of treatment.

Biochemical results:

Peak GH levels were significantly lower in the patients with hypopituitarism, along with a non-significant reduction in IGF-1. All 6 patients in this group had isolated GHD. Three of these patients had a suboptimal cortisol response to glucagon stimulation, however subsequently had normal synacthen tests. Basal cortisol levels were significantly lower in the patients with hypopituitarism despite their elevated BMI. No deficiencies in the other anterior pituitary hormones were found.

Quality of Life: All patients had significantly impaired quality of life with a QOL-AGHDA score >11. There was no difference in quality of life scores between those patients with and without hypopituitarism at baseline.

Follow up data in patients receiving GHR:

All 6 patients with severe GHD received GHR as all had impairment in QOL AGHDA scores. 6-month follow up data demonstrated that median QoL-AGHDA score reduced significantly from 18.5 to 10.5 (p=0.046, by Wilcoxon signed ranks test, figure 5.4). IGF-1 levels increased significantly (13 nmol/l pre vs. 24.5nmol/l post p=0.02) , however there were no other significant changes in other biochemical or auxiological parameters.

As a control group, 6 of the patients without GHD who received no treatment also completed QoL-AGHDA scores 6 months later. The pre and post scores in both groups were compared by ANCOVA. The difference in scores between groups was -4.26 (95% CI: -10.24, 1.73, p=0.14). The improvement in scores noted above was therefore not significantly different to the change in the control group.

	Hypopituitarism (n=6)	Normal (n=9)	P value
Age (years)	41.5(33,62)	51(36,61)	0.210
Gender	5F	6F	
BMI (kg/m^2)	35.5(27.5,44.5)	23.8(20.2,32.5)	0.03
Body fat (%)	43.5(26.1,52.1)	32.6(20.5,45)	0.11
Visceral Fat	12.5(7,16)	6.5(5,10)	0.16
Peak GH (μg/L)	1.61(0.2,2.9)	6.13(3.48,15.6)	0.008
IGF-1(nmol/l)	13(11,17)	18(14,27)	0.1
Basal cortisol (nmol/l)	280(181,345)	442(186,574)	0.045
Peak Cortisol (nmol/L)	459(380,713)	686(496,818)	0.25
Cholesterol (mmol/L)	5.1(3.8,5.8)	5.2(3.7,6.8)	0.53
QOL-AGHDA	18.5(14,25)	18.5(9,25)	0.648

Table 5.5 – *Differences between patients with growth hormone deficiency and patients with normal pituitary function at baseline.*

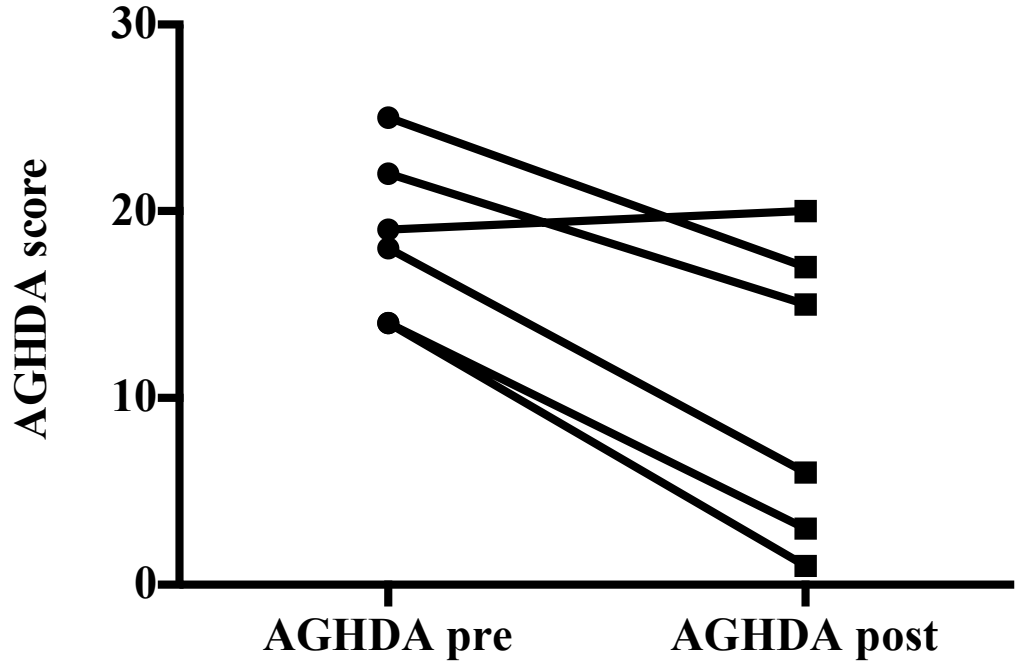


Figure 5.4 – *Change in QOL AGHDA score in patients with symptomatic GHD following SAH before and after 6 months of growth hormone replacement.*

Discussion

We have demonstrated that with the application of rigorous diagnostic criteria, using multiple dynamic function tests and BMI-specific cut-offs to define GHD, that hypopituitarism occurs in only 12% of patients 12 months post-SAH. Our observed prevalence is lower than the range (37.5-55%) reported by the systematic review by Schneider *et al*. (96), however is comparable to the results of the recent study by Lammert *et al* (359) which found a prevalence of GHD of 10%.

There are two significant differences between the current study and previous studies. The first difference relates to the use of multiple and confirmatory GH provocation tests. The importance of confirmatory testing in reliably diagnosing GHD and cortisol deficiency has already been highlighted in studies examining hypopituitarism in TBI (348). Klose *et al* (358) assessed 61 patients for GHD using the insulin tolerance test (ITT), or if contra-indicated, the GHRH-Arginine test. In cases where GHD was demonstrated, a confirmatory test was performed. With this approach, surprisingly, the authors did not identify a single case of hypopituitarism. Their approach is however consistent with the findings of Lissett *et al* (363) who demonstrate that a single GH provocation test can only reliably diagnose GHD in patients with two or three additional pituitary hormone deficits. For these reasons we used two different GH provocation tests to confirm the diagnosis of GHD in all patients as none of our patients had multiple pituitary deficiencies.

The second significant confounding factor affecting the outcome of endocrine testing in many of the earlier studies is a failure to account for the effects of obesity on GH secretion. Obesity has been clearly associated with impaired GH secretion: for each

unit increase in BMI, there is a 6% reduction in the daily GH secretion rate (36). This impairment is also evident in patients at risk for pituitary function deficit and thus is reflected in the results of a variety of GH provocation tests with negative correlation of GH peak with BMI (117, 118, 351, 356) including the insulin tolerance test (118, 364). BMI-specific GH cut off values to diagnose GHD using the GHRH-arginine test have been published(356). As yet however there have been no analyses of BMI-specific cut-offs to diagnose GHD when other stimulation tests are used.

Our Endocrine Unit uses the GST as the first line GH provocation test (104), which has been validated previously(365, 366) and has been shown to be equivalent to the Insulin tolerance test (102, 112). The Endocrine Society in its recent Guidelines acknowledged the role of the GST for the diagnosis of GHD, particularly in the face of the recent shortage of GHRH(105) and in cases where the ITT maybe contra-indicated (98, 360). Gomez *et al* who evaluated the GST in healthy controls found that none of their 46 volunteers failed to achieve a GH response <3µg/l, however the population used was lean. They did however note a significant correlation between BMI and peak GH response (103). We have demonstrated a negative correlation between peak GH and BMI in both healthy controls and in SAH patients; patients and healthy controls with a BMI > 30kg/m^2 frequently failed to achieve a peak GH level >3µg/l. Assuming our study conclusions had been based only on the outcome of a single test (the GST), without consideration of BMI or a second confirmatory test, the reported prevalence of hypopituitarism would have been very different, at 50%, broadly in line with the results of the early studies. This would suggest that the GST should not be used in isolation for diagnosis of hypopituitarism following SAH. Instead, using strict diagnostic criteria the prevalence of GHD was only 10%.

The second possible explanation for our much lower prevalence of neuroendocrine dysfunction may relate to the population studied. By offering follow up to all SAH survivors we had a large proportion of more favourable grade SAH patients, however Klose *et al* had more higher grade patients, and Lammert *et al* only included patients with fisher score 3/4, yet both groups found an prevalence similar or lower than ourselves. Our rate of vasospasm was lower than Lammert *et al* and comparable to Klose *et al*; however we had the greatest proportion of patients treated by endovascular coiling which may have been a significant factor. Kreitschmann-Andermahr *et al* (265) had speculated that open surgery may be associated with a higher incidence of hypopituitarism, and Bellebaum *et al*(367) had found a higher incidence of neuropsychological morbidity in patients who underwent clipping versus coiling.

One of the main strengths of our study is that our cohort of patients is a representative sample of all SAH patients, with all surviving patients invited and encouraged to undergo evaluation at 3 and 12 months post-SAH, including patients with significant disability. Participants and non-participants are of similar age with a similar severity of SAH (according to WFNS scores at presentation). Whilst caution must be exhibited in using cut off values from alternative assays, we have used commercial assays with good standardisation data, and have previously validated the cut of of 3micrograms/L for the GST in healthy controls. Furthermore the similar prevalence of GHD on GST compared with other studies using the GST alone is further reassurance of the appropriateness of this method.

We have also demonstrated that patients with confirmed hypopituitarism have significantly lower basal cortisol levels at 3 months and a tendency for lower levels at 12 months although they have acceptable peak cortisol responses following dynamic testing of the hypothalamic-pituitary-adrenal axis. This was again seen in our retrospective cohort were patients with GHD had lower basal cortisol than patients without GHD. These patients also tended to have lower quality of life scores (QOL-AGHDA), however this was not statistically significant. These findings of subtle defects of cortisol production, not apparent on stimulation testing, may suggest, disturbances in the circadian rhythm may be present as noted by Osterman (271) and lends support to the concept that these patients suffer pituitary insufficiency, rather than overt hypopituitarism, as is normally found in other causes of hypopituitarism.

Whilst these disturbances are not likely to lead to acute adrenal insufficiency, they may be responsible for the often-disabling lethargy seen in these patients. Unlike the prospective cohort, patients in the retrospective cohort with GHD did have elevated BMI with reduced basal cortisol levels whereas healthy obese patients would normally have elevated basal cortisol levels(368).

However given the complex interplay of both age and BMI on normal adrenal responses and ACTH sensitivity(369) these changes could simply represent normal physiological variance. This aspect certainly merits further investigation, and evaluation of the hypothalamo-pituitary-adrenal axis with 24-hour cortisol concentrations and diurnal variability may be informative.

With such a low prevalence we were unable to identify any clinical factors that may predict hypopituitarism. Kelly *et al* (264) had suggested the presence of clinical vasospasm as a possible aetiological factor, however only one of our patients with hypopituitarism had suffered this complication. Lammert *et al* (359) had also found a link between early hypogonadism and vasospasm; we did not find this, however the number of cases of vasospasm in that cohort was approximately double the number in our cohort. Likewise we found no link with the presence of hydrocephalus in our study.

There has been debate over the possible causation of hypopituitarism in SAH. Possible reasons postulated by Crompton *et al* included increased pressure in the chiasmatic cistern, direct damage of the perforating arteries by subarachnoid blood as they passed through the subarachnoid space, vasoconstriction, and subarachnoid blood passing up the sheaths of the perforating vessels then rupturing into the cerebral parenchyma. Other authors have suggested vasospasm(264) and damage done during craniotomy(265) as other possible explanations. Crompton *et al* (263) had also noted hypothalamic damage due to macro and micro haemorrhage in post mortem specimens from patients with SAH. This is significant as GHRH/Arginine testing does not act through the hypothalamus and therefore it is possible that use of this test may underestimate the presence of hypopituitarism if hypothalamic damage were the cause. Three of our patients had radiological evidence of infarction on follow up scanning however this was not associated with hypopituitarism. Unusually in this study we included three patients without an identifiable aneurysm (non-aneurysmal SAH), two of whom were found to have hormonal insufficiencies; this

has previously been reported (266) however recent studies have not included this patient group. This group may warrant further study.

In our retrospective cohort we demonstrated a higher incidence of pituitary dysfunction, as may be expected when studying a selected group. Only one patient with GHD had lasting neurological damage, the remainder having made a full physical recovery. It has been shown that patients with SAH have a persistent impairment of their quality of life unrelated to physical disability (367, 370), and that the symptoms demonstrated in SAH survivors bear close resemblance to those of patients with GHD. Whilst the data on treated patients in isolation suggest that there are improvements in QOL after GHR, when compared with the control group, this is not statistically significant. Our numbers are however small and it is likely that failure to achieve significance is at least in part because the study is underpowered. It is however possible that this improvement was down to placebo effect, or natural recovery from the SAH, although this is less likely as in most cases it was a considerable period of time since the initial insult.

It is still more difficult to explain the improvements in QOL seen in untreated patients, however in this group there may also be the effect of the reassurance of a normal test result on psychological well being. The QOL AGHDA score is also relatively crude as a measure of QOL and so subtle or domain specific changes will not be picked up. It will also not distinguish from other life factors apart from GHD which may affect QOL as demonstrated by the similar scores in both halves of the cohort at baseline. This was also found in the study by Kreitschmann-Andermahr who studied a cohort of patients following TBI and demonstrated a reduction in QOL

in patients with neuroendocrine dysfunction using other QOL tools but no significant difference in QoL-AGHDA scores in those patients with or without GHD. Kelly *et al* (278) also found reduced domain specific QOL in patients with post TBI hypopituitarism using other measures of QOL.

It remains unclear what role cortisol has to play in determining QOL. As previously mentioned we have seen lower basal cortisol levels in our retrospective patients with GHD.

Kreitschmann-Andermahr at al did find a correlation between QOL impairment and basal cortisol levels in patients following traumatic brain injury and it may be possible that subclinical defects in the HPA axis may partially explain the symptoms these patients describe. In our study we found no association between basal cortisol level and QOL AGHDA score, however given the limitations of cohort size and questionnaire type this aspect would certainly merit more attention in future studies.

In conclusion, we have demonstrated a prevalence of hypopituitarism in our prospective SAH cohort of 12% after 12 months follow up, consisting of isolated GHD in 10% and asymptomatic hypocortisolaemia in 2%. We did not identify any predictive factors for the development of hypopituitarism, however we have demonstrated evidence of subtle defects in the hypothalamo-pituitary axis that may suggest evidence of pituitary dysfunction. We believe therefore that universal screening for the presence of hypopituitarism is not justified, however targeted screening in patients who display evidence of delayed recovery from SAH in terms of energy, mood and quality of life could be performed. If screened, this should be performed at least 12 months after the SAH, when potentially reversible pituitary

dysfunction has resolved. Given the effect that confounders, such as obesity, have on dynamic testing for GH in particular, reference ranges, which take into account obesity, should be used, and in the presence of isolated deficits confirmatory testing should be undertaken. Given the findings of Karaca *et al* that new GHD cases may evolve up to three years post insult (371), and our own experience of evolving GHD (372), patients with on-going symptoms of suggestive of hypopituitarism may benefit from rescreening despite previously normal results. Whilst we have failed to demonstrate statistically significant improvements in QOL, there is a promising trend, which merits further study in larger cohorts with more detailed QOL testing applied.

Chapter 6

Growth Hormone Deficiency after Traumatic Brain Injury: cardio-metabolic profiles and long-term improvements in quality of life with GH therapy - analysis of the KIMS database

Abstract

Like SAH**,** hypopituitarism has been reported to be a frequent consequence of Traumatic Brain Injury, with patients with PTHP demonstrated as having a reduced QoL. Studies in these patients have shown short-term improvement in QoL and cognition with GH treatment, however long-term data are not yet available. We aimed to look for evidence of changes in QoL scores and body composition data to ascertain if changes were similar to patients with more conventional pituitary lesions. We analysed GH treated patients enrolled on KIMS (Pfizer International Metabolic Database). We compared 479 TBI patients with 3773 patients with non-functioning pituitary adenoma (NFPA). Cardio-metabolic indices at baseline and changes in QoL score were compared before and after GH initiation. As might be expected, patients with PTHP are younger but also have a higher incidence of GHD, higher peak GH concentration and a more favourable cardio-metabolic profile at baseline. Despite this, their baseline QoL is much lower, but has improved to a greater extent after 12 months (5 vs. 3.5 points, PTHP vs. NFPA; p=0.04). Improvements in QoL are sustained for at least eight years post treatment, although persistently remain worse than both NFPA and the general population over this period. We demonstrate that although the proportion of KIMS patients with PTHP on the KIMS database is increasing, absolute numbers remain low with an increased lag period (~2.4 years) between injury and GHD diagnosis, compared to NFPA patients.

Despite more profound impairments in QoL, with a worse baseline QoL score, GH replacement results in significant and sustained improvement in QoL in PTHP. The more favourable cardio-metabolic profile in PTHP suggests their disease may not confer the same increased cardiovascular risk as NFPA patients.

Introduction

In our previous study we failed to obtain sufficient power to demonstrate significant improvement in QoL in 6 patients with GHR post SAH, however improvements were seen. We therefore looked to utilise the Pfizer KIMS database to ascertain changes in QoL in a larger number of patients. The database however only holds details of 3 patients with GHR following SAH. We therefore looked to study a group with similar issues.

There is evidence to demonstrate that, following a traumatic brain injury (TBI), patients are at significant risk of developing anterior hypopituitarism, or post-traumatic hypopituitarism (PTHP) although estimated prevalence is highly variable(96, 264, 267, 373-383). Following TBI, patients have significantly reduced quality of life and cognitive impairments including memory impairment, reductions in executive functioning and decreased information processing speed(384, 385). Symptoms of fatigue are further exacerbated in the presence of GHD compared to patients with normal GH reserve(278). These functional outcomes, together with PTHP, have been associated with abnormal neuroimaging findings in TBI patients(386). Cognition and mood can be improved with GHR, with benefits shown after one year of therapy(387, 388), however little is known about changes in QoL following GHR in this population with only one study reporting improvements in QoL(389).

Studies to address how common this problem is have revealed widely differing estimates of the prevalence rates of PTHP, partly because of variation in the definitions of GHD, the use of different provocation tests to diagnose GHD, and

different time points for assessing pituitary function post-TBI(381, 390). Furthermore, with several dynamic function tests, results are dependant on the body mass index (BMI) of the patient(391).

Despite the high prevalence of PTHP reported in some studies, interrogation of the KIMS database by Casanueva *et al.* in 2005 highlighted only 51 KIMS patients with adult-onset PTHP compared with 688 KIMS patients with GHD following non functioning pituitary adenoma (NFPA)(392). Subsequent to this a further interrogation of the German KIMS database in October 2006 found only 69 cases of GHD for this indication(393). These data may suggest deficiencies in the neuroendocrine evaluation of patients after TBI and potentially a very high prevalence of undiagnosed and symptomatic GHD and hypopituitarism patients. It should be highlighted that only patients receiving GH replacement are followed in the KIMS database, and therefore, alternative explanations are either these patients do not receive GH therapy or have not been registered on the KIMS database.

The aim of the current study was to utilise the KIMS database to examine: i) the secular trends in numbers of KIMS patients with PTHP, ii) the cardio-metabolic profile and associated defects of patients with PTHP, and iii) the effect of GHR on QoL measures over an extended period to assess the degree of long-term response. We used patients treated for non-functioning pituitary adenoma (NFPA) as a reference group.

Patients and Methods

Patient groups (see figure 6.1)

In August 2012 the KIMS database (Pfizer International Metabolic Database; a pharmacoepidemiological survey of adult hypopituitary patients with GHD; total number of KIMS patients = 16266)(394) was interrogated for all patients with a diagnosis of TBI. In this study three different groups of patients were analysed: 1) all patients with TBI for the assessment of secular trends in the registrations of PTHP (Group 1, n=479); 2) patients with TBI, diagnosed with adult-onset GHD, never treated with GH before entry into KIMS and who had baseline and a minimum of 12 months of follow up data for the 1-year analysis of changes in cardio-metabolic profile and QoL (Group 2, n=161); 3) patients with TBI, diagnosed with adult-onset GHD, never treated with GH before entry into KIMS, from Belgium, France, Germany, The Netherlands, Spain, Sweden and the UK, and with reported QoL-AGHDA up to 8 years of follow-up for the cross-sectional analysis of QoL (Group 3, n=154). In addition patients with more than one pituitary diagnosis were excluded. Patients diagnosed with GHD in adulthood due to a TBI sustained in childhood were included.

Data from all patients with a diagnosis of adult-onset GHD caused by NFPA and no reports on cranial irradiation, who had baseline and a minimum 12 months of follow up data, were used as a reference group. There were 1268 eligible NFPA patients for 1-year longitudinal analysis and a maximum number of 1143 NFPA patients with QoL data at entry into KIMS for cross-sectional QoL analysis.

Assessments (see figure 6.1)

Three separate analyses were made:

In the first analysis, in order to ascertain whether number of KIMS patients with TBI were increasing, the database was interrogated for all registrations from inception to 2009. In order to ascertain relative proportions, this number was expressed as percentage of the total number of registrations. Additionally, in patients with an injury date/onset of pituitary disease, the age at onset was compared to age at diagnosis of GHD to ascertain the lag time from injury to diagnosis in both TBI and NFPA (Group 1).

In the second analysis, patients with TBI and NFPA were compared at baseline and 1 year follow-up (Group 2). Data analysed included demographic characteristics, number of associated pituitary deficiencies, stimulation test used, GH peak, IGF-I SDS , height, weight, body mass index (BMI), cholesterol, HDL-cholesterol (HDL), LDL-cholesterol (LDL), triglycerides, lean body mass (BIA), fat mass (BIA), systolic and diastolic blood pressure, fasting glucose, HBA1c and total QoL-AGHDA score.

In the third analysis, QoL-AGHDA scores in patients with TBI and NFPA were analysed cross-sectionally in relation to country-specific normative values up to 8 years of KIMS follow-up (Group 3).

The QoL-AGHDA, a disease-oriented instrument, consists of 25 items that evoke yes/no answers, acknowledging or denying certain problems(179). QoL-AGHDA score is scored from 0 (best QoL) to 25 (worst QoL). The instrument is reliable and valid with a high level of internal consistency(184). The normative reference values

for the QoL-AGHDA were derived from general population samples from 7 European countries(186-189).

The analysis was also performed for the following QoL-AGHDA dimensions: problems with memory and concentration (6 items), tiredness (7 items), tenseness (3 items), social isolation (5 items) and problems with self-confidence (4 items) and the results presented as proportion of items with impairment within each dimension(186).

Serum IGF-I and lipids were centrally analysed: at Kabi Pharmacia, Stockholm, Sweden (1994-1997), and thereafter at Sahlgrenska University Hospital, Gothenburg, Sweden. The following assay methods were used: until November 2002, radioimmunoassay after acid/ethanol precipitation of IGF-binding proteins (Nichols Institute Diagnostic, San Juan Capistrano, CA, USA); until September 2006, chemiluminiscence immunoassay (Nichols Advantage system); and after September 2006, Immulite 2500 (DPC Siemens)(395). Intra-assay, inter-assay, and total coefficients of variation were less than 9% in the concentration range of 125–1046 μg/L. The assay detection limit was 13.5 μg/L. Age and gender-specific reference ranges were used to determine an IGF-I SDS for each patient(396). Serum total cholesterol, HDL-cholesterol, and triglycerides were measured by standardized methods and serum LDL-cholesterol was calculated according to the Friedewald formula(397). Waist and hip measurements were conducted according to KIMS Guidelines(397), circulated to all participating physicians, and BMI was calculated as body weight (kg)/height (m^2).

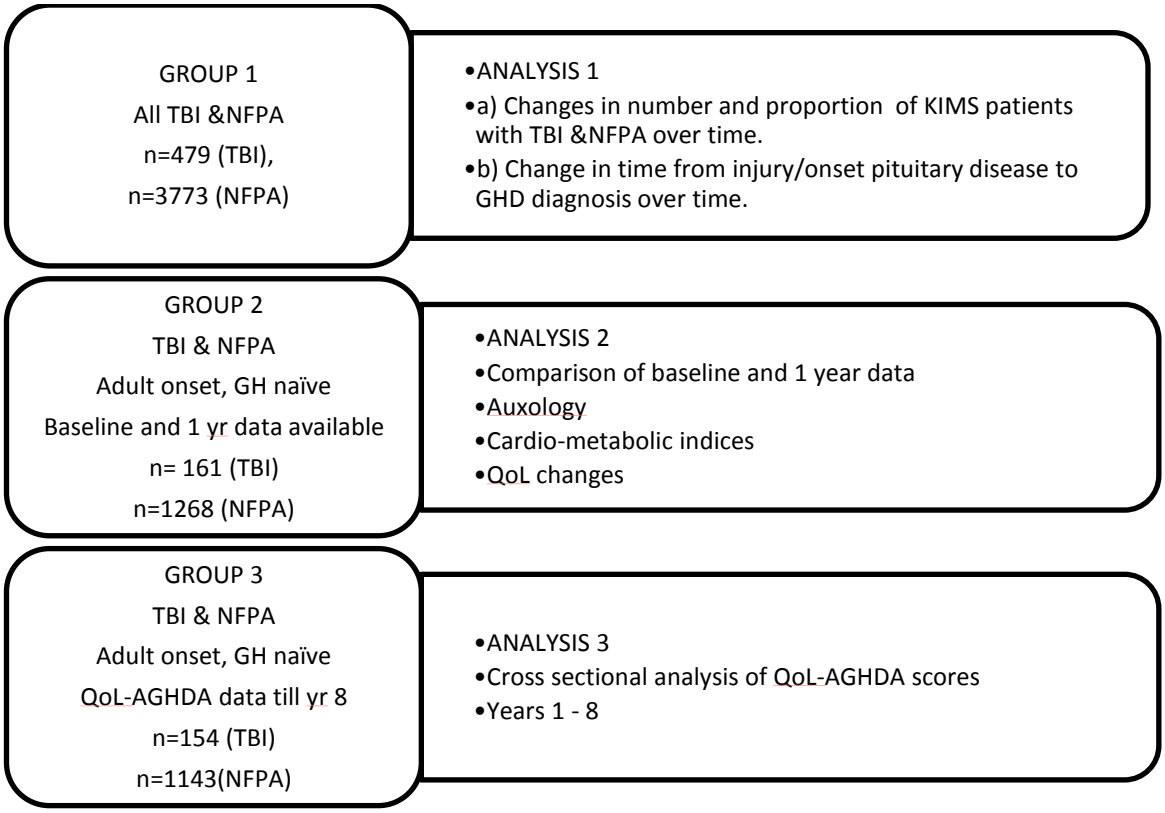


Figure 6.1 - *Investigation algorithm demonstrating inclusion criteria and analyses performed.*

Statistical Analysis

Descriptive statistics were presented and analysed with means and SD, or proportions, depending on the type of variable. Regression analysis on time between injury date (TBI) or date of diagnosis of NFPA and diagnosis of GHD was adjusted for age (numerical), gender, calendar-year (numerical) and were performed with PROC REG, SAS version 9.2.

The statistical analyses for baseline outcome variables and 1-year change outcome variables were performed by covariance analyses for unbalanced designs (PROC GLM, SAS version 9.2). Group comparisons were adjusted, in general, for age at measurement, and gender, except for the analysis of GH peak, which additionally were adjusted for type of stimulation test. Adjusted means are presented at age 45 years and equal gender distribution. For the analyses of dimensions of QoL-AGHDA, mixed-linear regression was used as the within-patient dimension scores are expected to be correlated. The number of items differed by dimension, so standardisation was necessary and this was achieved by computing the percentage of items within a dimension that a patient expressed problems with. The covariance structure was estimated with an unstructured variance-covariance matrix. Adjustments were made for age and gender. Analyses were performed at yearly visits (cross-sectionally).

Confidence intervals were Wald based. Statistical significance was set to $p<0.05$.

Results

Analysis 1:

KIMS registrations

The proportion of registrations due to TBI increased over time, with the highest proportion being recorded between 2005 and 2009 (Table 6.1).

Time from injury to GHD diagnosis

Based on all available reports, injury date was recorded in 141 TBI patients. Mean age of injury was 25.9 (SD 13.8) years. 10 patients were below the age of 19 years when the injury occurred. There was a trend over the calendar year for a longer lag time between injury date (TBI) or diagnosis of pituitary disease (NFPA) and GHD diagnosis. In order to understand the significant factors implicated in the delay between diagnosis (onset of pituitary disease in KIMS)/injury date and treatment, a regression analysis was undertaken with years between injury diagnosis/injury as the dependant variable, and diagnosis group, age at GHD diagnosis, gender, calendar-year of GHD diagnosis as explanatory variables. The time between pituitary disease onset and GHD diagnosis was 4.6y in NFPA patients vs. 7.0y for TBI patients (as estimated at calendar-year 2000 and males age 45 at diagnosis). Thus TBI patients were generally diagnosed on average 2.4 years later (95% CI: 1.36 to 3.55 years; p=<0.0001). Females were diagnosed 0.45 years (95% CI: 0.03 to 0.88; p=0.035) later than males and time increased on average by 0.14 years by each calendar year (95% CI: 0.11 to 0.18; p<0.0001). Time to diagnosis decreased by 0.19 year per increased year of age (95% CI: -0.20 to -0.17; p<0.0001).

	<1980	*1980-1989*	*1990-1994*	*1995-1999*	*2000-2004*	*2005-2009*	*2010 - 2012*
Total no registrations for TBI	15	24	24	68	190	138	19
% of all registrations per interval	2.16	1.58	1.25	1.68	3.75	5.91	3.94
Total no registrations for NFPA	42	190	467	1209	1157	606	126
% of all registrations per interval	6.03	12.50	24.40	29.87	22.86	25.95	26.1

Table 6.1 - *Registrations on KIMS database attributed to traumatic brain injury up until 2012.*

Analysis 2:

Differences in baseline profiles

For the analysis of cardio-metabolic profile and QoL on true naïve adult onset patients (161 TBI and 1268 NFPA patients) description and comparison of baseline characteristics are shown in Table 6.2. TBI patients were significantly younger, however there was no significant difference in gender distribution with a male preponderance in both groups. TBI patients were significantly shorter than NFPA patients by 0.9cm (1.8cm when corrected for age and gender; 95% CI: -3.0 to -0.6 cm; p=0.004).

Body composition was similar in both groups with the exception of a reduction in lean mass in the TBI group. Cardio-metabolic indices were more favourable in the TBI group even after correction for age and gender with lower systolic and diastolic blood pressure, and total cholesterol and LDL (Table 6.2).

Despite similar IGF-1 SDS in both groups, peak GH was significantly greater in the TBI patients after adjustment for age and gender (p=0.03), but not after additional adjustment for type of stimulation test (p=0.12). There was a non-significant trend to a higher peak GH level in TBI patients regardless of test used. Proportions of tests by group are shown in figure 6.2.

TBI patients had significantly fewer associated pituitary deficiencies (1.6; 95% CI: 1.4 to 1.8 for TBI vs. 2.4; 95% CI 2.3 to 2.5 for NFPA) and a higher prevalence of isolated GHD (24.9% TBI vs. 6.4% NFPA). The most frequent additional deficiency was ACTH deficiency in both groups, followed by TSH, LH/FSH and ADH in TBI

patients, and LH/FSH, TSH and ADH in NFPA. QoL-AGHDA score was significantly worse in TBI patients compared with NFPA. This was consistent across all QoL-AGHDA subdomains of memory, tiredness, tenseness, socialising and self-confidence (data not shown).

Variable	*TBI (mean, 95% CI)*		*NFPA (mean, 95% CI)*		*p value*
Age (y)	42.6	40.8, 44.5	53.2	52.5, 53.8	<0.0001
Gender (%Male)	58		62		NS
BMI(kg/m^2)	29.0	28.2, 29.8	28.9	28.6, 29.3	NS
Height (cm)	169.9	168.8, 171.0	171.7	171.2, 172.2	0.004
Waist Hip Ratio	0.93	0.91, 0.94	0.92	0.92, 0.93	NS
Lean Mass(kg) BIA	54.7	52.0, 57.5	58.1	56.9, 59.3	0.02
Fat mass (kg) BIA	25.7	22.7, 28.7	26.9	25.6, 28.3	NS
Fasting glucose (mmol/L)	5.0	4.8, 5.3	4.9	4.8, 5.0	NS
HBA1c (%)	5.3	5.2, 5.5	5.2	5.2, 5.3	NS
Diastolic BP(mmHg)	79	77, 80	81	80, 81	0.02
Systolic BP (mmHg)	125	122,128	128	127, 129	0.03
Cholesterol (mmol/L)	5.37	5.06, 5.68	5.76	5.64, 5.88	0.02
LDL (mmol/L)	3.25	2.99, 3.51	3.55	3.45, 3.65	0.03
HDL (mmol/L)	1.27	1.18, 1.36	1.27	1.23, 1.30	NS
Triglycerides (mmol/L)	1.81	1.41, 2.20	2.19	2.04, 2.34	NS
Peak GH (μg/L)*	3.22	1.55, 4.89	1.79	1.08, 2.49	NS(0.12)
IGF-1 SDS	-1.27	-1.63, -0.90	-1.52	-1.66, -1.38	NS
Additional deficiencies	1.6	1.4, 1.8	2.4	2.3, 2.5	<0.0001
QoL-AGHDA score	13.9	12.5, 15.4	10.4	9.8, 10.9	<0.0001
QOL-AGHDA dimensions:					
Memory	0.63	0.56, 0.70	0.46	0.43, 0.49	<0.0001
Tiredness	0.66	0.59, 0.73	0.50	0.47, 0.53	<0.0001
Tenseness	0.65	0.57, 0.72	0.50	0.47, 0.53	0.0004
Socialising	0.38	0.32,0.45	0.30	0.28,0.33	0.02
Self Confidence	0.42	0.35,0.48	0.27	0.25,0.30	<0.0001

Table 6.2 - *Baseline data for 161 TBI patients and 1268 NFPA patients demonstrating age and gender adjusted mean and 95% confidence intervals with between group differences (* Peak GH adjusted for age, gender, and type of stimulation test). QoL-AGHDA dimensions are shown as age and gender adjusted mean proportion of items that patients have problems with.*

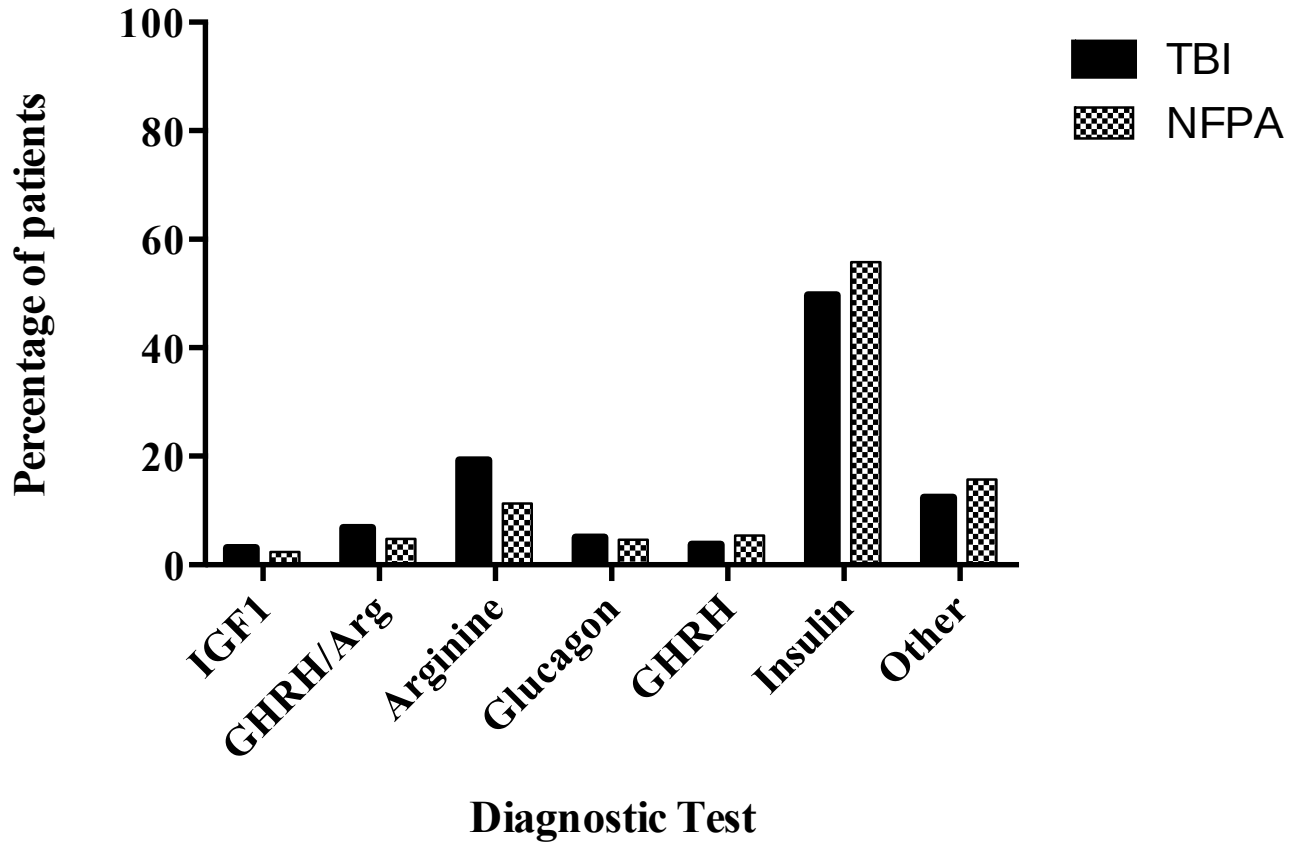


Figure 6.2 - *Growth hormone stimulation tests used for diagnosis of GHD in 161 TBI patients and 1268 NFPA patients*

1 year follow up data

Follow up data for both TBI and NFPA patients are shown in Table 3. Improvements were seen in IGF-I SDS and waist hip ratio in both groups, however improvements were also seen in body composition in the NFPA group with an increase in lean mass, which was not seen in the TBI group. Likewise NFPA patients saw marginal increases in fasting glucose and HBA1c with reductions in total cholesterol, LDL and triglycerides. There were no significant between group changes in any of these variables. Mean GH dose over the 12-month period somewhat higher in the TBI group (0.37 mg/day vs. 0.33 mg/day for NFPA patients (mean difference: 0.04mg/day 95% CI: 0.01 – 0.07; p=0.006; adjusted for age and gender)

Variable	*TBI (mean, 95% CI)*		*NFPA(mean, 95% CI)*		*Between Groups*
BMI (kg/m^2)	-0.19	-0.46, 0.08	-0.04	-0.16, 0.08	NS
Waist Hip Ratio	-0.01	-0.02, 0.0	-0.01	-0.01, 0.0	NS
IGF-1 SDS	1.59§	1.19, 1.98	1.90§	1.76, 2.04	NS
Lean Mass (kg) BIA	0.12	-1.56, 1.80	0.96§	0.17, 1.75	NS
Fat mass(kg) BIA	-1.91	-3.88, 0.06	0.87	-1.8, 0.05	NS
Fasting glucose (mmol/l)	0.17	-0.12, 0.46	0.17§	0.06, 0.28	NS
HBA1c (%)	0.1	-0.04, 0.24	0.1§	0.05, 0.15	NS
Diastolic BP(mmHg)	0.15	-1.71, 2.02	-0.32	-1.12, 0.48	NS
Systolic BP (mmHg)	1.71	-1.33, 4.75	-1.07	-2.38, 0.23	NS
Cholesterol (mmol/l)	-0.06	-0.34, 0.21	-0.20§	-0.31, -0.10	NS
LDL (mmol/l)	-0.12	-0.32, 0.09	-0.16§	-0.24, -0.08	NS
HDL (mmol/l)	-0.03	-0.10, 0.05	0.02	-0.01, 0.04	NS
Triglycerides (mmol/l)	0.08	-0.30, 0.46	-0.15§	-0.29, -0.01	NS
GH Dose (mg/day)	0.37	0.35, 0.40	0.33	0.32, 0.34	0.006
QoL-AGHDA score	-5.0§	-3.7, -6.4	-3.3§	-3.0, -4.0	0.04
QOL-AGHDA dimensions:					
Memory	-0.17§	-0.23, -0.1	-0.13§	-0.15, -0.10	NS
Tiredness	-0.22§	-0.3, -0.15	-0.21§	-0.23, -0.18	NS
Tenseness	-0.21§	-0.3, -0.13	-0.14§	-0.17, -0.11	NS
Socialising	-0.14§	-0.2, -0.08	-0.09§	-0.12, -0.07	NS
Self Confidence	-0.2§	-0.27, -0.14	-0.08§	-0.11, -0.06	0.0008

Table 6.3 - *12 month data for 161 TBI patients and 1268 NFPA patients demonstrating age and gender adjusted change within groups between baseline and 1 year, together with significant changes between groups at 1 year. (§ significant difference within group between baseline and 1 year.)*

Analysis 3 *Improvements in Quality of Life*

Year one Data

At baseline TBI patients had significantly worse QoL-AGHDA scores compared to NFPA patients (13.8 vs. 9.7). When reassessed 12 months after treatment commenced, QoL-AGHDA score had improved significantly in both groups, with the improvement being greater in the TBI patients (5.0 vs. 3.5 points; p=0.04) however the overall score remained worse in TBI patients.

QoL-AGHDA dimensions

Data on dimensions at baseline are shown in Table 2. Responses at baseline showed significant impairment in all dimensions in both TBI and NFPA patients. TBI patients had greater impairment in all areas compared with NFPA. TBI patients showed greatest impairment in tiredness, followed by tenseness, memory, self-confidence and lastly socialising.

At one year both groups of patients improved significantly in all areas. Self-confidence was the only dimension where patients with TBI improved to a significantly greater extent than patients with NFPA (table 6.3).

Changes in QoL-AGHDA scores over time

QoL-AGHDA scores for eight years post GH start were available in both sets of patients. In the TBI patients the initial improvement in QoL seen after 12 months is sustained over the eight-year period, however remains significantly worse than the NFPA group (Figure 6.3A). When compared to data from the normal population,

TBI patients have a significantly worse QoL score up until year eight when this difference becomes non-significant.(Figure 6.3B).

Analysis of QoL-AGHDA dimensions shows that TBI patients saw normalisation of socialisation compared with the normal population after 1 year. Self-confidence returned to within the range of the normal population by year 6 as did tenseness, although small numbers lead to wider confidence intervals by this point. Impairment in tenseness and memory never returned to the level of the normal population.

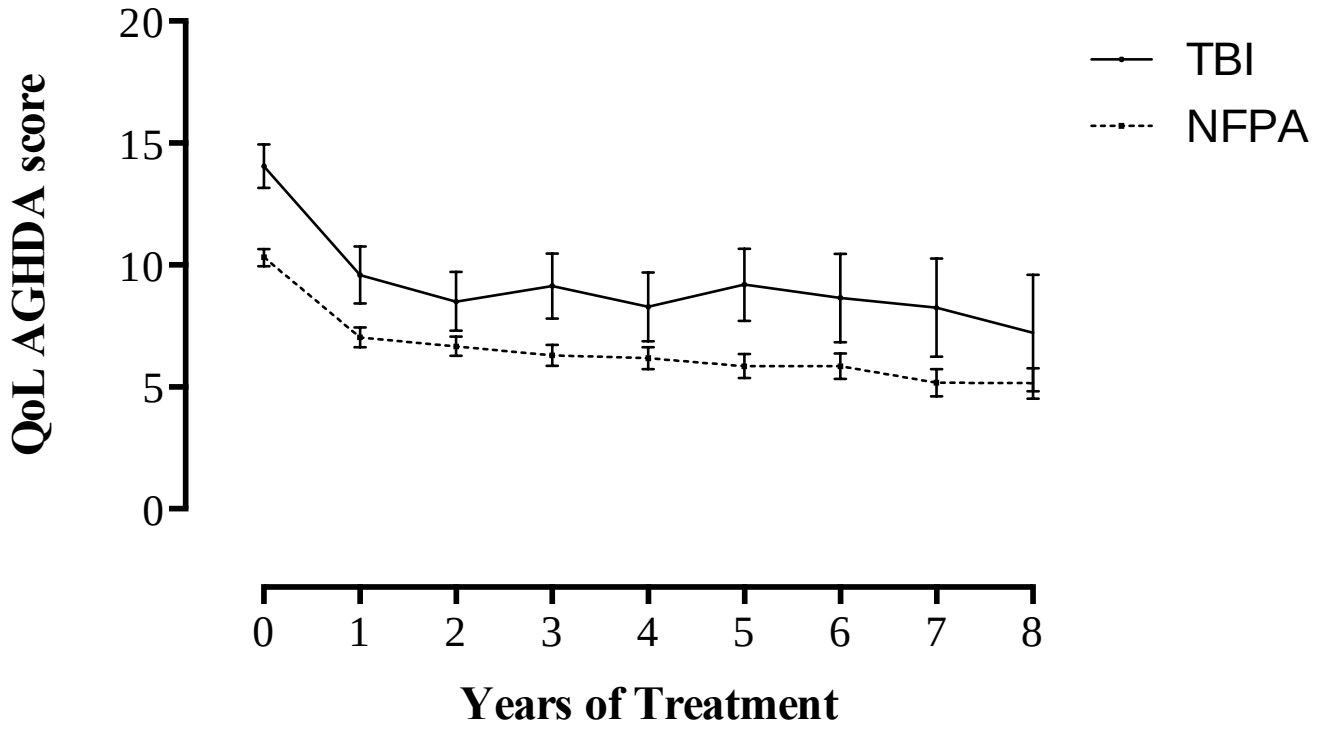


Figure 6.3A - *Changes in QoL-AGHDA score over time demonstrating sustained reduction in score for both TBI and NFPA patients but with significantly worse scores for TBI compared with NFPA until 8 years post treatment initiation.*

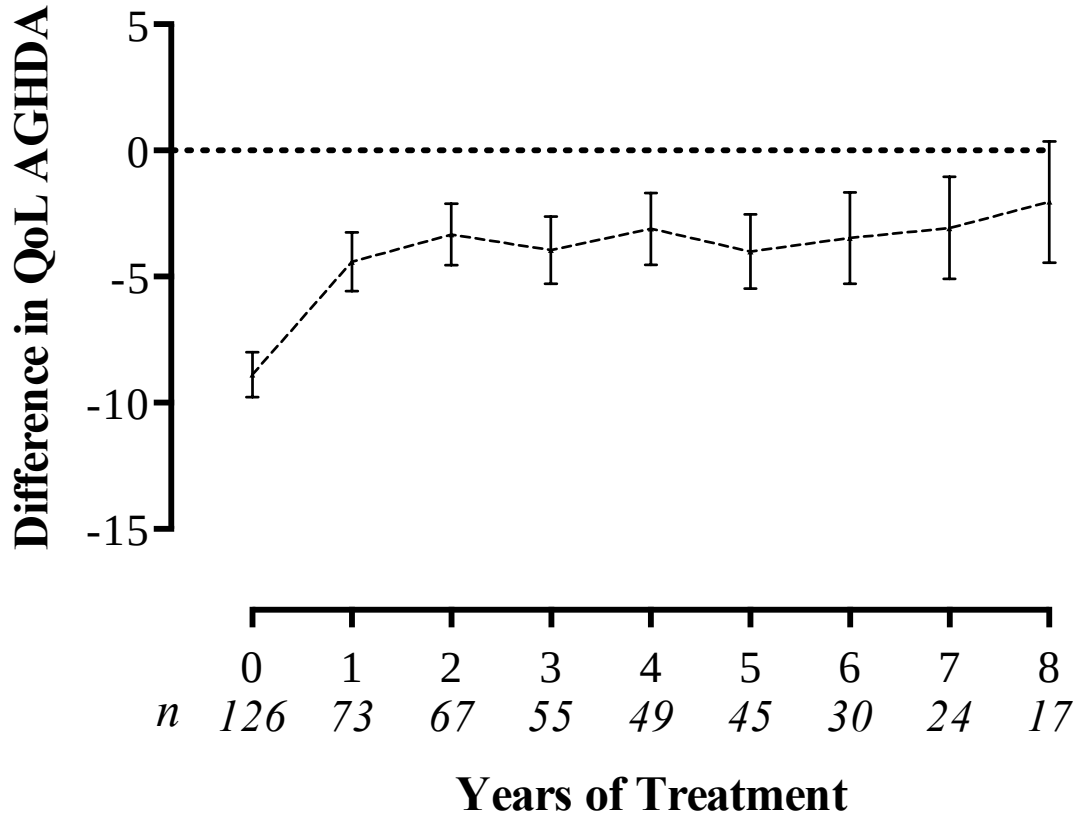


Figure 6.3B - Changes *in QoL-AGHDA score over time demonstrating significantly reduced QoL in TBI patients compared with the normal population up to 8 year follow up. Dotted line indicates level of normal population, n equals number of TBI patients available for follow up at each point.*

Discussion

In this analysis we have examined the largest cohort of patients following traumatic brain injury with one year follow up data on a range of variables. We have additionally presented quality of life data for eight years post GHR and compared this, not only to other patients with hypopituitarism due to another cause (NFPA), but also to the general population.

We had speculated that after the recent interest in PTHP, registrations for this cause of GHD would have increased. Whilst the proportion of registrations for this indication is increasing, absolute numbers remain low. The incidence of hospitalised TBI patients is 235 per 100,000 population(398). Considering an incidence of GHD after TBI between 25-50%, this would suggest that a large number of undiagnosed patients with GHD exist, perhaps due to lack of screening programs or poor awareness of clinicians.

Our second hypothesis, that with increased awareness, the lag time from injury to GHD diagnosis would have reduced, was not confirmed. We demonstrated once again a significant lag time between injury date and diagnosis of GHD, with patients with TBI diagnosed over 2 years later than NFPA patients. This lag was observed in the two previous KIMS studies(392, 393). It may be that a reduction in the lag time is observed in future studies.

Our analysis of the cardio-metabolic profile and nature of PTHP in this population has reconfirmed that the TBI population is younger, and of similar BMI to NFPA patients. Our analysis shows that the nature and risk of PTHP differs from that seen

with NFPA. There is a higher incidence of isolated GHD, and a consequent reduction in the number of associated pituitary deficiencies, suggestive of less severe hypopituitarism than in NFPA, supported by the finding of a trend to higher peak GH to stimulation in GHD patients although this can partially be explained by differences in stimulation testing, with no significant difference in peak GH observe after correction for stimulation test and similar pre-treatment IGF-1-SDS between the groups.

The body composition and cardio-metabolic data of PTHP suggest a lower cardiovascular risk than in patients with NFPA, with lower cholesterol and LDL concentrations, and lower systolic and diastolic blood pressures even after correction for age and gender. The only marker of adverse body composition in the TBI group is the finding of lower lean muscle mass, which may represent disuse atrophy.

Similar to previous KIMS data, patients post TBI were shorter than counterparts with NFPA. Subgroup analysis has shown that this difference can be accounted for by patients whose injury date was before 19 years of age (data not shown). This suggests that head injury in childhood may in some cases account for reductions in growth, although the numbers involved are small. However, Bellone *et al* demonstrated an association between reduced height velocity in children post TBI and PTHP in this group(399).

Significantly, this study has provided strong evidence on the beneficial and sustained effect of GHR on QoL in this cohort over eight years. It has been shown in many studies that TBI patients have reduced QoL, and that TBI patients with PTHP have a

worse QoL and greater fatigue than compatriots without PTHP(278, 388, 400, 401), although this is not universally agreed(402). GHR is known to improve QoL in patients with GHD(186), and this is reconfirmed by the improvements in QoL seen in the NFPA patients in our group. Short-term studies and case reports have shown GHR to improve QoL and cognition in TBI patients with GHD up to 12 months(403, 404). Kozlowski *et al* demonstrated improvements in cognition, particularly verbal memory and in QoL after 12 months treatment(389), whilst Maric *et al* demonstrated improved cognitive abilities particularly in verbal and non verbal memory, and improvements in psychiatric functioning with subsequent worsening in 3 patients who discontinued treatment for 12 months(388). Our data show significant improvement in QoL-AGHDA score after 12 months, however additionally we have demonstrated sustained improvement over a number of years.

Similar to previous data on changes in QoL-AGHDA dimensions in GHD(186), we have shown a faster improvement in problems with socialising, and a much slower improvement in problems with memory and tiredness. As would be expected QoL scores remain significantly lower than both NFPA patients and the normal population, most likely reflecting other consequences of injury in these patients. Recently it has been shown by Tanriverdi *et al* that hypopituitarism improves in a proportion of patients when pituitary function is rechecked 5 years post injury(377). This factor as well as overall physical recovery may be responsible for the continued gradual improvement in QoL after the initial 12-month period.

This study does have weaknesses. This data covers only patients with GHD rather than the full spectrum of pituitary deficiencies without GHD. This also only includes

patients commencing GH, which would indicate that patients, particularly in the UK would need to be symptomatic – this could lead over reporting of poor QoL scores compared with the overall population of patients with PTHP.

In conclusion our findings suggest that there are likely to be a large number of undiagnosed patients with PTHP. We have shown that whilst the 'phenotype' of PTHP is less severe than PTHP, there are very clinically relevant improvements in QoL, which are sustained, in the long term in this population. These important findings provide an evidence base for screening and treating affected patients with PTHP.

Chapter 7

Discussion and Final Conclusions.

GHD is a clear physiological entity with a definite phenotype. It is clear that GHD is associated with adverse body composition and increased cardio-metabolic risk, and that GHR in cases of pathological GHD results in clear benefit in terms of QoL and improvements in body composition and cardio-metabolic risk factors. This thesis in all its components examines the complex interplay between GH secretion and fat mass and the implications both for fat deposition but also quality of life and response to GHR.

Chapter 3 described the proportion of GHD patients with elevated IHCL compared with control volunteers matched for age and BMI. We did not see a difference in the proportion of patients with NAFLD based on MRS in the 2 groups, and in hindsight based on subsequent studies in this thesis it maybe tempting to speculate on whether these 'GHD' patients really did have true GHD given that the majority were diagnosed by the GST alone as is the clinical practice rather than using confirmatory testing. If this were to be the case, it would not have been surprising to see no difference. There are however compelling arguments against this view. Firstly patients with GHD did have significantly lower IGF-I levels. Secondly body composition was very different between the two groups with raised VAT and SAT in the GHD group which, due to matching cannot simply be accounted for by BMI per se.

In this study we then followed the response to treatment of symptomatic GHD patients with GHR. Although this observational study was small, we did as previously seen show significant reductions in VAT and SAT suggesting that this group behave in similar fashion to other GHD patients studied in the literature. The

fact that there was a non-significant reduction in liver fat may therefore suggest that the liver is the least sensitive of the 3 fat depots to the effects of growth hormone. This study raises questions about the concept of 'physiological' vs. 'pathological' GHD. GH is known to be significantly reduced in obesity with reductions of 75% seen in obese patients compared with age-matched controls. Although we did not measure peak GH to stimulation in our control population in this study, we do know from our subsequent study that a majority of otherwise healthy controls with BMI >30kg/m^2 have a peak GH >3μg/L. Given that patients with GHD are known to have insulin resistance, the question raised by this study is whether treatment of 'physiological' GHD results in similar improvements in body composition. i.e. regardless of the cause of GHD, does replacement lead to improvement in cardio-metabolic and body composition outcomes. Since our data has been published, a further study by Bredella *et al* looking at young men with abdominal adiposity without GHD has been published. This compared the effect of administration of GHD vs. placebo to this group and demonstrated the same improvements in body composition and IHCL in GH treated participants as demonstrated in our GHD group. Similar to our outcomes, a reduction in VAT/SAT ratio was seen implying a greater sensitivity of VAT to the effects of GH.

Future studies in this area will need to investigate the role of GH on the liver and on physiological outcomes in more detail. Our small study failed to show significant reductions in liver fat most likely due to small patient numbers. Larger multicentre studies would be needed in order to achieve this, however the implications of reductions in liver fat on the subsequent progression to the complications of NASH would be clinically important. Given that Bredella *et al* noted an inverse relationship

between many markers and IGF-I, it may be necessary to include GH dosing adjustments as part of this work. As such future studies would need to include a marker of NASH rather than simply IHCL, together with other cardio-metabolic markers such as hsCRP, adiponectin and apolipoprotein estimation.

Chapter 4 allowed us to look in more detail at the GH response of healthy volunteers. We saw a negative correlation between peak GH response to the GST and BMI, which mirrored the findings in the earlier chapter, and confirms the concept of 'physiological' GHD. We were able to confirm our methodology as appropriate and validate this against international cut offs.

The problem of diagnostic testing in adult GHD is taking on a new significance with the combination of the newer indications for testing which do not necessarily have demonstrable structural lesions in the pituitary gland, coupled with changes in the general population meaning that more people are becoming obese. The clinician needs to be aware of these diagnostic pitfalls and testing strategies to minimize false diagnosis need to be developed. The GST does have a valuable place in diagnosis of GHD however as demonstrated in chapter 4 it is not fool proof. It is heavily affected by BMI as are other tests, and the cortisol response in particular is unreliable. Other groups have showed this also. Future strategies could include validation of the GST at different levels of BMI as has been done with the GHRH/Arginine test, or the introduction of routine second testing for patients without demonstrable pituitary lesions on scanning as happens in the paediatric population currently. The role of IGF-I in this situation has potential. Although affected by obesity and age, standardized SDS scores are becoming available and could prove valuable as a

'sense check' in interpreting dynamic function testing results. Together with this higher resolution 3T MRI scanning has the potential to enable pituitary and hypothalamic lesions to be more readily recognized, again aiding in the decision around which test to choose and the interpretation of findings. Finally it is clear that the GST should not be relied on as a marker of cortisol reserve.

Chapter 5 allowed us to move the theories developed in chapter 4 into a clinical context. In this study we looked at a population of patients with SAH and screened for hypopituitarism using robust testing with confirmation of deficiencies. The advantage of conditions such as SAH and TBI when attempting to evaluate the influence of obesity, is that there is a definite point of onset, unlike many other causes of pituitary disease in adulthood when patients may have lived with conditions and the associated GHD for a number of years. The effects of GHD on longitudinal weight gain have been clearly shown(108, 405) , however in SAH we were able to evaluate patients and record their BMI before any long-term effect took hold.

In the study we demonstrated a clear influence of BMI on the outcome of the GST in patients with SAH. This is an effect that was not present in the patients with multiple pituitary hormone deficits studied in chapter 4. We also demonstrated that with confirmatory testing the overall incidence of hypopituitarism at 12 months fell to 12% of which GHD made up 10%. As supportive evidence we did not see significant changes in weight in this cohort between 3 and 12 months, which is different to studies of patients with confirmed GHD secondary to other pituitary conditions, and we did not see differences in QoL AGHDA score between patients with and without

hypopituitarism. Whilst overt hypopituitarism is uncommon in this group, such robust testing is at risk of overlooking subtle changes in pituitary function. Basal cortisol levels were noted to be significantly lower in patients with hypopituitarism at 3 months and non-significantly lower at 12 months. Impairment in QoL, which has been blamed on GHD, previously could indeed be due to alterations in the HPA axis in these patients. Future studies could look to estimating cortisol reserve more elegantly looking to alterations in diurnal variability of cortisol or changes in total daily production of cortisol in the quest to evaluate whether neuroendocrine dysfunction rather than deficiency may play a part in the reduced QoL seen in these patients.

In this chapter we did evaluate the effects of GHR in a small number of patients with GHD diagnosed by standard methods following SAH. Whilst there was a significant improvement in the group over the 6-month period, this lost significance when comparison with changes in scores in an untreated group was added in. This study demonstrates that with such small numbers of SAH patients treated for GHD it is difficult to draw meaningful conclusions around the effect of GHR. We therefore turned to the Pfizer KIMS database for a larger number of SAH patients to study. Despite such prominence in the medical literature over recent years the Pfizer database contained only three patients treated with GHR following SAH. It was therefore decided to look at a separate patient group where similar diagnostic conundrums are faced, and where there is currently a gap in the literature as to outcome, namely patients with traumatic brain injury.

Chapter 6 - In this chapter we evaluated the single largest group of patients in the project so far. Using the Pfizer KIMS database we evaluated data on a total of 479 patients with TBI and 3773 patients with NFPA. Our primary outcome was to ascertain whether GHR in this population, which is similar to SAH patients in many ways, (definite date of onset, often no demonstrable pituitary lesion, other injuries etc. complicating QoL assessment) improved QoL in patients with GHD. In addition given the questions raised by this thesis as to the reliability of diagnosis in patient groups such as this, we aimed to look for evidence of the 'severity' and 'phenotype' of GHD in this population when compared with patients with NFPA, long considered the archetypal pituitary lesion.

We demonstrated evidence of a less severe phenotype, with higher peak GH levels, higher incidence of isolated GHD, and less severe cardio-metabolic aberrations after correction for age and gender, despite there being no significant difference in BMI. We did however demonstrate significant and sustained improvements in QoL AGHDA dimensions over eight years post commencement of GH. It must be considered due to this being a GH database, only patients wishing to commence GH, or in the UK patients with impairment in QoL were included, this is likely to bias the findings towards the more symptomatic patients. Future studies would ideally include a randomized RCT of GH vs. placebo in patients with SAH and TBI however given the clear benefits of GHR replacement in reducing cardio-metabolic risk and QoL in patients with GHD, such studies may be difficult to justify on ethical grounds.

Chapter 8

Publications

Publications

Gardner CJ, Javadpour M, Stoneley C, Purthuran M, Biswas S, Daousi C, MacFarlane IA, Cuthbertson DJ. Low prevalence of hypopituitarism after subarachnoid haemorrhage using confirmatory testing and with BMI-specific GH cut-off levels. Eur J Endocrinol. 2013 Mar 15;168(4):473-81. doi: 10.1530/EJE-12-0849. Print 2013 Apr. PubMed PMID: 23258271.

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Gardner CJ, Richardson P, Wong C, Polavarapu N, Kemp GJ, Cuthbertson DJ. Hypothyroidism in a patient with non-alcoholic fatty liver disease. BMJ. 2011 Jan 6;342:c7199. doi: 10.1136/bmj.c7199. PubMed PMID: 21212123.

Appendix 1- QoL-AGHDA Questionnaire

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	☐	☐

QoL-AGHDA English
2000-02-17
OR 7064-01

	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.

QoL-AGHDA English
2009-02-17
OR 7064-01

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