Neuroendocrine Consequences of Childhood Traumatic Brain Injury

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Declaration

I declare that this submission is my own work.

The research developed from an idea initiated by Professors Christopher Kelnar and Robert Minns. I wrote the study protocol, obtained ethical approval to carry out the study and compiled the database from which participants were recruited. I performed the baseline and dynamic endocrine investigations and compiled and evaluated the body composition data. I evaluated the results of quality of life questionnaires completed by subjects and/or their parents/guardians and extracted head injury information from subjects' medical records. I was not involved in the analysis of physiological monitoring data from computerised intensive care records.

Analysis of the endocrine data and statistical comparisons were performed with assistance from Ms. Catriona Graham and advice from Dr. Patricia Crofton.

I confirm that all of the research work was undertaken whilst in post in Edinburgh and that I have not submitted the thesis in candidature for any other degree, postgraduate diploma or professional qualification.

Dr. Sophie N. Khadr

Date: 1st September 2009

Dedication

This thesis is dedicated to Leo and my family, who support me through everything I do.

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Abstract

Objectives: 1) To determine the prevalence, aetiology and clinical significance of pituitary dysfunction after moderate or severe childhood traumatic brain injury (TBI); and 2) to examine its impact on quality of life (QoL) and body composition.

Subjects: Retrospective observational study of 33 survivors of accidental TBI (27 males) and two of inflicted TBI (both males). Accidental TBI group: mean (SD) age at study was 13.4y (3.7y) and interval since injury, 4.1y (1.6y). King's Outcome Scale for Childhood Head Injury (KOSCHI) rating: 15 good recovery, 16 moderate disability, 2 severe disability. Inflicted TBI group: ages at study were 5.0 and 3.7 years at 4.9 and 3.3 years post-injury with good recovery and moderate disability respectively.

Methods: Early morning urine samples were obtained for osmolality. Basal hormone evaluation (0800-1000h) was followed by the gonadotropin-relasing hormone (GnRH) and insulin tolerance (ITT, n=26) or glucagon tests (if previous seizures, n=9). Subjects were not primed. Body composition was evaluated using bioelectrical impedance analysis. Standardised quality of life (QoL) questionnaires were completed. Head injury details were extracted from patient records.

Results: There were no abnormal findings in the two survivors of inflicted TBI. Among the accidental TBI group, no subject had clinical evidence of impaired growth: mean height standard deviation score (SDS) was +0.5 (range -1.6 to +3.0 SD). Median peak growth hormone (GH) response to stimulation was 7.9 μg/L. Six peri-pubertal males had suboptimal GH responses (<5 μg/L). Their height SDS at study ranged from -1.5 to +1.4; one had slow growth on follow-up. GH response was borderline low in one post-pubertal male (3.2 μg/L).

Median peak cortisol responses were 538 nmol/L (ITT) and 562 nmol/L (glucagon). 9/25 (ITT) and 2/8 (glucagon) subjects had sub-optimal responses. In two cases (one ITT, one glucagon test), basal cortisol levels were high (624 and 722 nmol/L). For the rest, in 6/9, further testing or no action was advised; in 3/9, steroid cover was recommended for moderate or severe illness or injury. None required routine glucocorticoid replacement. No subject had diabetes insipidus. Thyroid function, IGF-I, oestradiol/testosterone, and baseline and GnRH-stimulated LH and FSH were appropriate for age, sex and pubertal stage. One male was prolactin deficient (<50 mU/L).

Abnormal endocrine findings were unrelated to severity of TBI, nature of primary or secondary brain injury, or KOSCHI rating. No significant difference in QoL was observed between those with normal or abnormal pituitary function <16y. QoL was poorer in the post-pubertal male with GH deficiency than in other subjects >16y.

Conclusions: Whilst mild pituitary 'dysfunction' was common (39%), no unequivocal clinically significant endocrinopathies were found, although the GH and hypothalamo-pituitary-adrenal axes may be vulnerable.

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List of Abbreviations

ACTH	Adrenocorticotrophic hormone
ADH	Anti-diuretic hormone
ADHD	Attention-deficit/hyperactivity disorder
AIS	Abbreviated Injury Scale
BIA	Bioelectrical impedance analysis
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BRIEF	Behaviour Rating Inventory of Executive Function
CPP	Cerebral perfusion pressure
CRH	Corticotrophin releasing hormone
CI	Confidence intervals
CT	Computed tomography
DAI	Diffuse axonal injury
DI	Diabetes insipidus
DXA	Dual X-ray absorptiometry
EPTS	Early post-traumatic seizures
EQ-5D	European Quality of Life – 5 Dimensions Questionnaire
ESPE	European Society of Paediatric Endocrinology
FFM	Fat-free mass
FM	Fat mass
FSH	Follicle-stimulating hormone
GCS	Glasgow Coma Scale
GH	Growth hormone
GHD	Growth hormone deficiency
GHRH	Growth hormone-releasing hormone
GHRP-6	Growth hormone-releasing peptide 6
GHRS	Growth Hormone Research Society
GnRH	Gonadotropin-releasing hormone
GOS	Glasgow Outcome Scale
GP	General Practitioner
hCG	Human chorionic gonadotrophin

HH	Hypogonadotrophic hypogonadism
HPA axis	Hypothalamo-pituitary-adrenal axis
HPG axis	Hypothalamo-pituitary-gonadal axis
HPT axis	Hypothalamo-pituitary-thyroid axis
HT	Height
HT^2/R	Resistance Index
ICD-10	International Classification of Diseases-10
ICP	Intracranial pressure
IGF1	Insulin-like growth factor 1
IGFBP-3	Insulin-like growth factor binding protein 3
IM	Intramuscular
IQ	Intelligence quotient
ISD	Information and Statistics Division
ISS	Injury Severity Score
ITT	Insulin tolerance test
IV	Intravenous
KIGS	Pfizer International Growth database
KIMS	Pfizer International Metabolic database
LBM	Lean body mass
L-dopa	3,4-dihydroxy-L-phenylalanine
LH	Luteinising hormone
LHRH	Luteinising hormone releasing hormone
MREC	Multi Research Ethics Committee
MRI	Magnetic resonance imaging
MSH	Melanocyte-stimulating hormone
NAHI	Non-accidental head injury
NICE	National Institute of Health and Clinical Excellence
NIH	National Institute of Health
NISS	New Injury Severity Score
NHP	Nottingham Health Profile
PedsQL	Pediatric Quality of Life Inventory
PICU	Paediatric intensive care unit
PTI	Pressure Time Index
PTIcpp	PTI values derived from CPP
PTIicp	PTI values derived from ICP

PTS	Pediatric Trauma Score
QLS-H	Questions on life satisfaction – hypopituitarism questionnaire
QoL	Quality of life
Qol-AGHDA	Quality of Life - Assessment of Growth Hormone Deficiency in Adults questionnaire
RHSCE	Royal Hospital for Sick Children, Edinburgh
ROC analysis	Receiver operating characteristic analysis
RTA	Road traffic accident
RTS	Revised Trauma Score
SD	Standard deviation
SDQ	Strengths and Difficulties Questionnaire
SDS	Standard deviation score
SF-36	Short Form-36 Health Survey
T3	Triiodothyronine
T4	Thyroxine
TBI	Traumatic brain injury
TBW	Total body water
TRH	Thyrotropin-releasing hormone
TRISS	Trauma Injury Severity Score
TSH	Thyroid stimulating hormone
UK	United Kingdom
USA	United States of America
WGH	Western General Hospital
WHO	World Health Organisation
WT	Weight

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Chapter 1: Introduction: Table of Contents

Chapter 1: Introduction

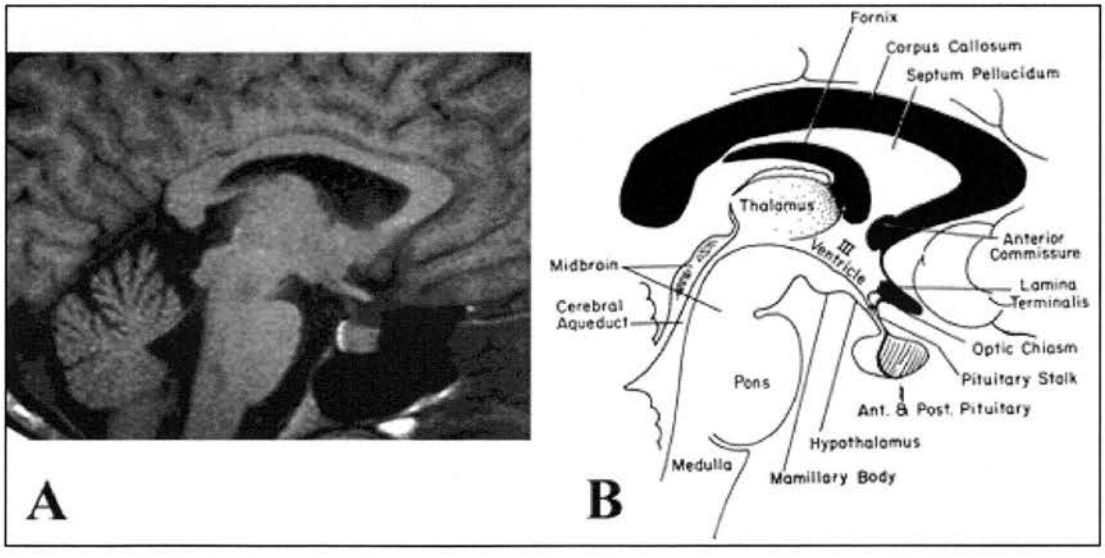
Hypopituitarism is a recognised complication of traumatic brain injury (TBI). In the last decade, much work has been done to quantify and characterise neuroendocrine sequelae in adult TBI survivors. Evidence is emerging to suggest that children too may develop pituitary dysfunction after TBI. However, as yet, there is limited understanding of the likelihood, causative mechanisms and impact of pituitary dysfunction in this group.

Children's quality of life is impaired after TBI. Detailed cognitive, behavioural and neurodevelopmental studies are confirming that the outcome following severe TBI in early childhood is worse than the outcome for older children and adults. These outcomes may be further influenced by a diagnosis of post-traumatic hypopituitarism. Systematic identification and management of pituitary dysfunction may be required for survivors of childhood TBI (an already compromised group) to achieve their fullest potential in terms of physical and psychosocial development.

1.1 Introduction to the pituitary gland

The pituitary gland, or hypophysis, is situated within the sella turcica, a bony recess in the sphenoid bone, in the middle cranial fossa (Asa et al 2002). It is enclosed within a capsule continuous with the dura mater. The infundibular (or pituitary) stalk attaches the pituitary gland to the hypothalamus at the base of the brain (Figure 1a).

Figure 1a: (A) Magnetic resonance image (MRI) & (B) corresponding diagram of the hypothalamus & pituitary gland seen in sagittal section (adapted from Lechan 1987)



The pituitary gland comprises the adenohypophysis and the neurohypophysis. The adenohypophysis derives from Rathke's pouch, an up-growth of pharyngeal ectodermal cells. It comprises the anterior and intermediate pituitary lobes, both of which consist of hormone-secreting cells. The anterior lobe of the pituitary synthesises growth hormone (GH), adrenocorticotrophic hormone (ACTH), prolactin, thyroid-stimulating hormone (TSH) and the gonadotrophins (follicle-stimulating hormone [FSH] and luteinising hormone [LH]). The intermediate lobe comprises a thin layer of cells that produce pro-opiomelanocortin, the precursor of melanocyte-stimulating hormone (MSH). The neurohypophysis or posterior lobe of the pituitary derives from a downwards out-pouching of neural tissue cells from the floor of the third ventricle. The posterior lobe stores and releases anti-diuretic hormone (ADH, also known as vasopressin) and oxytocin, both synthesised by the hypothalamus.

1.1.1 Pituitary hormone actions

GH promotes linear growth in children, lean body (or fat-free) mass gain and reduction in fat mass (Mehta & Dattani 2005, also section 1.12.1). It is anabolic, lipolytic and diabetogenic. GH also has an indirect effect on bone by stimulating synthesis of insulin-like growth factor 1 (IGF1). GH secretion is pulsatile and maximal overnight, increasing during times of stress, exercise, hypoglycaemia, and after a protein meal. ACTH stimulates the adrenal glands to produce glucocorticoids (especially cortisol) and adrenal androgens. Cortisol mobilises body carbohydrate stores and has additional roles in regulation of blood pressure, immune function and the body's inflammatory response. ACTH secretion follows a circadian rhythm and increases during periods of physiological stress.

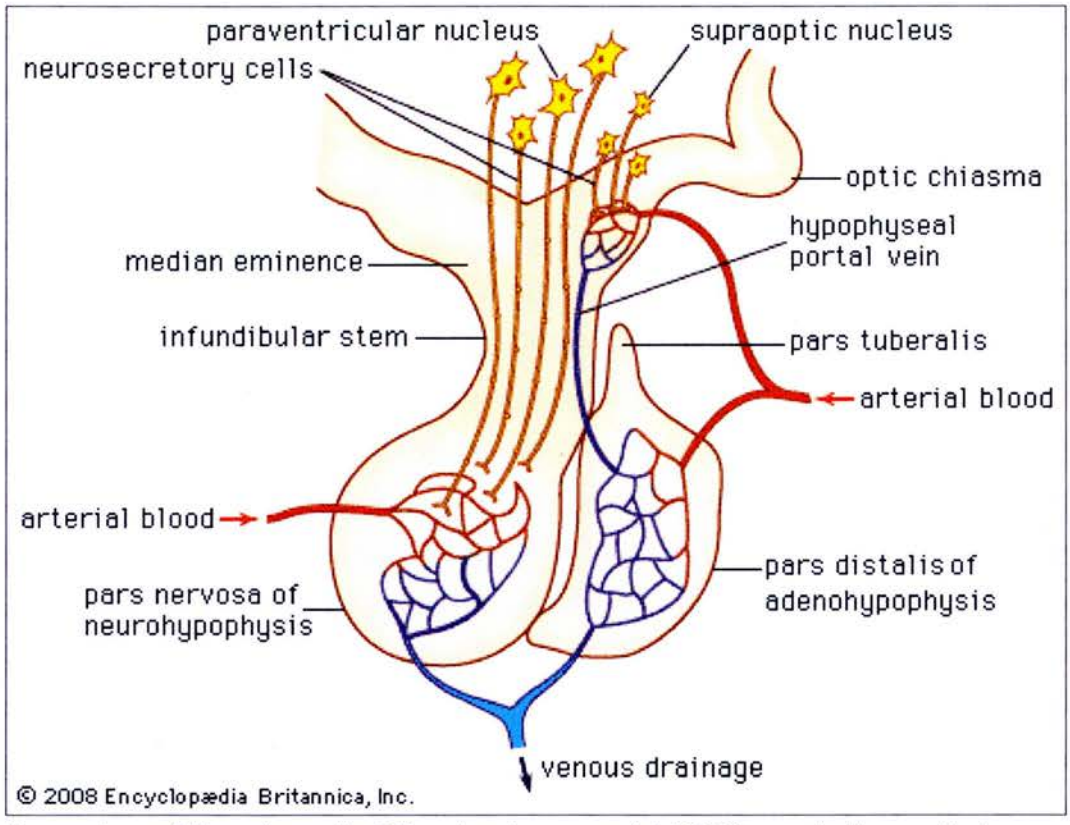
TSH stimulates the thyroid gland to secrete triiodothyronine (T3) and thyroxine (T4). The thyroid hormones regulate energy metabolism and also influence growth and development during childhood. The gonadotrophins influence fertility and the development of secondary sexual characteristics through action on the ovaries or testes. FSH regulates gametogenesis and LH influences gonadal steroid secretion. Prolactin regulates breast development and is required for the initiation and maintenance of lactation in females. ADH regulates water balance. Oxytocin is required during parturition and lactation.

1.1.2 Hypothalamo-pituitary communication and vascular supply

The hypothalamus modulates anterior and posterior pituitary hormone secretion through different mechanisms (Asa et al 2002). Communication with the anterior lobe is via a

vascular link: the hypophysial portal system. Hypothalamic-releasing hormones and inhibitory factors are secreted into capillary vessels that stem from a branch of the hypophyseal artery and feed into the hypothalamic-hypophyseal portal veins. The latter travel down the infundibular stalk and branch into a second capillary bed in the anterior pituitary lobe, delivering hypothalamic hormones to target cells. In contrast, the hypothalamus communicates directly with the posterior pituitary via neurones that extend down from the paraventricular and supraoptic nuclei and terminate there (Figure 1b).

Figure 1b: Mechanisms of hypothalmo-pituitary communication



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1.2 Epidemiology of pituitary dysfunction

Pituitary dysfunction may be congenital or acquired. In some cases, no cause is found. Congenital hypopituitarism may arise from birth complications, or be developmental/genetic in origin, sometimes occuring as part of a syndrome. Acquired causes of hypopituitarism include pituitary or other brain tumours; rarely, metastatic disease; neurosurgery or radiotherapy; inflammation or infection (e.g. meningitis, encephalitis, abcess, sarcoidosis,

autoimmune disease); infiltration (e.g. Langerhans cell histiocytosis, haemochromatosis); vascular incidents and trauma.

Pituitary dysfunction (involving any pituitary hormone) is thought to be uncommon in the general population. However, studies determining its incidence and prevalence are few. Regal et al (2002) conducted two cross-sectional surveys of the prevalence of hypopituitarism in a population of approximately 146,000 adults residing in South Galicia in Spain. The prevalence was 29 per 100,000 in 1992, rising to 45.5 per 100,000 in 1999. The average annual incidence of hypopituitarism between January 1993 and December 1999 was 4.21 cases per 100,000, remaining stable throughout the study period.

1.2.1 Epidemiology of pituitary dysfunction in children

Most paediatric prevalence studies have focused on growth hormone deficiency (GHD) because it is the commonest pituitary hormone problem seen in children. Internationally published prevalence figures for GHD in school age children (occurring alone or in combination with other hormone abnormalities, and with or without an obvious cause), range from one in 3,500 to one in 8,650 (Bao et al 1992, Preece et al 1982, Thomas et al 2004, Lindsay et al 1994, Vimpani et al 1977). The prevalence of GHD in Scottish children is approximately one in 4,000 (Vimpani et al 1977).

The exact prevalence of other pituitary hormone deficiencies in children is unclear, but they are considered uncommon. Estimates of the incidence of hypogonadotrophic hypogonadism range from one in 10,000 to one in 86,000 (Mehta & Dattani 2005). It is four times more common in males than females. Isolated deficiencies of ACTH or TSH are rare (Mehta & Dattani 2005). Both are more likely to co-exist with other pituitary hormone abnormalities. ACTH deficiency has been reported in between 4% and 11% of children with idiopathic GHD and TSH deficiency in between 9% and 29% (Walvoord et al 2004, Rona & Tanner 1977, August et al 1990). In a study of 310 childhood cancer survivors, 18% were observed to have ACTH deficiency, all but one of whom also had GHD and/or central hypothyroidism (Rose et al 2005).

Studies investigating the long-term prevalence of pituitary dysfunction after TBI are discussed in sections 1.6 and 1.9.

1.3 Epidemiology of traumatic brain injury

TBI results from an external mechanical force to the brain that causes temporary or permanent neurological dysfunction and may lead to impairment of cognitive, physical and psychosocial function (Khan et al 2003). TBI is a major public health concern and the leading cause of death and disability in young adults (Klasbeek et al 1980, National Institute of Health [NIH] 1998). The incidence of TBI amongst all ages in Europe ranges from 90 to 550 per 100,000 per year, typically estimated from hospital admissions (Bondanelli et al 2005). Many mild head injuries are not included in these statistics. In the United States of America (USA), there were an estimated 1.5 million cases of TBI in 2003, 290,000 hospitalisations and 51,000 deaths (Rutland-Brown 2006).

Each year, between 70,000 and 90,000 Americans are left with significant disability following TBI (NIH 1998). The lifetime cost of supporting TBI survivors who require assistance with activities of daily living ranges from US$ 600,000 to US$ 1.9 million per person (Masel 2004). In the USA, 50% of all TBIs result from road traffic accidents (RTAs) involving pedestrians, cyclists, motorcyclists or car passengers. Falls account for between 20% and 30% of cases and acts of violence, the remaining 20% (NIH 1998). Twice as many males as females sustain TBI, although this gender difference is growing smaller (Masel 2004).

1.3.1 Epidemiology of childhood TBI

Although there has been a marked decline in head injury-related deaths in children in the UK (Parslow et al 2005), childhood TBI remains a significant cause of long-term morbidity and disability (see section 1.10). Causes of TBI include child abuse, falls, RTAs and sports injuries (Acerini et al 2007). United Kingdom (UK) hospitalisation rates for childhood TBI range from 180 to 290 per 100,000 per year (Brookes et al 1990, Hawley et al 2003, Parslow et al 2005). The incidence of TBI doubles between five and 14 years of age (Acerini et al 2007). There is an earlier peak in young children, with those under two years accounting for nearly a fifth of TBI admissions (Hawley et al 2003). Non-accidental head injury in children less than one year of age has an annual incidence of 24.6 per 100,000 (Barlow & Minns 2000).

The Glasgow Coma Scale (GCS) is often used to describe the severity of TBI in children (Teasdale & Jennett 1974, Table 1.i). A modified version exists for use in children aged less than five years (James & Trauner 1985, Table 1.ii). The GCS can be applied at various time points from the time of injury. The post-resuscitation GCS score is considered most relevant prognostically compared with pre-resuscitation scores (Marshall et al 1991a). Scores for eye opening (E), and verbal (V) and motor (M) responses are added together to give a cumulative score of between three and 15. Scoring should reflect the best response elicited. Scores of 13-15 denote minor TBI and 9-12, moderate TBI. Scores of 3-8 indicate severe TBI with coma, providing that the following also apply: E1, $V \leq 2$ and $M \leq 5$ (Miller 1992). Failure to fulfil these criteria suggests moderate TBI.

Approximately 85% of children admitted with TBI have minor brain injuries (Hawley et al 2003). UK-wide, the incidence of severe TBI requiring admission to paediatric intensive care is 5.6 per 100,000 per year (Parslow et al 2005). Almost a third of children admitted to intensive care require neurosurgical evacuation of a subdural or extradural haematoma (Tasker et al 2006).

Table 1.i: The Glasgow Coma Scale

Score	**Eye opening**	**Verbal Response > 5 years**	**Motor Response > 5 years**
1	None	None	No response to supraocular pain
2	To pain	Incomprehensible sounds	Extension to supraocular pain
3	To sound/voice	Inappropriate words	Flexion to supraocular pain
4	Spontaneous	Confused	Withdraws from nail bed pressure
5		Orientated	Localises pain
6			Normal spontaneous movement
Maximum Score	**4**	**5**	**6**

Table 1.ii: The modified Glasgow Coma Scale

Score	**Eye opening**	**Verbal Response < 5 years**	**Motor Response < 5 years**
1	None	None	No response to supraocular pain
2	To pain	Whimpers/moans	Extension to supraocular pain
3	To sound/voice	Cries inappropriately	Flexion to supraocular pain
4	Spontaneous	Less than usual ability; spontaneous, irritable cry	Withdraws from nail bed pressure
5		Alert, babbles, coos, words - to usual ability	Withdraws from touch
6			Normal spontaneous movement
Maximum Score	**4**	**5**	**6**

1.4 Pathophysiology of trauma-related pituitary gland injury

The hypothalamus and pituitary are vulnerable to vascular and mechanical injury as a consequence of trauma. Most of the available data regarding pathophysiology pertain to adult TBI although children have featured in some studies. Direct injury to the pituitary gland and/or hypothalamus has been reported following basal skull fracture in both adults and children (Bistritzer et al 1981, Crompton 1971). Vascular injury to the hypothalamus has been observed in autopsy studies, affecting hypothalamic nuclei (Crompton et al 1971). Shearing injury may damage hypothalamic axons projecting down to the pituitary via the infundibular stalk. Poomthavorn et al (2008) hypothesised that even patients with minor head injury may have significant pituitary stalk injury secondary to contra-coup mechanisms without losing consciousness. Contra-coup injuries occur when the moving head strikes a stationary object, setting the brain in motion and causing it to collide with the opposite side of the skull.

Stalk injuries causing rupture of the hypophysial portal veins compromise the blood supply to the anterior pituitary lobe, resulting in ischaemia and necrosis (Acerini 2007). Vasospasm has been shown to be another potential mechanism of ischaemic injury in adults (Oertel et al 2005). Secondary pituitary insult in adult TBI survivors may be caused by hypoxia, hypotension, anaemia, or raised intracranial pressure (Kelly et al 2000). The pituitary and its blood supply are vulnerable to compression from brain and/or pituitary gland swelling within the confines of the sella turcica (Bondanelli 2005). The significance of secondary brain insult is discussed in more detail in section 1.13.

Benvenga et al (2000) summarised the results of early autopsy studies describing injuries to the hypothalamus and pituitary after TBI. Structural damage to the pituitary gland was seen in between 26% and 86% of cases across four studies involving 496 cases in total. Haemorrhage and/or necrosis of the anterior or posterior lobes, capsule or infundibular stalk were all observed. In another series, Harper et al (1986) reported anterior lobe infarcts in 38 of 100 (mostly adult) cases following non-missile head injury, often in association with haemorrhage (Harper et al 1986). Most recently, Salehi et al (2007) examined the pituitary glands of 42 victims of RTAs who died within a week of injury. The specimens were grouped according to whether the victim died immediately (n=12) or after an interval of up to seven days post-injury (n=30). No pituitary infarcts were observed in the first group,

whereas in the latter group, acute infarcts were seen in 13 of 30 cases. These ranged from focal insults to subtotal necrosis involving 90% of the anterior pituitary lobe.

Benvenga et al (2000) have also attempted to summarise the types of hypothalamo-pituitary lesions seen in *survivors* of TBI with pituitary dysfunction. They reviewed the cranial computed tomography (CT) scans or magnetic resonance imaging (MRI) in 76 such cases (age range not specified), finding lesions in 93% (Benvenga et al 2000). Infarction of the anterior pituitary was seen in 25%, and haemorrhage of the posterior pituitary or hypothalamus in 26% and 29% respectively. In 7%, no hypothalamo-pituitary abnormalities were seen.

The apparent susceptibility of some hormone axes and not others to dysfunction after TBI may be explained by the functional anatomy of the anterior pituitary. The laterally placed GH- and gonadotrophin-secreting cells are particularly vulnerable to hypophysial portal vein damage (Daniel et al 1959, Kelly et al 2000). In contrast, ACTH- and TSH-secreting cells are positioned more ventrally, within reach of arteries supplying the posterior lobe. Factors influencing emergence of late neuroendocrine dysfunction are unclear. The effects of damage sustained may take time to become functionally apparent (Tanriverdi et al 2006).

Regeneration of the portal vessels may permit some resumption of anterior lobe function over time (Daniel et al 1959). Recovery may also be seen as oedema subsides (Tanriverdi et al 2006, Agha et al 2004a). Young children, in whom overall outcomes after TBI appear to be worse than for other age groups (section 1.10), could demonstrate less potential for recovery of pituitary gland function than adults.

1.5 Acute changes in pituitary function following adult TBI

Pituitary dysfunction following TBI can be divided into transient changes during the acute phase post-injury and long-term hypopituitarism. Acute changes in pituitary function after TBI are well recognised. Elevated cortisol levels after brain trauma have been demonstrated in several studies (Barton et al 1987, Feibel et al 1983, Steinbok et al 1979). Activation of the hypothalamo-pituitary-adrenal (HPA) axis occurs in the context of hypovolaemia, emotional stress and tissue damage (Steinbok et al 1979). This and lowering of cortisol binding protein concentrations during the first week of critical illness result in increased

circulating levels of free cortisol (Klose et al 2007a). Tissue sensitivity to glucocorticoids also increases.

Pituitary stalk injury may cause elevated prolactin levels due to disruption of the negative feedback system between the hypothalamus and the pituitary gland (Mehta & Dattani 2005). Prolactin secretion also increases in times of physical or psychological stress (Agha et al 2004a). In contrast, the hypothalamo-pituitary-gonadal (HPG) axis down-regulates during critical illness, possibly to conserve utilisation of metabolic substrates by vital organs (Agha et al 2004a). Recent prospective studies of pituitary function in the acute phase following TBI have reported low gonadotrophin concentrations in between 40% and 80% of subjects (Agha et al 2004a, Tanriverdi et al 2006, Klose et al 2007a). Acute phase TSH insufficiency has been observed in between 2% and 33% of subjects (Agha et al 2004a, Tanriverdi et al 2006, Klose et al 2007a). Klose et al (2007a) questioned the classification of these early changes as 'abnormalities' rather than adaptive physiological mechanisms regulating the inflammatory response after TBI.

Abnormalities of GH and ACTH secretion in the acute phase are harder to measure and to interpret. Low basal GH levels do not preclude normal function as GH secretion is pulsatile and best evaluated using a stimulation test. IGF1 concentrations do not always reflect true GH status and are most useful as a guide only (Murray et al 2000, Hoeck et al 2000, Lissett et al 2003, Sizonenko et al 2001). Baseline early morning cortisol concentrations do not provide a reliable indicator of maximal HPA axis capacity (Agha et al 2004b, Agwu et al 1998). Furthermore, during acute illness, the circadian rhythm of ACTH and cortisol secretion may be altered (Steinbok et al 1979), confounding the interpretation of basal levels.

Stimulation testing of the HPA axis is hindered by the fact that the insulin tolerance test (ITT) is contraindicated in patients with neurological disturbance. The ITT is the most reliable test for evaluating the integrity of the entire HPA axis. It is described in detail in the Methods chapter (section 3.4.2.1) and discussed in section 5.2.4.2. The glucagon test, also described in the Methods chapter, fails to elicit a cortisol response in approximately 10% of healthy individuals (Agha et al 2004a, Rao & Spathis 1987). Other options include the short Synacthen and corticotrophin releasing hormone (CRH) tests. Synacthen (tetracosactide) is an analogue of ACTH, comprising its first 24 amino acids ($ACTH_{1-24}$). The standard (250 μg) Synacthen test is poorly sensitive and specific for secondary causes of adrenal insufficiency (Cunningham et al 1983, Orme et al 1996, Lindholm & Kehlet 1987, Maghnie

et al 2005, Soule et al 1996). The low dose (1 μg) Synacthen test may correlate more closely with the ITT than the standard test (Orme et al 1996, Agwu et al 1999). The CRH test is not widely used and reliable reference data are lacking (Tetlow & Clayton 2005). It can only be used to assess pituitary-adrenal function and is thus of no use in the evaluation of pituitary stalk lesions. The various methods of evaluating the HPA axis are discussed in more detail in section 5.2.4.

1.5.1 Studies of acute phase pituitary dysfunction following adult TBI

The challenges of assessing the GH and HPA axes are reflected in the varied design of acute phase studies. Tanriverdi et al (2007) evaluated basal endocrine function in 104 subjects within 24 hours of TBI. Nine percent were reported to have ACTH deficiency and 20%, GHD, on the basis of low basal cortisol and IGF1 concentrations respectively. Cohan et al (2005) measured basal cortisol and ACTH samples twice daily (at 6 a.m. and 4 p.m) for up to nine days post-TBI in 80 subjects. Some degree of acute phase adrenal insufficiency was reported in 53% of subjects. This was defined as any single serum cortisol level less than 5 μg/dL (140 nmol/L, regardless of time of day) or two serum cortisol levels less than 15 μg/dL (420 nmol/L, equivalent to the 25th percentile amongst comparator group of non-TBI trauma patients). However, serum cortisol concentrations <140 nmol/L are not uncommon in the afternoon or evening in healthy individuals. Typically, the 5th or 10th percentile is used to define cut-offs derived from control group data.

Agha et al (2004a) evaluated 50 consecutive neurosurgical admissions with moderate or severe trauma at a median of 12 (range 7 to 20) days following injury. They used the glucagon test to assess GH and ACTH secretion, comparing responses with those of 31 healthy matched controls. Eighteen percent had GH responses below the 5 μg/L cut-off established from control data. Only 6% had GH responses less than 3 μg/L, the accepted cut-off for diagnosis of severe GHD using the ITT (Growth Hormone Research Society [GHRS] 1998). There were no significant differences in IGF1 concentration, age, body mass index (BMI) or severity of injury between those with normal and abnormal GH responses. Inadequate cortisol responses to glucagon (<450 nmol/L) were seen in 16% of subjects, with low baseline early morning cortisol levels in 6%. Insufficient cortisol responses to glucagon were also seen in 10% of controls.

Dimopoulou et al (2004) reported ACTH deficiency in 15% of subjects requiring intensive care following moderate or severe TBI, evaluated using the low dose (1 μg) Synacthen test.

They found associations between ACTH deficiency and both raised interleukin-6 levels and prior vasopressor dependency. Klose et al (2007a) evaluated 46 subjects within 12 days of injury, detecting suboptimal cortisol responses to the standard (250 μg) Synacthen test (30-minute cortisol level < 500 nmol/L) in two (4%). Early abnormalities were significantly associated with severity of injury but not with the risk of late pituitary dysfunction. In spite of this, Klose et al (2007a) hypothesised that a low or low-normal circulating cortisol level might be an indicator of risk for future HPA insufficiency. However, three subjects who developed long-term HPA insufficiency all had basal cortisol levels overlapping those of subjects who remained healthy.

Diabetes insipidus (DI), resulting from ADH deficiency, has been reported in between 22% and 26% of TBI patients in the acute phase (Agha et al 2004c, Agha et al 2005a). It is often transient and may be caused by oedema that later resolves.

1.6 Long-term pituitary dysfunction after TBI in adulthood

1.6.1 Case series in survivors of TBI

Cyran (1918) reported the first case of persistent hypopituitarism following TBI 90 years ago. TBI seemed, from an early case series, to be an infrequent cause of pituitary dysfunction, accounting for only four of 595 cases reviewed (Escamilla and Lisser 1942). Larger case series followed, with Edwards and Clark (1986) identifying 53 cases in the literature and Benvenga et al (2000), 367.

In Benvenga's cohort, the male:female ratio was 5:1 and 74% were survivors of RTAs. Peak age at injury was 20 to 29 years (35%). Twenty-five percent were aged less than 20 years at injury; the majority (21%), adolescents aged 11 to 19 years. There was a history of coma in 93% of cases, typically lasting days or weeks (where information regarding duration was available). Skull fracture featured in just over 50% of cases.

Seventy percent of Benvenga's cohort developed symptoms early and pituitary dysfunction was diagnosed within a year of TBI. In 15%, the diagnosis was made five or more years after head injury, and not until 36 and 42 years afterwards in two cases. Gonadotrophin deficiency was identified in nearly 100% of patients tested. Deficiencies of TSH, ACTH, GH and prolactin were present in 44%, 53%, 24% and 4% respectively. Diabetes insipidus featured in 30%. Pituitary dysfunction was rarely transient.

Whilst symptoms of hypopituitarism are often vague, signs associated with HPG axis dysfunction are more specific and may prompt referral to an endocrinologist for investigation. This may explain the predominance of gonadotrophin deficiency in the aforementioned case series. An alternative explanation is that gonadotrophin deficiency is truly the commonest neuroendocrine consequence of TBI and this will be explored in due course.

Although the link between TBI and pituitary dysfunction has now been recognised, TBI is still rarely listed as a cause of hypopituitarism in patients under follow-up. Su et al (2005) reviewed the medical records of 635 patients diagnosed with hypopituitarism between 1982 and 2000: TBI was identified as the cause in only 18 (2.8%). Routine screening of other at-risk groups, for example, survivors of brain cancer who have received cranial irradiation, is well established. TBI survivors seem to be under-investigated and risk delays in diagnosis of hypopituitarism. GH-deficient TBI survivors appear to wait longer from onset of symptoms to treatment, than patients with GHD from other causes, as indicated by time to inclusion on the Pfizer International Metabolic (KIMS) database (Casanueva et al 2005).

1.6.2 Studies of long-term pituitary dysfunction after TBI

Case series comprise self-selecting patients (who present with symptoms) and some patients in whom post-traumatic pituitary dysfunction is an incidental finding. They provide little or no information about affected TBI-survivors who are asymptomatic or those who do not develop pituitary dysfunction at all. To define the extent and nature of the problem more clearly, several studies have been conducted in survivors of TBI in adulthood over the last decade. Persistent hypopituitarism has been observed in between 11% and 69% of subjects. The results are summarised in table 1.iii.

1.6.2.1 Cross-sectional studies

In early cross-sectional studies by Kelly et al (2000) and Lieberman et al (2001), the interval between TBI and investigation for hypopituitarism ranged from three months to 23 years. Kelly's subjects underwent basal and dynamic anterior pituitary function tests, including the ITT for evaluation of the GH and HPA axes. All subjects with pituitary dysfunction (34%) had GCS scores of 10 or less at the time of TBI. Hypoxic or hypotensive insults were more common and affected subjects were more likely to have had signs of diffuse swelling on cranial CT scan than TBI survivors with normal endocrine function (Kelly et al 2000).

Table 1.iii: Studies of long-term hypopituitarism after TBI in adulthood

Author	Design	Severity of head injury	Subjects (males)	Interval TBI-assessment	Any axis affected	>1 axis affected	Axis affected				
							GH	ACTH	LH/FSH	TSH	ADH
Kelly et al 2000	C, MC	MIN, MOD & SEV	22 (18)	0.3 - 23 y	36%	9%	18%	5%	23%	5%	NS
Lieberman et al 2001	C	SEV in 32 of 38 in whom GCS known*	70 (46)	0.1 - 23 y (median 13 m)	69%	17%	10%	7%	0%	22%	NS
Agha et al 2004b & c	C, MC	MOD & SEV	102 (85)	6 - 36 y (median 17 m)	28%	6%	9%	13%	12%	1%	7%
Bondanelli et al 2004	C	MIN, MOD & SEV	50 (40)	12 - 64 m	54%	12%	8%	0%	14%	10%	0%
Leal-Cerro et al 2005	C	SEV	99 (NS)	1 - 5 y	42%	15%	10%	11%	29%	10%	3%
Aimaretti et al 2005a	P	MIN, MOD & SEV	70 (50)	12 m	23%	10%	20%	7%	11%	6%	3%
Agha et al 2005a & b	P, MC	MOD & SEV	50 (38)	12 m	NS	NS	10%	18%	12%	2%	6%
Tanriverdi et al 2006	P	MIN, MOD & SEV	52 (43)	12 m	51%	10%	38%	19%	8%	6%	NS
Schneider et al 2006	P, MC	MIN, MOD & SEV	78 (52)	12 m	36%	3%	10%	9%	21%	3%	NS
Herrmann et al 2006	C	SEV	76 (53)	22 ± 10 m (median 20 m)	24%	NS	8%	3%	17%	3%	NS
Bondanelli et al 2007	C	MOD & SEV	72 (56)	180 - 365 d (median 212 d)	31%	7%	14%	4%	14%	4%	3%
Klose et al 2007a	P+, MC	MIN, MOD & SEV	46 (33)	12 m	11%	7%	11%	7%	1%	1%	1%
Bavisetty et al 2008	P, MC	MIN, MOD & SEV	70 (57)	6 - 9 m	21%	4%	16%	0%	11%	0%	1%

C, cross-sectional; P, prospective; MC, matched controls; P+, prospective cohort nested within a larger cross-sectional study (results for larger study not tabulated); MIN, minor; MOD, moderate; SEV, severe; y, years; m, months; d, days; NS, not specified; *GCS unknown in the other 32/70 subjects

Lieberman et al (2001) recruited subjects from a population of TBI survivors residing in a community-based brain injury rehabilitation facility. All had some degree of cognitive impairment resulting from their TBI. The prevalence of pituitary dysfunction observed amongst participants was high (69%). In addition to basal testing, the standard Synacthen test (250 μg) was carried out to investigate the HPA axis. Dynamic testing of the GH axis was performed in 69% of subjects. Of these, twenty underwent both glucagon and L-dopa (3,4-dihydroxy-L-phenylalanine) stimulation tests; and 28, the glucagon test only. The L-dopa test is considered unreliable in the evaluation and diagnosis of adult GHD (Agha et al 2004b). For this reason, only subjects who failed the glucagon test (n=7) were reported to be GH-deficient: 15% of those who underwent a glucagon test, or 10% of the whole cohort. Abnormalities of the thyroid axis were reported in 22% of subjects but only 12% had low or inappropriately normal TSH concentrations in the presence of low thyroxine levels.

In another cross-sectional study, Leal-Cerro et al (2005) identified 249 survivors of severe TBI admitted over a 5-year period and invited them to complete two screening questionnaires by post. These comprised an unvalidated hypopituitarism questionnaire and the Quality of Life - Assessment of Growth Hormone Deficiency in Adults (QoL-AGHDA) measure. 170 subjects consented to take part in the study. From their questionnaire results, investigators decided that 57 subjects did not require pituitary hormone evaluation. Fourteen others declined testing. Thus, basal endocrine tests were carried out in 99 subjects. Dynamic function tests were only performed if basal results were low, in spite of the fact that GHD can exist in the context of normal IGF1 levels (Murray et al 2000, Lissett et al 2003) and that individuals with normal baseline early morning cortisol levels can have suboptimal responses to physiological stressors (Agha et al 2004b, Clark et al 1998). However, where dynamic testing was carried out, three different tests were used to confirm GHD.

Bondanelli et al (2004) evaluated 50 TBI patients admitted over a five-year period, at least one year after head injury in all cases. Injury severity ranged from severe to minor. The GH-releasing hormone plus arginine (GHRH-arginine) test was used to evaluate the GH axis. No other stimulation tests were performed. Results for the HPA axis, reported as normal in all subjects on the basis of baseline early morning cortisol levels greater than 200 nmol/L, should be interpreted with caution. There was a significant relationship between pituitary dysfunction and head injury severity, but no relationship to other head injury

characteristics, interval since injury or Glasgow Outcome Score was found. Abnormalities on cranial MRI were seen in only two of 27 (7%) subjects with pituitary dysfunction. The authors hypothesised that post-traumatic hypopituitarism may result from hypoxic insult leading to functional damage of the hypothalamus and pituitary.

In a second study, Bondanelli et al (2007) investigated pituitary function in 72 survivors of moderate or severe TBI during rehabilitation, between 180 and 365 days after head injury. The low dose synacthen test (1 μg) was performed in subjects with baseline early morning cortisol concentrations between 98 nmol/L and 285 nmol/L. Baseline cortisol concentrations below 98 nmol/L were presumed to indicate HPA insufficiency; concentrations greater than 285 nmol/L were presumed to indicate normal HPA function. GH cut-offs for the GHRH-arginine test were stratified according to body mass index (BMI) because blunted GH responses are a recognised feature of obesity (Corneli et al 2005). Outcome after TBI was evaluated using the Level of Cognitive Functioning Scale, the Disability Rating Score and the Functional Independence Measure and compared between groups with normal versus abnormal pituitary function. These findings are discussed in section 1.7. Multiple regression analyses identified GCS and peak GH response to the GHRH-arginine test as independent predictors of outcome.

Hermann et al (2006) also investigated subjects during rehabilitation following TBI. Seventy-six survivors of severe TBI underwent testing at a median of 20 months post-injury. The GHRH-arginine test was used to evaluate the GH axis. GHD was defined as a peak GH response less than 9 μg/L and was confirmed by ITT (peak GH response <3 μg/L). Dynamic testing of the HPA axis was only performed in subjects undergoing the ITT to confirm GHD.

Agha et al (2004b, 2004c) evaluated pituitary function in 102 survivors of moderate or severe TBI at a median of 17 months (range 6-36) following injury. Healthy volunteers were recruited to provide control data. Dynamic testing of the GH and HPA axes involved the glucagon test and either the ITT or GHRH-arginine test plus standard Synacthen test (250 μg). Only 9% of subjects were diagnosed with what would be considered to be severe GHD (GH peak <3 μg/L during ITT or <9 μg/L during the GHRH-arginine test). GH-deficient subjects had significantly higher BMI and lower IGF1 concentrations than those with normal GH responses. Suboptimal cortisol response to stimulation was associated with lower baseline early morning cortisol levels in four of 13 subjects. There were no relationships between pituitary dysfunction and severity of injury or any other head injury features. The

posterior pituitary was evaluated using the water deprivation test, using a cut-off of 700 mOsm/kg to diagnose DI (based on measurements in 27 healthy controls). Seven percent of subjects had evidence of permanent DI, all of whom responded to desmopressin therapy. Subjects with DI were more likely to have had a severe head injury. The authors suggested that permanent DI might be due to irreversible damage to the supraoptic or periventricular nuclei or their projections into the posterior pituitary.

1.6.2.2 Prospective studies

In a follow-up to the previous study, Agha et al (2005a, 2005b) evaluated 50 of 69 (73%) patients admitted with moderate or severe TBI at baseline, six and 12 months post-injury. Control data were obtained from healthy volunteers. Acute phase deficiencies, described section 1.5, resolved in many subjects. However, new abnormalities emerged over time. Gonadotrophin deficiency, present in 80% acutely, resolved in 85% of affected subjects by the 12-month assessment. Of nine cases of GHD at baseline, four recovered within six months, two new cases emerged and a fifth case recovered by the 12-month assessment. Four of eight subjects with HPA insufficiency in the acute phase improved within six months but five new cases emerged. Nine subjects had persistent HPA insufficiency at 12 months, as evaluated using the glucagon test. One case of TSH deficiency at baseline resolved and one new case emerged.

Other investigators report similar patterns of changing pituitary hormone status in the months following TBI. Tanriverdi et al (2006) investigated 52 subjects' pituitary function at baseline and a year post-TBI. Baseline hormone abnormalities (section 1.5) resolved in 58% of subjects by the 12-month assessment. New abnormalities emerged in 52%, particularly affecting the GH and HPA axes. However, dynamic testing was only performed at the 12-month assessment so the accuracy of baseline diagnoses of GHD and HPA insufficiency is unclear. A high proportion of subjects (38%) developed GHD (GH peak <10 μg/L), diagnosed using the combined GHRH and GH-releasing peptide 6 (GHRP-6) test. No control data were collected. GH-deficient subjects were older than GH-sufficient subjects, and had higher BMIs. There was no correlation with IGF1 levels. The low dose Synacthen test (1 μg) was used to evaluate the HPA axis. There was no correlation between pituitary dysfunction at 12 months and severity of head injury.

Aimaretti et al (2005a) evaluated pituitary function in 70 TBI patients at three and 12 months post-head injury. Thirty-three percent had abnormalities in at least one hormone axis at

three months, falling to 23% at a year. Half of subjects with multiple abnormalities had panhypopituitarism. Abnormalities resolved in several subjects with isolated deficiencies but none with panhypopituitarism. New abnormalities emerged in four subjects (12%) during the study period, in two cases in subjects who already had isolated deficiencies at three months. The GHRH-arginine test was used to diagnose GHD. Severe GHD (peak GH response < 9 μg/L) was diagnosed in 20%. Fifty percent of GH-deficient subjects had other hormone abnormalities. Dynamic testing of the HPA axis was not performed so the incidence of HPA insufficiency may have been underestimated.

In a similar study by Schneider et al (2006), 78 subjects were investigated for pituitary dysfunction at three and 12 months post-TBI. The GHRH-arginine and standard Synacthen tests were used to evaluate the GH and HPA axes respectively. Dynamic function tests were performed in 77 subjects at three months and pituitary hormone abnormalities were identified in 56%. Only subjects with pituitary dysfunction at three months or abnormal basal results at 12 months underwent repeat dynamic function testing at 12 months (n=32) so it is possible that GH or HPA insufficiency were missed in some cases. Pituitary dysfunction was reported in 36% of subjects at 12 months.

Klose et al (2007a) assessed 46 subjects at baseline, three, six and 12 months after TBI, comparing results with data from 30 healthy controls. Nearly 50% had had minor head injuries. Dynamic function testing was performed at three and 12 months post-head injury. Basal pituitary hormone abnormalities were seen in 76% of subjects at baseline (section 2.5), resolving in the majority by three months. Thirteen percent had evidence of pituitary dysfunction at three months, falling to 11% at 12 months, all of whom had GHD. GHD was diagnosed using the ITT (peak GH response <3 μg/L) or the GHRH-arginine test (peak GH response <9 μg/L) where the ITT was contraindicated. Subjects with abnormal results were re-tested to confirm the diagnosis. The HPA axis was evaluated using the ITT or the standard Synacthen test (peak or 30-minute cortisol cut-offs 500 nmol/L respectively). More than one axis was affected in three (7%) subjects. There were no significant associations between pituitary dysfunction and severity of injury or raised intracranial pressure but those with hormone abnormalities were older. Quality of life findings are discussed in 1.11.3.

This prospective cohort was part of a larger cross-sectional study (Klose et al 2007b) of 104 subjects assessed at 10-27 (median 13) months post-injury. Similar proportions of hormone abnormalities were seen amongst the larger group: GHD in 15% of subjects, HPA

insufficiency in 5%, gonadotrophin deficiency in 2%, central hypothyroidism in 2% and DI in 2%. Pituitary dysfunction was seen in 15% of subjects, with multiple deficits in 4%. In the larger group, there were significant associations between pituitary dysfunction and female gender, older age, higher BMI, severe TBI and raised intracranial pressure (ICP). However it is unclear how representative the sample was as only 25% of patients enrolled at the time of injury were subsequently evaluated. Limited information was provided about the characteristics of the 75% who were excluded or withdrew prior to testing.

Sample bias may also have featured in a study by Bavisetty et al (2008). Pituitary function was evaluated in 70 of 206 TBI subjects at between six and nine months following TBI. Of the remainder, 45 patients died, 15 were excluded and over 76 were either lost to follow-up or withdrew from the study prior to the 6-month evaluation. Fifty-seven subjects who completed the study were also assessed three months post-TBI. The GHRH-arginine and low dose Synacthen tests were used to investigate the GH and HPA axes. Based on fifth and tenth percentile data from healthy volunteers, severe GHD and GH insufficiency were defined as peak GH responses less than 5 μg/L and 10 μg/L to the GHRH-arginine test, respectively. The percentage with GH responses less than 10 μg/L is quoted in Table 1.iii). Pituitary dysfunction at three months resolved in 50% of affected subjects (n=14) by their next assessment. New abnormalities emerged in six subjects with no prior record of pituitary dysfunction. Subjects with pituitary dysfunction six to nine months post-TBI were more likely to have had abnormal findings on initial CT scan, diffuse brain swelling and intracranial haemorrhage evacuation. They also had higher BMI at assessment and a worse Disability Rating Scale. Mental health and quality of life findings are discussed in full in 1.7 and 1.11.3.

Aimaretti et al (2005b) have conducted the only study of pituitary dysfunction in survivors of TBI occurring in the transition phase between childhood and adulthood. Twenty-three young people aged between 16 and 25 years were evaluated at three and 12 months post-injury. Pituitary dysfunction was present in 34.6% at three months and 30% at 12 months post-injury. These findings are discussed in more detail in section 5.2.6.

1.6.2.3 Summary and consensus guidelines

The aforementioned studies have demonstrated persistent hypopituitarism in between 11% and 69% of subjects following adult TBI. GHD is most common and has been observed in between 8% and 38% of subjects. HPA insufficiency has been reported in between 0 and

19%, gonadotrophin deficiency in between 0% and 29%, central hypothyroidism in between 0 and 22% and DI in between 0% and 7% of survivors.

It is difficult to draw comparisons between the findings of individual studies due to significant differences in design. There have been wide variations in the interval between TBI and endocrine evaluation, tests employed and test cut-offs used. Some studies have used two stimulation tests to confirm GHD or HPA insufficiency where others have drawn conclusions from basal hormone results. However it is clear from longitudinal studies that many early pituitary hormone abnormalities resolve in the months after TBI, and some new abnormalities emerge.

Appropriate information regarding numbers of patients excluded from taking part or who declined to participate is not always presented. This can make it difficult to assess whether or not the cohort investigated is representative of the wider study population. This is particularly relevant to studies where conclusions are drawn regarding associations between post-traumatic pituitary dysfunction and baseline characteristics like gender, age or head injury severity.

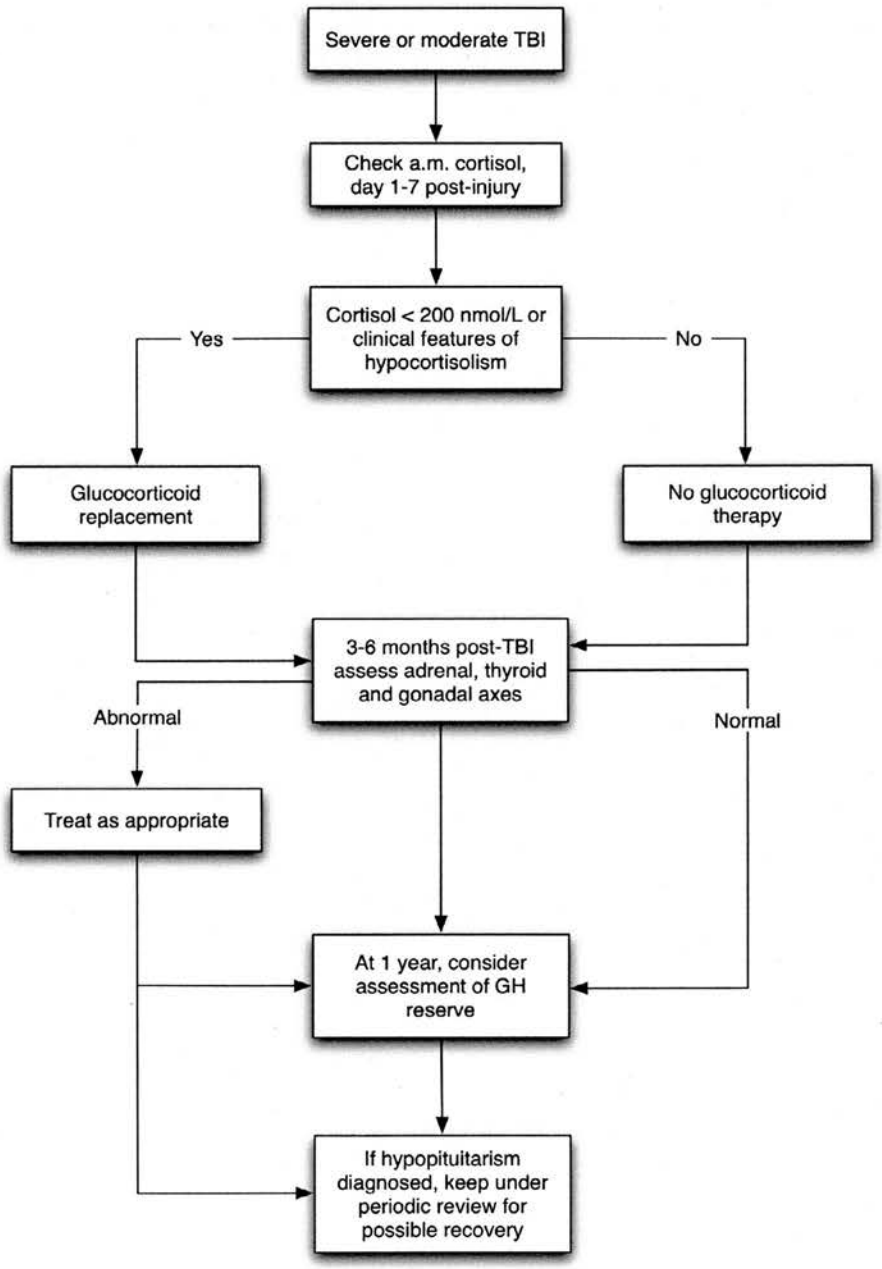
In a recent meta-analysis of existing studies (Schneider et al 2007), the pooled prevalence of anterior pituitary dysfunction at least five months following TBI was 27% (95% confidence intervals [CI], 23% to 29%). The pooled prevalence of hypopituitarism was greater in survivors of severe TBI than mild or moderate TBI. It was suggested that there be a lower threshold for investigation of patients with basal skull fractures, diffuse axonal injuries, raised intracranial pressure and prolonged intensive care admissions.

Consensus guidelines published in 2005 suggest conducting baseline tests to screen TBI survivors for pituitary dysfunction three and 12 months post-injury (Ghigo et al 2005). However, as previously discussed, this approach may result in cases of GHD and/or HPA insufficiency being missed. In a variation on the consensus algorithm, Behan et al (2008) recommend dynamic function testing of GH reserve 12 months post-injury (Figure 1c). They also advocate use of a stimulation test to evaluate the HPA axis at three to six months.

Recent consensus guidelines on adult GHD have recommended that testing should be extended from hypothalmo-pituitary disease and cranial irradiation to include survivors of

traumatic brain injury (Ho et al 2007). Because early post-traumatic hormone abnormalities may resolve, it is suggested that GH testing is performed at least 12 months after injury.

Figure 1c: Suggested algorithm for assessment of adult TBI survivors (adapted from Behan et al 2008)



1.7 Significance of long-term pituitary dysfunction after adult TBI

The potential impact of hypopituitarism after TBI is considerable. Pituitary dysfunction may compromise the general health and wellbeing of survivors and impede rehabilitation (Agha et al 2004b). TSH deficiency is associated with lethargy, muscle weakness and neuropsychiatric symptoms. ACTH deficiency may have life-threatening consequences during acute illness or injury and additionally causes lethargy, muscle weakness and poor exercise tolerance. Testosterone and oestrogen deficiencies impair sexual and reproductive function in men and women respectively, reduce bone mineral density (BMD) and, in the case of testosterone, also lean body mass (LBM). Adult GHD is associated with decreased strength, reduced mobility, reduced LBM, impaired cardiac function and reduced BMD (Agha et al 2004b, Lieberman et al 1996, Carroll et al 1998). Other features of adult GHD include sleep disturbance, social isolation, mood changes and a reduction in general wellbeing. There may be cognitive impairment, particularly in the domains of attention, memory and executive function (Falleti et al 2006).

Many TBI survivors suffer from similar neuropsychological problems to those seen in adult GHD (Mazaux & Richer 1998, Colantonio et al 1998, Morton & Wehman 1995). These similarities are summarised in Table 1.iv. Neurological and psychological symptoms emerge weeks and months after TBI (MacKenzie et al 2002). Clinically, there is a period in which brain function is stable, followed by evidence of later deterioration. Some of the deterioration in function may relate to evolving tissue damage following the head injury. Human and animal studies suggest that there may be progressive white matter degradation following TBI (MacKenzie et al 2002, Rodriguez-Paez et al 2005). MacKenzie et al found brain abnormalities on imaging supporting the idea of a chronic post-head injury syndrome in adults, with decreasing brain volume following mild and moderate TBI.

Unrecognised pituitary dysfunction may compound these problems. Growing evidence suggests that the GH-IGF1 axis in particular has an important role in the recovery of the central nervous system from experimental injury. Neuro-protective and regenerative effects of the GH–IGF1 system have been demonstrated in different animal models of brain injury (Scheepens et al 2001, Bondanelli et al 2007). GH and IGF1 have effects on vascular reactivity and tone and seem to be involved in central nervous system repair processes. Both the GH-IGF1 and hypothalamo-pituitary-thyroid axes also influence postnatal and adult neurogenesis. Zhang et al (2009) observed that pharmacologically inducing hypothyroidism

in rats led to downregulation of neurogenesis in the dentate gyrus of the hippocampus, a region where neurogenesis occurs throughout adulthood. Aberg et al (2000) demonstrated selective induction of neurogenesis in the same region following a peripheral infusion of IGF1.

Table 1.iv: Symptoms in adult survivors of moderate or severe TBI (adapted from Ghigo et al 2005)

Problem	Moderate or severe TBI	Adult-onset GHD
Memory impairment	√	√
Concentration impairment	√	√
Decreased IQ	√	√
Impaired judgement / problem solving	√	X
Poor organisational skills	√	X
Decreased quality of life	√	√
Fatigue	√	√
Anxiety	√	√
Depression	√	√
Social isolation	√	√
Deterioration of sex life	√	√
Increased unemployment	√	√

IQ, intelligence quotient

In terms of effects on cognitive function, experimental studies have shown that GH/IGF1-deficient animals exhibit reduced glucose metabolism in many brain regions, especially those involved in the hippocampus-dependent processes of learning and memory (Sonntag et al 2006). Increases in neurotransmitters implicated in memory function and attentional performance have been seen in treated GH-deficient individuals (Popovic et al 2005). GH replacement also leads to changes in the levels of neurotransmitters in the cerebrospinal fluid that are similar to those seen in patients treated for depression (Popovic et al 2005).

Springer and Chollet (2001) highlighted the potential impact of pituitary dysfunction on prognosis after TBI. They described the case of a man presenting with severe cognitive impairment and flat affect two months after a RTA in which he sustained a TBI. Post-traumatic dementia was diagnosed. Severe GHD was diagnosed two years later on the basis of a low peak GH response (<0.15 μg/L) to an unspecified stimulation test. Other pituitary hormone abnormalities were also identified. Improvements were seen in the patient's cognitive abilities after initiation of hormone replacement therapy including GH. This is consistent with studies of (non-head-injured) GH-deficient adults that have shown some improvement in cognitive function with GH replacement therapy (Falleti et al 2006, van Dam 2005).

In a retrospective study, Popovic et al (2004) evaluated endocrine, neurological, neuropsychological and psychiatric status in 67 TBI survivors at least a year after injury. Nearly 50% of subjects were found to have mild or moderate depression, regardless of endocrine status. Neuropsychological testing revealed significant positive correlations between peak GH response in the combined GHRH and GHRP-6 test and verbal learning and short-term memory abilities. Paranoid ideation and somatisation were negatively correlated with peak GH response.

Bondanelli et al (2007) reported worse scores at discharge from rehabilitation in three outcome measures (the Level of Cognitive Functioning Scale, Disability Rating Score and Functional Independence Measure) amongst TBI survivors with pituitary dysfunction than in subjects with normal pituitary function. Scores in the Level of Cognitive Functioning Scale and Disability Rating Score measures correlated with peak GH response to the GHRH-arginine test. The authors argued that GH-deficient adults should be treated during rehabilitation on this basis. Bavisetty et al (2008) found that TBI survivors with GHD had worse Disability Rating Scale scores and were at greater risk of depression than subjects with normal pituitary function. Further details of both these studies can be found in section 1.6.2. Quality of life (QoL) findings from the Bavisetty study are discussed in section 1.11.3.

1.8 Acute phase pituitary dysfunction after childhood TBI

Although adolescents have been included in some 'adult' TBI studies, younger children have not been represented. However, a body of evidence is accumulating to suggest that children too may be at risk of pituitary dysfunction after TBI. Einaudi et al (2006) measured basal endocrine function in 30 children within 72 hours of TBI. Abnormalities of thyroid or water haemostasis were seen in seven subjects, including DI in one case. Low cortisol concentrations were detected in four subjects and low prolactin levels in four others but as treatment had been instituted in all eight prior to proper evaluation, the true nature of these abnormalities could not be confirmed. All hormone abnormalities resolved fully.

In another study (as yet only published in abstract form), Thomas et al (2007) investigated ten consecutive TBI patients within 48 hours of admission to the paediatric intensive care unit (PICU). Basal endocrine tests were conducted, in addition to 24-hour cortisol profile. Diminished cortisol and ACTH levels were reported in two children, DI in one and central hypothyroidism in one. Acute post-traumatic DI has previously been associated with poor

outcome. In a series of 19 children with severe brain insult and DI (12 with a history of trauma), only three survived (Barzilay et al 1988).

1.9 Long-term pituitary dysfunction after childhood TBI

1.9.1 Case series in survivors of childhood TBI

There are few early reports of long-term pituitary dysfunction following childhood TBI. Goldman and Jacobs (1945) reported a case of DI presenting in a 15 year-old girl who died aged 21 following development of anterior pituitary failure. In another example, Klachko et al (1968) describe how a 39 year-old male was diagnosed with anterior pituitary dysfunction 35 years after sustaining a basal skull fracture.

Recently, Acerini et al (2006) reviewed 20 cases of post-traumatic pituitary dysfunction published between 1976 and 2006. The details are summarised in Table 1.v. GHD was the commonest abnormality seen in the Acerini series, in 85% of cases (Acerini et al 2006). Most children had abnormalities of more than one pituitary hormone axis. There were only two cases of permanent DI: one, isolated, the other, associated with anterior pituitary dysfunction (Mariani et al 1996). Hypopituitarism resulting from TBI was reported across a range of ages and via a range of mechanisms. In six cases, the preceding head injury was mild with no loss of consciousness (Benvenga et al 2004, Mariani et al 1996, Yamanaka et al 1993). Others developed pituitary dysfunction following severe brain injury associated with subdural haematoma or skull fracture (Paxson & Brown 1976, Girard & Marielli 1996, Grossman & Sanfield 1994, Valenta & de Feo 1980). The mechanism in three children was thought to be child abuse sustained as infants featuring subdural haematoma (Miller et al 1980).

Diagnosis was delayed by several years in the majority of cases. Indeed, three individuals were diagnosed with pituitary dysfunction as adults. Median time to diagnosis was five years (range one to 43 years). The commonest presenting features of pituitary dysfunction were poor growth and pubertal delay or regression. Hypothalamic injury was thought to underlie hypopituitarism in several cases, on the basis of pituitary hormone responses to gonadotropin releasing hormone (GnRH) or thyrotropin releasing hormone (TRH) (Grossman & Sanfield 1994, Yamanaka et al 1993, Valenta & de Feo 1980). Pituitary stalk transection was seen on MRI in several children (Mariani et al 1996, Barbeau et al 1998, Yamanaka et al 1993).

Table 1.v: Summary of clinical details & pituitary hormone deficiencies following paediatric traumatic brain injury (adapted from Acerini et al 2006)

Author	Cases (n)	Sex	Age at TBI (y)	Type of injury	Pituitary hormone axis					Presenting symptoms	Interval injury to diagnosis (y)
					GH	TSH	ACTH	LH/FSH	ADH		
Benvenga et al 2004	4	F, M, M, NS	7 – 16	Fall, RTA, RTA, fall	2	4	1	4	-	Apathy, libido, amenorrhoea	5, 41, 42, 22
Eichler et al 1988	1	M	12	NS	1	-	-	-	-	Poor growth	1
Paxson & Brown 1976	1	F	14	RTA, skull fracture	1	1	1	1	-	Pubertal regression	1.2
Girard & Marelli 1977	1	M	3	RTA	1	1	1	-	-	Poor growth	3.1
Miller et al 1980	3	F, M, M	0.1 – 0.3	Abuse	3	2	3	3	-	Poor growth	12, 12, 6
Mariani et al 1996	3	F, M, M	2, 8, 9	RTA, judo, fall	2	2	2	2	2	Poor growth, pubertal delay	10, 1, 5
Barbeau et al, 1998	3	M, M, F	0.5 – 3	NS	3	2	2	2	-	Poor growth	5, 5, 1
Grossman & Sanfield 1994	1	F	0.8	RTA	1	1	1	1	-	Pubertal delay	16
Yamanaka et al 1993	2	M, M	3, 11	Falls	2	1	-	2	-	Poor growth	6.5, 2
Valenta & de Feo 1980	1	F	16	Skull fracture	1	1	-	1	-	Pubertal regression	2
Total (n)	**20**				**17**	**15**	**11**	**16**	**2**		
Percentage					**85**	**75**	**55**	**80**	**10**		

M, male; F, female; NS, not specified; y, years;

Inappropriate activation of the HPG axis is another potential complication of childhood TBI. Central precocious puberty has been reported in more than ten children following TBI (LaFranchi 1979, Shaul et al 1985, Sockalosky et al 1987, Blendonohy et al 1991), occurring at an interval ranging from 0.4 to 1.6 years after injury. The majority of cases described have been female. This is comparable with other causes of central precocious puberty, where the prevalence is approximately ten times as high in girls as in boys (Teilmann et al 2005). The most common mechanism is thought to be early activation of pulsatile GnRH secretion. This may result from hypothalamic tumors or lesions, but in most cases remains unexplained (Carel & Leger 2008).

1.9.2 Introduction to prevalence studies

The case reports described in the previous section provide evidence of long-term post-traumatic pituitary dysfunction in children. However, they represent a highly selective group of patients who presented to medical services with signs and symptoms clearly warranting endocrine investigation. It is not possible, from these examples, to determine the incidence or prevalence of hypopituitarism amongst survivors of childhood TBI generally. The natural history of pituitary dysfunction occurring after TBI also needs further clarification, including risk factors for and time to development of hormone abnormalities, and clinical implications for those affected.

It could be presumed from the limited number of case reports on the subject that post-traumatic pituitary dysfunction is uncommon. A departmental survey conducted 30 years ago reported trauma as the cause of hypopituitarism in only 3.7% of paediatric patients (Gnehm et al 1979). McDonald et al (2008) examined the Pfizer International Growth (KIGS) database and found that a surprisingly small number of children were registered with post-traumatic GHD (n=141) compared with idiopathic GHD (n=23,722). TBI survivors were more likely than patients with idiopathic GHD to have multiple pituitary deficiencies. The KIGS database is an international pharmaco-epidemiological database holding data on children and adolescents treated with recombinant human GH since 1987. These data may not be representative of all GH-treated children and young people. Furthermore, the database provides no insight into the number and characteristics of GH-deficient people who are not prescribed replacement therapy.

Thus, the KIGS data are unlikely to reflect the true prevalence of TBI-induced GHD and provide little information about TBI-survivors with other pituitary hormone deficiencies

(unless GHD also features). The data raise questions about the likelihood of investigation of TBI-survivors for endocrine problems and the likelihood of endocrinologists linking new diagnoses of GHD to a previous TBI. Children with faltering growth rates may not be referred for investigation if their height measurements are within normal range or the possibility of an endocrinopathy is not considered. Endocrinologists making a diagnosis of GHD may fail to ask about previous TBI.

1.9.3 Prevalence studies of long-term post-traumatic pituitary dysfunction

Preliminary prevalence studies have varied considerably in their methodology and findings. Niederland et al (2007) investigated 26 survivors of childhood TBI (19 males) 30 ± 8.3 months after injury in a retrospective study. It was not clear how subjects were selected to take part in the study. The growth hormone axis was assessed using the insulin tolerance and L-dopa tests. GHD was diagnosed in 11 subjects (42%) on the basis of peak GH responses of less than 7 μg/L in both tests. Results were compared with those of 21 age-matched controls but it does not appear that control data were used to establish reference ranges for the hormone responses measured. The 7 μg/L cut-off may have been an inappropriately high cut-off for L-dopa, a weak GH secretagogue (Behan et al 2008). No significant difference in height standard deviation scores (SDS) was observed between GH-deficient and -sufficient TBI subjects.

Low basal cortisol levels were reported in 34% of subjects in the Niederland study but the reference range used was not provided. It was not made clear whether any subjects had sub-optimal cortisol responses to insulin-induced hypoglycaemia, or what cut-off was used. The HPG axis was not evaluated in this study on the basis that normal hormone ranges were hard to define in pre-pubertal or pubertal children (Niederland et al 2007). In all, pituitary hormone abnormalities were reported in 61% of TBI subjects. There was no association between severity of TBI and pituitary dysfunction.

Poomthavorn et al (2008) conducted another retrospective study. Fifty subjects were recruited from a cohort of 113 survivors of severe TBI, admitted to hospital over a ten-year period. All 113 were aged between 0.1 and 18 years at the time of injury and had CT evidence of brain injury. Recruited subjects were asked to complete two child health questionnaires and the QoL-AGHDA. The latter has not been standardised for use in children. Subjects with responses suggestive of hypopituitarism were asked to attend for a physical and endocrine assessment.

Twenty-nine of 50 subjects underwent basal hormone evaluation between 0.9 and 8.5 years after TBI (median 4.5 years). The remaining 21 were presumed healthy, on the basis of the questionnaire results, and telephone interviews regarding their growth, development and wellbeing. The glucagon test was performed in eight subjects demonstrating poor growth velocity and low IGF1 concentrations. All had normal GH responses to glucagon (defined as a peak response >3.3 μg/L, a lower than average cut-off in children). Cortisol responses were suboptimal in three subjects (<450 nmol/L) but as previously discussed (section 1.5), glucagon is not always a reliable stimulus for the HPA axis. Baseline early morning cortisol had not been measured in any of these patients. Other results included one case of precocious puberty and one case of possible mild central hypothyroidism in a child with a neurodegenerative disorder.

The results of four children who had previously been investigated for (and found to have) post-traumatic pituitary dysfunction (Poomthavorn & Zacharin 2007) were also included in the study findings. Of these, two had multiple pituitary hormone abnormalities, the third, isolated GHD and the fourth, isolated HPG insufficiency. Thus, pituitary dysfunction was observed in nine of 54 patients (17%), although less than two thirds had any endocrine testing and only a fifth underwent any stimulation tests. A higher proportion of subjects with pituitary dysfunction sustained basal skull fractures at injury (three of nine) compared with three of the other 45 subjects.

Einaudi et al (2006) have provided the only prospective data on long-term post-traumatic pituitary dysfunction in children to date. Thirty subjects underwent basal hormone evaluation within 72 hours of TBI, with follow-up in 26 and 20 subjects at six and 12 months post-injury respectively. The GHRH-arginine test was performed in subjects with height velocities below the 25th percentile in the presence of low or normal IGF1 levels. Nocturnal GH secretion was also evaluated. Subjects with low baseline early morning cortisol levels or symptoms suggestive of HPA insufficiency underwent the glucagon test. Precocious or delayed puberty was investigated using the GnRH stimulation test.

The acute data were discussed in section 1.8; all pituitary abnormalities resolved by the six-month assessment. One subject was diagnosed with HPA insufficiency six months post-injury (peak cortisol response to glucagon <500 nmol/L) and confirmed on re-testing at 12 months. Another was diagnosed with GHD at 12 months post-injury (peak GH response to

GHRH-arginine <20 μg/L, based on a paediatric cut-off established by Ghigo et al [1996a]). In both cases, the TBI sustained was considered minor (GCS scores of 14).

Einaudi and colleagues also evaluated 22 subjects retrospectively (from a cohort of 98 patients admitted over an 8-year period). Median time since TBI was 3.9 years (range 0.7 to 7.3 years). Precocious puberty was diagnosed retrospectively in one male subject (aged 10.5 years), whose parents had noticed the first signs of puberty two years previously. Hypogonadotrophic hypogonadism was diagnosed in another male with a pre-pubertal response to the GnRH test at 13 years (bone age 14 years, uncharacteristically) who remained pre-pubertal on examination at 13.5 and 14 years. One subject in the retrospective cohort had multiple anterior pituitary hormone abnormalities and another had isolated GHD. Of the 48 subjects who were evaluated in total (26 prospectively and 22 retrospectively), hormone abnormalities were observed in 13%. However, few subjects underwent dynamic function testing of the GH and HPA axes and a third of the prospective cohort had defaulted follow-up by the 12-month assessment.

Some data are also available from three studies that have only been published as abstracts. Zapletalova et al (2007) measured baseline endocrine function in 40 patients between 26 and 36 months after TBI. Unspecified dynamic function tests were performed in subjects with abnormal findings on examination or baseline test results. Two children were diagnosed with GHD (one who also had TSH deficiency), two males with precocious puberty and one adolescent female with central hypogonadism. Kaulfers et al (2007) prospectively investigated 25 children three, six and 12 months following TBI. Only ten children (40%) completed the 12-month assessment. Three children were diagnosed with precocious puberty aged less than 10 years (precise ages at diagnosis were not provided). A fourth subject had a suboptimal cortisol response to the low dose synacthen test and another was diagnosed with multiple pituitary hormone deficiencies. Lastly, Suarez-Ortega et al (2008) presented preliminary findings from a prospective study. Three of 11 TBI survivors (27%) had peak GH responses less than 10 μg/L to the glucagon and L-dopa tests three months post-injury. No other hormone abnormalities were detected. The authors plan to conclude the study with a 12-month assessment.

1.10 Significance of long-term pituitary dysfunction after childhood TBI

1.10.1 Physical, cognitive and behavioural outcomes after childhood TBI

The scope of recovery following childhood TBI is wide, and dependent on the cause and severity of the injury. Severe accidental TBI results in persistent neurological impairment in the form of physical, intellectual and emotional deficits in approximately 42% of survivors (Scott-Jupp et al 1992). Specific deficits of motor function include limb weakness (particularly hemiplegia or diplegia) and dyskinesia. Epileptic seizures may feature. Communication problems seen following TBI include impaired speech production (articulation and pronunciation difficulties) and other disorders of language.

Cognitive dysfunction is well recognised after childhood TBI (Scott-Jupp et al 1992, Lehnung 2001, Verger et al 2000, Ewing-Cobbs 1998, Ewing-Cobbs et al 2006, Anderson & Catroppa 2005, Anderson et al 2005a). Survivors of severe TBI exhibit the greatest recovery of executive function in the two years following injury, but remain the worst affected compared with mild and moderate TBI groups (Anderson & Catroppa 2005). Academic achievement scores are lower in survivors of severe TBI than in healthy control groups (Ewing-Cobbs et al 2006) or survivors of mild or moderate TBI (Ewing-Cobbs 1998). Between a third and half of children with severe TBI are identified as needing special educational support or fail a school grade (Scott-Jupp et al 1992, Ewing-Cobbs et al 2006).

Behavioural problems are seen in 36% and 22% of children who survive severe or moderate TBI respectively, compared with 10% of orthopaedic trauma patients (Schwartz et al 2003). Impairments of memory, attention and information processing are also frequently observed (Anderson et al 2005b, Lehnung 2001, Verger et al 2000; Yeates et al 2005). Yeates and colleagues found that long-term attention problems were significantly more common in children with severe TBI than orthopaedic trauma patients (Yeates et al 2005). Group differences were most pronounced for children with pre-morbid attention problems, suggesting that TBI exacerbates pre-existing neurobehavioural difficulties. Keenan et al (2008) have suggested that TBI before two years of age may not be causal in the development of attention-deficit/hyperactivity disorder (ADHD) but rather a marker for a future diagnosis of ADHD.

Non-accidental head injury (NAHI) has even more severe consequences, with barely 10.9% normal at follow-up (Minns et al 2005). Prospective longitudinal studies have demonstrated worse outcomes in children with inflicted injuries than in survivors of accidental TBI (Goldstein et al 1993, Keenan et al 2006a). Cumulative literature sources show that the mortality is approximately 20.6% in this group, with severe and moderate disability resulting in 34% and 25% of survivors respectively (Minns et al 2005). Motor deficits are seen in 38%, epileptic seizures in 30% and acquired smaller-than-expected head size in approximately 51% (Minns et al 2005). Blindness is a consequence in 15% of survivors of childhood NAHI, with some degree of visual impairment seen in 45% (Minns et al 2005). Overall, intellectual and cognitive deficits are seen in 54% and behavioural or emotional problems in 38% (Minns et al 2005).

The natural history of recovery following TBI has been studied in some detail. Children improve in many aspects of neuropsychological functioning over the first year post-injury, after which recovery plateaus (Yeates et al 2002, Jaffe et al 1995, Keenan et al 2006b, Ewing-Cobbs 1998). Fay et al (2009) observed that severe TBI predicts an increased likelihood of persistent deficits in a range of neuropsychological, behavioural, adaptive and academic domains compared with moderate TBI or orthopaedic trauma.

1.10.2 Effects of TBI on brain growth and development

Brain growth slows after severe childhood TBI. Survivors of both inflicted and accidental TBI show smaller-than-expected brain volume for head circumference (Lo et al 2003, Tasker et al 2005), a consequence of global cerebral atrophy in more than 50% (Lo et al 2003). It was previously held that young children's brains were more adaptable after severe insult, a view supported by the relative sparing of language after infantile hemiplegia or hemispherectomy (Levin et al 1982). This theory of neural or functional "plasticity" has been challenged in recent years, as comparisons of cognitive recovery after diffuse brain insult have shown that young children are particularly vulnerable to residual impairment after infectious disease and cranial irradiation (Levin et al 1982, Ewing-Cobbs 2006).

In animal studies, Fineman et al (2000) demonstrated that diffuse brain injury is capable of inhibiting both anatomical and cognitive manifestations of experience-dependent cortical plasticity in rat pups. In children, despite dynamic neural processes regulating axonal and dendritic development and connectivity during infancy and the pre-school years, there appear to be significant limits on neural and cognitive plasticity after early TBI. Less

complete recovery in young children may reflect damage to a limited repertoire of mechanisms involved in learning and memory, which interferes with the acquisition of new information and skills (Ewing-Cobbs et al 2006). Detailed cognitive, behavioural and neurodevelopmental studies are confirming that the outcome following severe TBI in early childhood is worse than the outcome for older children, young people and adults (Verger et al 2000, Taylor & Alden 1997, Anderson et al 2005a, Kieslich et al 2001).

1.10.3 Potential consequences of pituitary dysfunction after childhood TBI

On the basis of the preceding arguments and given the multiplicity of mechanisms of head injury in children, it would be surprising if there were not neuroendocrine consequences of TBI along with other acquired disturbances of brain function. The diagnosis of pituitary deficiency may fundamentally influence the outlook for such children. GH, TSH and the gonadotrophins play important roles in growth and development during childhood and adolescence (see sections 1.1.1, 1.12.1 and 5.2.6). Hypopituitarism after TBI may have adverse effects on these processes. Unrecognised pituitary dysfunction may hinder physical and neurological rehabilitation after TBI (section 1.7) and may even contribute to later neurological deterioration (Popovic et al 2005).

There has been little study of the influence of pituitary dysfunction on children's neuropsychological function. Cognitive deficits have been reported in children with multiple pituitary hormone deficiencies, in addition to deficits in visualmotor integration, spatial orientation and attention (Ross 2005). However isolated GHD does not appear to affect cognition (Meyer Bahlburg et al 1978) and no significant benefits have been seen with GH replacement therapy (Meyer Bahlburg et al 1978, Sanberg et al 1998). GH may have a positive impact on attentional outcomes in deficient children (Smith et al 1985). Higher rates of depression and anxiety (Wiren et al 2001), in particular, social phobia (Stabler et al 2001), have been reported in GH-deficient young people in whom treatment was discontinued after attainment of adult height.

1.11 Quality of life (QoL) outcomes

As the previous section illustrates, the long-term consequences of TBI for survivors are wide-ranging. Co-existing pituitary dysfunction may exacerbate the physical and neuropsychological sequelae of TBI. However, no paediatric studies to date have measured this impact. Establishing the clinical significance of pituitary dysfunction in survivors of

childhood TBI is complex. The impact of hypopituitarism on growth and pubertal development is important but will not necessarily reflect wider effects on overall health and wellbeing. There is growing appreciation of the impact of psychological and social dimensions on children's QoL and the utility of QoL as an outcome measure (Ravens-Sieberer et al 2002).

Health-related QoL measures the impact of illness or its treatment on a person's emotional, social and physical wellbeing and their ability to function in daily life (Ravens-Sieberer et al 2002). Questions pertaining to specific aspects of daily living (e.g. physical, emotional, cognitive or social functioning) are grouped into domains. QoL questionnaires may be generic or disease-specific and developed for use in paediatric or adult populations. Their reproducibility and validity must be established. The reproducibility reflects the likelihood of eliciting similar responses between comparable study populations or in the same population over time (assuming that their QoL is unchanged). The validity reflects how accurately the tool in question measures what it is supposed to measure.

Quality of life research demands the perspective from the view of the person under study (Ravens-Sieberer et al 2002). For this reason, self-report questionnaires are desirable in studies involving children rather than use of parent-report or 'proxy' versions alone. Developmentally appropriate questionnaires are required. Adult-orientated QoL measures may fail to explore aspects of QoL that are important to children and need modification for children's language and cognitive skills (Eiser & Morse 2001). Varni et al (2007a) have reported that children as young as five years can reliably self-report their QoL when provided with an age-appropriate instrument.

A recent review of generic paediatric QoL measures concluded that the Pediatric Quality of Life Inventory (PedsQL), KIDSCREEN-52 and DISABKIDS questionnaires were most suitable in the long-term follow-up of children after major trauma (Janssens et al 2008). All are applicable to a large age range, have good psychometric properties and cover many sections of the World Health Organisation (WHO)'s International Classification of Functioning, Disability and Health (WHO 2001, Janssens et al 2008). Self-report and proxy versions of the PedsQL version 4.0 generic core scales have been validated for use in both healthy and patient populations (Varni et al 2007b, Varni et al 2001, Upton et al 2005). The KIDSCREEN-52 measure examines more domains than the PedsQL version 4.0 questionnaire and was developed using community samples (Ravens-Sieberer et al 2005).

The DISABKIDS questionnaire was developed using a population of children with seven different chronic medical conditions (Schmidt et al 2006). It is designed for use in chronic illness.

1.11.1 QoL after childhood TBI

There is no paediatric QoL questionnaire specific to either TBI survivors or children with hypopituitarism. However, the proxy version of the PedsQL has been validated in survivors of childhood TBI. McCarthy et al (2005) measured parent-reported QoL status in 302 patients at baseline, three and 12 months post-injury, comparing the results with those for 89 children with extremity fractures. Severity of TBI was categorised using the New Injury Severity Score (NISS), rather than the post-resuscitation GCS score. Proxy versions of the PedsQL (version 4.0) generic and cognitive functioning scales and the Behaviour Rating Inventory of Executive Function (BRIEF) measure were used. The PedsQL tool appeared to be more sensitive to differences in QoL and cognitive function among children with different types and severities of TBI. However, the authors also concluded that parts of the generic PedsQL scales needed refinement to more accurately reflect unique dimensions of QoL.

In a follow-up to the validation study, the same study group (McCarthy et al 2006a) measured parent-reported QoL status in 330 survivors of TBI at the same time points as previously. They reported a negative impact on QoL in survivors of moderate or severe (compared with mild) TBI that did not improve significantly over time. QoL scores were adversely affected by pre-existing psychosocial conditions, impaired family functioning, inferior health insurance status and single parent household status.

Stancin et al (2002) also linked risks for poorer QoL outcomes after TBI to social disadvantage and poor pre-injury child behavioural and academic functioning. The Child Health Questionnaire Parent and Child Forms were used in this study. Parents and children reported worse health-related QoL following severe TBI than after orthopaedic trauma only. However, QoL status as reported by adolescent subjects did not differ significantly between the two groups, in spite of poorer communication and daily living skills and general adaptive functioning in the severe TBI group. Assessments were conducted at baseline, three and 12 months and at a mean of four years post-injury.

In another study of the long-term impact of TBI on QoL, Horneman et al (2002) found that survivors of serious TBI had more medical and mental health problems than groups of

children with other medical conditions. Functional limitations were still extensive ten years post-injury. Cattelani et al (1998) also reported social maladjustment and poor QoL many years following childhood TBI, related to behavioural and psychosocial disorders rather than day-to-day functioning.

1.11.2 QoL in GH-deficient children and adults

There has been little study into the impact of GHD or GH replacement therapy on children's QoL. The QoL benefits of GH therapy in children may vary depending on the aetiology of the GHD (Sheppard et al 2006). A recent review of QoL amongst GH-deficient adolescents in the transition phase from childhood to adulthood concluded that GH therapy has minimal effect on QoL during this period (Gleeson & Clayton 2007). However one study suggested that domains related to age-specific psychological problems are worse in untreated GH-deficient subjects than healthy young people, and appear to respond positively to GH therapy (Attanasio et al 2005). Evidence for the effects of GH therapy on QoL in GH-deficient adolescents is discussed in more detail in section 5.2.6. Comprehensive evaluation of QoL aspects of GHD in children is required.

Poor QoL is a well-recognised feature of adult-onset GHD and must be demonstrated for deficient adults to qualify for GH therapy under current National Institute of Health and Clinical Excellence (NICE) guidance (NICE 2003). A NICE review found that the cost effectiveness of adult GH therapy was driven by the day-to-day improvements in QoL rather than longer-term health consequences (Bansback et al 2002). Enduring QoL benefits have been demonstrated in long-term observational studies (NICE 2003, Rosilio et al 2004, Svensson et al 2004a) although the pooled data from short-term randomised controlled trials are more equivocal (NICE 2003).

Increased reporting of psychological and physical symptoms has been demonstrated when adult GH therapy is discontinued, correlating with a reduction in IGF-1 concentration (McMillan et al 2003). However, QoL appears to be less impaired in adults with childhood-onset GHD than patients with adult-onset disease (Attanasio et al 1997, Rosilio et al 2004). This may reflect a lack of sensitive tools for measuring QoL in GH-deficienct children and adolescents. Alternatively, if GHD is diagnosed at a young age, a child may not perceive associated changes in their QoL in the same way that an adult might notice a difference. Attanasio et al (1997) observed no sustained QoL benefits with GH therapy in subjects with

childhood-onset GHD using the NHP to evaluate QoL status. In contrast, Rosilio et al (2004) demonstrated improvements in QoL using the QLS-H questionnaire to monitor change.

Commonly used adult generic QoL measures include the Nottingham Health Profile (NHP) (Hunt & McKenna 1991) and the Short Form-36 Health Survey (SF-36) questionnaire (Ware & Sherbourne 1992; McHorney et al 1993). Reliability estimates for the NHP are slightly higher than those for the SF-36 (Doward et al 2004) but the SF-36 has been shown to be the more discriminatory of the two in evaluation of QoL in GHD (McMillan et al 2003). Generic measures are by definition not specific to the issues encountered by GH-deficient individuals and are not particularly sensitive to changes in QoL that may occur during GH therapy (Blum et al 2003).

Two disease-specific measures have been developed for evaluation of QoL in adult GHD. These are the Questions on Life Satisfaction - Hypopituitarism (QLS-H) measure (Blum et al 2003) and the Quality of Life - Assessment of GH Deficiency in Adults (QoL-AGHDA) questionnaire (McKenna et al 1999). Construct and scoring are outlined in more detail in the Methods Chapter (section 3.4.4.2). A QoL-AGHDA score of 11 (out of 25) or higher is required to be eligible for adult GH therapy in UK (NICE 2003). Reassessment of QoL status is required nine months after commencing GH therapy with demonstration of at least a 7-point improvement in Qol-AGHDA score to justify ongoing treatment (NICE 2003).

1.11.3 QoL outcomes in survivors of adult TBI with pituitary dysfunction

Several recent studies have examined differences in QoL amongst adult TBI survivors with normal versus abnormal pituitary function. In a retrospective study by Wachter et al (2009), pituitary function was evaluated in 53 adult subjects at between 12 and 36 months post-TBI. These constituted only 32% of the total number invited to take part in the study. Outcome was assessed using the SF-36 and a non-validated pituitary questionnaire. Neuropsychological complaints were observed in two thirds of subjects and were associated with intracerebral haemorrhagic lesions and not pituitary insufficiency. Only subjects with abnormal basal hormone screens (taken in the afternoon) or one or more positive responses to a non-validated pituitary questionnaire underwent dynamic testing. This selection bias complicates interpretation of the study results.

Kelly and colleagues measured QoL in 70 of 206 TBI survivors at three and between six and nine months post-injury (Bavisetty et al 2008). Full details of the study can be found in

section 1.6.2.2. Eleven of 70 subjects were found to be GH-deficient or -insufficient. These were defined as peak GH responses to the GHRH-arginine test below the fifth (6 μg/L) or tenth (12 μg/L) centile respectively using reference data obtained from healthy controls. Compared with GH-sufficient subjects, the 11 with suboptimal results were more likely to be depressed and score poorly on the Disability Rating Scale. Reduced QoL was demonstrated in three domains of the SF-36: energy and fatigue, emotional wellbeing and general health.

In another previously described study (section 1.6.2.2), Klose et al (2007c) evaluated 104 subjects at a median of 13 months after TBI, comparing them with healthy controls. Health-related QoL was assessed using the NHP and European Quality Of Life-5 Dimensions (EQ-5D) questionnaires and the QoL-AGHDA. The 12-month age-adjusted EQ-5D visual analogue scale scores (measuring overall health), Qol-AGHDA scores, and NHP scores in the dimensions of emotional reaction, sleep pattern, energy level and physical mobility were worse in head injury survivors than controls. Qol-AGHDA score and NHP scores in the dimensions of sleep, energy and social isolation were worse in TBI subjects with hypopituitarism relative to those with normal pituitary function. Findings were independent of age, TBI severity and BMI. Dimensions of physical mobility, pain and emotion were similar between groups. Baseline ('pre-injury') scores were available for a cohort of 46 prospective patients 'nested' within the wider study. These were comparable with control data, indicating normal baseline QoL pre-injury.

In summary, QoL is reduced after childhood TBI but it is not clear whether pituitary dysfunction further impairs QoL outcomes. There are some early data to suggest that pituitary dysfunction has a negative impact on QoL in adults after TBI. This requires investigation in children.

1.12 Impact of pituitary dysfunction on body composition and BMI

Data from adult TBI studies also suggest that post-traumatic pituitary dysfunction may affect BMI. In studies by Agha et al (2004b) and Tanriverdi et al (2006), BMI was significantly higher in GH-deficient TBI survivors than GH-sufficient subjects. Bavisetty et al (2008) observed that BMI was higher in subjects with pituitary hormone abnormalities than those without. Klose et al (2007b) also reported higher BMI values in subjects with pituitary dysfunction; all had GHD, some with additional hormone deficiencies. Full details of these studies can be found in section 1.6.2.2. No such data are available from studies of TBI in

childhood. Indeed Moon et al (2009) highlighted the lack of any sort of growth monitoring following childhood TBI. They observed that height and weight were only documented at 33% of follow-up appointments.

1.12.1 GH effects on body composition and BMI

The most obvious marker of GH sufficiency in children is appropriate linear growth. In addition, GH promotes increases in lean body or fat-free mass (FFM) and decreases in fat mass (FM). GHD in children is associated with above average subcutaneous fat and reduced muscle width (Tanner et al 1971). Differences in subscapular and triceps skinfold measurements indicate relative truncal obesity (Zachmann et al 1980). GH therapy decreases skinfold thickness and increases muscle bulk (Tanner et al 1971, Schoenle et al 1995). Greater decreases are seen in subscapular than upper limb skinfold thickness (Wit et al 1988). Gregory et al (1991) demonstrated a mean increase in FFM of 1.37 kg and a mean decrease in FM of 0.41 kg after six weeks of GH therapy. Boot et al (1997) observed continued FFM gains over a two to three year treatment period whereas FM losses stabilised after six months of GH. GH treatment is associated with decreases in BMI (Baars et al 1998, Schoenle et al 1995), most pronounced in children with higher initial BMI (Baars et al 1998).

The tendency towards truncal obesity in GHD, together with associated tendencies towards insulin resistance and adverse lipid profile, may increase cardiovascular risk in the long term (Colao et al 2006). Left unchecked, GHD may have adverse effects on future cardiovascular and metabolic outcomes. Pituitary insufficient adults have a reduced life expectancy, with a two-fold higher risk of death related to cardiovascular risk (Colao et al 2006). Yet in GH-treated individuals, a large retrospective Swedish study concluded that the overall mortality was similar to the general population, with apparent protection from myocardial infarction (Svensson et al 2004b). It is unclear to what degree GH exposure during childhood and adolescence influences these outcomes. Nonetheless, evaluation of body composition may be a useful adjunct in the evaluation and monitoring of pituitary function (particularly, GH status) following childhood TBI.

1.12.2 Measurement of body composition

BMI is easily calculated but does not distinguish between increased mass in the form of fat, lean muscle or bone (McCarthy et al 2006b). There is the potential for misclassification of large-framed and/or muscular children. For this reason, measurement of body composition

is preferable for evaluation of body fatness. Established reference methods for evaluating body composition include underwater weighing, isotope dilution and ^{40}K measurements, all of which are expensive, time-consuming and require specially trained staff (Pietrobelli et al 2003). MRI and CT are also highly accurate but are expensive and the latter involves significant exposure to ionising radiation. Dual X-ray absorptiometry (DXA), although accurate (Gately et al 2003) and used frequently to evaluate body composition, is not suitable for use as a bedside tool. The cost and exposure to ionizing radiation also make DXA unsuitable for frequent, repeated measurements.

Bioelectrical impedance analysis (BIA) offers a rapid, inexpensive alternative (McCarthy et al 2006b). The procedure is rapid, painless and can be performed at the bedside. A low energy, high frequency electrical signal (50 kHz, 800 μamps) is passed through the body and a measurement of body resistance (ohms) to the flow of the electrical current taken using a bioimpedance analyzer. The electrical current is restricted to the extracellular fluid, which is roughly accounted for by lean body (muscle) mass, since adipose tissue and bone contain little in the way of water or conducting electrolytes. Biolectrical impedance correlates well with DXA (Shaikh et al 2007, Pietrobelli et al 2003) and provides more accurate results than other bedside tests such as anthropometric measurements (e.g. skinfold thickness) (Tyrrell et al 2001, Gutin et al 1996). The accuracy of BIA is discussed in more detail in section 5.3.1.1.

In summary, pituitary dysfunction may have an impact on BMI in adult TBI survivors. GHD in childhood is known to affect BMI and body composition. These require evaluation in survivors of childhood TBI.

1.13 Causative mechanisms of pituitary dysfunction after childhood TBI

Greater understanding is needed of factors that predispose children to pituitary dysfunction following TBI. This would enable identification of at-risk TBI survivors. Bondanelli et al (2004) observed an association between severity of injury and emergence of pituitary dysfunction. This has not been substantiated by other adult (Agha et al 2004b, Tanriverdi et al 2006, Klose et al 2007a) or paediatric studies (Niederland et al 2007, Einaudi et al 2006). However DI has been observed to be more common after severe TBI (Agha et al 2004b).

Although the aetiology is sometimes clear (section 1.4), post-traumatic pituitary dysfunction is not always associated with obvious abnormalities on scan such as hypothamalo-pituitary haemorrhage or pituitary stalk transection (Benvenga et al 2000, Bondanelli et al 2004). Indeed, Bondanelli et al (2004) identified abnormalities on cranial MRI in only two of 27 (7%) subjects who developed pituitary dysfunction after TBI. Kelly et al have demonstrated a relationship between diffuse brain swelling and emergence of pituitary dysfunction in two separate adult TBI studies (Kelly et al 2000, Bavisetty et al 2008). Klose et al (2007b) observed an association between raised intracranial pressure (ICP) and development of pituitary hormone abnormalities. Bavisetty et al (2008) found an association with subjects requiring evacuation of intracranial haemorrhage. Poomthavorn et al (2008) suggested a link with basal skull fracture in children. Schneider et al (2007) concluded that TBI survivors with basal skull fractures, diffuse axonal injury (DAI), raised ICP and prolonged intensive care admissions may be at greater risk of post-traumatic pituitary dysfunction.

1.13.1 Defining primary and secondary brain injury

The consequences of TBI result from both the primary brain injury and the effects of secondary brain insult (Blumbergs 2005a). Primary brain injury may be diffuse or focal and is determined by the mechanism of injury and the nature and severity of impact. Diffuse axonal injury (DAI) results from shearing forces produced by acceleration and deceleration of the brain within the cranium. Contusions and lacerations are examples of focal brain damage. Focal vascular injury may result in intracerebral, subdural, extradural or subarachnoid haemorrhage, depending on the anatomical region of the brain affected. Subarachnoid haemorrhage (Pillai et al 2001) and DAI and have been associated with poorer outcome after childhood TBI (Ong et al 1996).

Secondary brain damage may also be diffuse or focal and manifests as hypoxic-ischaemic damage or brain swelling. Secondary insult is caused by systemic or intracranial abnormalities resulting from breathing or circulatory insufficiency, temperature or biochemical imbalance, seizures, infection, vasospasm and importantly, raised ICP (Miller et al 1977, Marmarou et al 1991, Chambers et al 2001). Raised ICP affects cerebral perfusion pressure (CPP), which reflects the pressure of blood flow to the brain. CPP is defined as mean arterial pressure minus ICP.

1.13.2 Assessment tools

The ability to measure primary and secondary brain injury may help to delineate the mechanisms of neuroendocrine derangement occurring after childhood TBI. However, many of the assessment tools used in the acute setting do not differentiate between primary and secondary injury, instead providing an estimate of overall TBI severity. Most were developed to predict immediate outcome after TBI, including the likelihood of mortality. Some of these tools are described below.

Use of the GCS to grade TBI severity has been described previously (section 1.3.1). The GCS correlates well with outcome after severe TBI in children (Ducrocq et al 2006, Chiaretti et al 2002, Pillai et al 2001, Chung et al 2006). The Injury Severity Score (Baker et al 1974) is an anatomical scoring system that has been shown to correlate with outcome after severe TBI in children in some (Ducrocq et al 2006) but not all studies (Prasad et al 2002). It is described in more detail in the Methods chapter (section 3.1.1). The Marshall score is another anatomical assessment tool (Marshall et al 1991b, section 3.5.1.). It was developed in adults and provides a framework for grading diffuse injury on cranial CT scans. Marshall et al (1991b) observed a higher mortality rate with increasing severity of diffuse injury, the latter correlating positively with risk of elevated ICP. The correlation with ICP may not be as well defined in children (Figaji et al 2008).

Other examples of scoring systems include the Pediatric Trauma Score (PTS; Tepas et al 1985), the Revised Trauma Score (RTS; Champion et al 1989, Champion et al 1981) and the Trauma Injury Severity Score (TRISS; Boyd et al 1987). These are outlined in more detail in the Methods chapter (section 3.5.1). Both the RTS and the TRISS were developed for use in adults. The RTS is a physiological scoring system that is heavily weighted towards the GCS (Chawda et al 2004). The RTS informs the TRISS, which predicts the probability of survival after trauma. Two disadvantages of these scoring systems are that they do not take into account duration of physiological derangement (Chawda et al 2004) and cannot be applied accurately in patients who are intubated. These limit their utility for predicting secondary brain insult.

Pupillary responses (normal vs. unilaterally or bilaterally abnormal) have correlated with outcome in some studies (Ong et al 1996, Pillai et al 2001) but not others (Massagli et al 1996, Prasad et al 2002).

1.13.3 Measurement of physiological variables and ICP monitoring

Several paediatric studies have examined the impact of individual physiological variables on outcome after TBI. Ong et al (1996) observed that hypoxia on admission was associated with poor outcome. In another study, hypotension was found to be an independent predictor of outcome after severe TBI (Ducrocq et al 2006). Chiaretti et al (2002) reported that hypoxia, hypotension, disordered clotting, hyperglycemia and early post-traumatic seizures were all bad prognostic features. The retrospective nature of these studies may have precluded accurate assessment of the duration and severity of abnormal physiological findings. Many outcome studies have also been limited by a lack of intracranial pressure monitoring data (Lam & MacKerskie 1999).

A series of prospective head injury studies conducted in Edinburgh have sought to address these issues (Jones et al 2003, Adelson et al 1997, Chambers et al 2006). Secondary insult data collected on children aged between two and 16 years admitted to hospital with moderate or severe TBI revealed that CPP is particularly important. The total duration of disturbed CPP was most predictive of outcome after head injury with respect to mortality and ill health (Adelson et al 1997). Further studies have enabled identification of critical CPP threshold values for children aged 2-6, 7-10 and 11-15, in relation to outcome after head injury (Chambers et al 2006).

Secondary brain damage, unlike the primary injury, can to some extent be limited or prevented. Greater understanding of risk factors associated with long-term pituitary dysfunction after TBI could be used to inform strategies to modify outcomes.

1.14 Summary

TBI is an important cause of death and disability in children and adults. The hypothalamus and pituitary gland are vulnerable to vascular and mechanical injury as a consequence of trauma. Cross-sectional and prospective adult studies have demonstrated acute and chronic pituitary dysfunction following TBI. The GH axis appears to be particularly vulnerable. Many acute-phase disturbances of pituitary function after TBI resolve. However, others persist and new abnormalities may emerge many months after TBI has occurred. Persistent hypopituitarism has been demonstrated in between 11% and 69% of survivors of adult TBI. Pituitary dysfunction may compromise the general health and wellbeing of TBI survivors

and impede their rehabilitation. Consensus guidelines recommend screening adults for pituitary dysfunction after TBI, with stimulation testing of the GH axis no earlier than 12 months after injury.

Detailed cognitive, behavioural and neurodevelopmental studies are confirming that the outcome following severe TBI in early childhood is worse than the outcome for older individuals. Prospective longitudinal studies have also demonstrated worse outcomes in young children with inflicted injuries than in survivors of accidental TBI. It would be surprising if there were not neuroendocrine consequences of TBI along with other acquired disturbances of brain function. The results of preliminary studies indicate that there is a significant risk of long-term pituitary dysfunction after childhood TBI.

A diagnosis of hypopituitarism may hinder physical rehabilitation after TBI and may have a lasting impact on growth and development during childhood and adolescence. QoL is reduced after childhood TBI but it is not clear whether pituitary dysfunction further impairs QoL outcomes. There are some early data to suggest that pituitary dysfunction has a negative impact on QoL in adults after TBI. This requires investigation in children. Adult TBI studies also suggest that post-traumatic hypopituitarism may affect BMI but this has not been evaluated in children. Greater understanding is needed of factors that predispose children to pituitary dysfunction following TBI. The ability to measure primary and secondary brain injury may help to delineate the mechanisms of neuroendocrine derangement occurring after childhood TBI.

Chapter 2: Aims and Objectives

2.1 Study hypotheses

Persistent post-traumatic hypopituitarism is observed in between 11% and 69% of adult TBI survivors (section 1.6). Consensus guidelines recommend screening adult TBI survivors for pituitary dysfunction after TBI, including stimulation testing of the GH and HPA axes. Little is known about the incidence and prevalence of long-term pituitary dysfunction following childhood TBI (section 1.9). The mechanisms of hypothalamo-pituitary injury as a result of trauma do not differ between children and adults (section 1.6). Case reports in the literature and preliminary studies investigating pituitary function after childhood TBI have identified endocrine abnormalities affecting a range of hormone axes (section 1.9). These findings indicate that children, like adults, may be vulnerable to post-traumatic hypopituitarism.

Previously held views that children's brains were more adaptable than adults' after severe injury due to functional plasticity have changed (section 1.10.2). Comparisons of recovery after TBI have shown that young children are particularly vulnerable to residual physical and cognitive impairment, particularly in the context of diffuse brain injury. Studies are confirming that the outcome following severe TBI in early childhood is worse than the outcome for older individuals and that outcomes may be poorest in survivors of non-accidental injury. My hypothesis was therefore that post-traumatic pituitary dysfunction would be as prevalent among survivors of childhood TBI as in adult studies.

Post-traumatic pituitary dysfunction may compromise the health and wellbeing of TBI survivors and have implications for physical, cognitive and social rehabilitation after injury (section 1.7 and 1.10.3). Despite a multitude of studies investigating post-traumatic hypopituitarism in adult TBI survivors, few have examined the clinical significance of abnormalities identified in terms of QoL, body composition or other parameters influencing the necessity to treat. The requirement for treatment of hormone deficiencies is implicit but may not be applicable, particularly in the case of GHD. The results of these and comparable paediatric studies need to be put in context.

The clinical significance of post-traumatic pituitary dysfunction in children and its impact on parameters such as growth, QoL and body composition are unknown (sections 1.10.3 and 1.11). Niederland et al (2007) found no difference in height SDS between TBI survivors with normal or abnormal pituitary function (section 1.9.3). QoL appears to be poorer after TBI in childhood (section 1.11.1) but it is unclear whether this is exacerbated by pituitary dysfunction. A small number of adult TBI studies have suggested that post-traumatic hypopituitarism may have a negative impact on QoL. Similarly, several adult studies have suggested that BMI may be elevated in TBI survivors with pituitary dysfunction. Further hypotheses were that QoL would be poorer and body composition measurements more adverse in survivors of childhood TBI with pituitary dysfunction than those with normal pituitary function. For body composition this hypothesis related specifically to GH-deficient TBI survivors.

Predictive risk factors for developing hypopituitarism after childhood TBI are also unclear (section 1.13). There has been some indication that certain head injury characteristics may increase the risk of post-traumatic hypopituitarism (section 1.13). Pituitary dysfunction does not, on the whole, appear to be associated with TBI severity but may have a higher incidence following primary brain injuries like basal skull fracture and secondary insults like diffuse brain swelling and raised intracranial pressure. The ability to measure primary and secondary brain injury may help to explain the pathogenesis of post-traumatic hypopituitarism and has implications for prevention or early identification of pituitary dysfunction in at-risk groups. I hypothesised that post-traumatic hypopituitarism would not be associated with TBI severity but may be associated with particular primary or secondary brain injury characteristics.

2.2 Aims and objectives

The primary aim of this pilot study was to establish whether post-traumatic hypopituitarism is a common occurrence after childhood TBI in the UK. My objective was to investigate this by determining the prevalence of pituitary dysfunction amongst a cohort of subjects who had been admitted to Edinburgh hospitals with head injuries as children.

Secondary aims were:

(i) To determine the clinical significance of any pituitary hormone abnormalities identified. The objective was to achieve this through critical evaluation and interpretation of results, where possible in relation to (locally) validated assays and age- and pubertal stage-related reference ranges. I also proposed to evaluate the significance of any abnormal endocrine findings by evaluating their effects on measurable parameters such as QoL and body composition (see below).

(ii) To establish whether pituitary dysfunction has a negative impact on QoL *beyond* any effects that might be attributable to TBI itself. I planned to investigate this by comparing the results of standardised QoL questionnaires completed by subjects with normal pituitary function with those of subjects with pituitary dysfunction.

(iii) To try to identify risk factors for pituitary dysfunction following TBI by determining whether post-traumatic hypopituitarism is related to the severity of TBI or primary or secondary brain injury characteristics. My objective was to compare the prevalence of different head injury characteristics between subjects with normal and abnormal pituitary function.

An exploratory aim was to determine whether post-traumatic hypopituitarism affects body composition, particularly GHD. The objective was to compare body composition measurements (obtained using BIA) between subjects with normal and abnormal GH responses to stimulation.

Chapter 3: Materials and Methods: Table of Contents

Chapter 3: Materials and Methods

I obtained Scotland 'A' Multi Research Ethics Committee (MREC) approval to carry out a retrospective observational study investigating long-term neuroendocrine sequelae after childhood TBI. The MREC stipulated that this should be a pilot study as there was little evidence that children were at the same (or greater) risk of post-traumatic hypopituitarism as adults. (The ethics application preceded publication of two of the three studies described in section 1.9.3.) The Committee had reservations about contacting subjects who were not be under any hospital follow-up, and the potential for causing unnecessary anxiety in these individuals. Permission to recruit 25 to 30 subjects was later revised to permit recruitment of up to 45 subjects to the study. The impact of ethical constraints on study design is discussed in sections 5.1.2 and 5.2.5.3 of the Discussion chapter.

3.1 Study population

The study population comprised subjects with previous accidental TBI, aged <16 years at time of injury and admitted to Edinburgh hospitals between 1st January 2001 and 31st August 2007.

3.1.1 Inclusion criteria

Subjects were eligible for inclusion if they were alive and had been admitted at least 12 months previously with head injuries classified as a) moderate or severe TBI, or b) minor TBI with injuries incurring an Injury Severity Score (ISS) ≥16 (see below and overleaf). Survivors of both accidental and suspected non-accidental TBI were eligible to take part.

Post-resuscitation GCS scores were used to grade TBI severity to determine study eligibility. Moderate and severe TBI were defined respectively as head injuries with post-resuscitation GCS scores of 9–12 or <9 (including E1, V2 or less and M5 or less - see section 1.3.1). Minor TBI was defined as head injury with a post-resuscitation GCS score of 13 to 15. An ISS score of ≥16 was chosen to enable inclusion of 'minor' TBI subjects with extradural or subdural haemorrhages who were intubated and ventilated for neurosurgery before their GCS score fell below 13. It was felt that the elevated GCS score in these subjects belied significant underlying pathology. In both cases, deterioration in conscious level can occur late. Furthermore, both can occur as a consequence of contra-coup injury and/or cause

raised intracranial pressure, features that may be associated with post-traumatic pituitary dysfunction (sections 1.4, 1.13 and 5.5.2).

The ISS is an anatomical scoring system that provides an overall score from 0 to 75 for patients with multiple injuries (Baker et al 1974). Discrete injuries are given a score of 1 (minor) to 6 (fatal) using the Abbreviated Injury Scale (AIS; Copes et al 1990) and grouped according to the body region injured (Table 3.i). Only the highest AIS score for each body region is used. Squaring the highest scores for the three most severely injured regions and adding these together produces the ISS. An ISS of 75 is automatically assigned when there is one or more AIS score of 6.

Table 3.i: Calculation of the ISS using the AIS

Region	**Injury description(s)**	**AIS (1 to 6)**	**Square top 3 AIS scores**
Head & Neck			
Face			
Chest			
Abdomen			
Extremity			
External			
Injury Severity Score:			

3.1.2 Exclusion criteria

Exclusion criteria included death during or since admission, residence outside Scotland or northeast England, missing case notes, or any history of neuro-developmental delay, cerebral palsy, learning disability or pituitary dysfunction prior to TBI.

Individuals with significant physical and/or learning disability as a consequence of TBI were not excluded from the study. It was considered unethical to deny these subjects the same opportunity as other TBI survivors for investigation and treatment of any post-traumatic pituitary dysfunction. Eligible subjects without capacity to consent were permitted to take part provided that informed consent could be obtained from a parent or legal guardian believed to be acting in their best interests.

3.2 Identification of potential participants

3.2.1 Royal Hospital for Sick Children, Edinburgh (RHSCE)

The RHSCE is a tertiary paediatric hospital serving the city of Edinburgh and southeast Scotland. It is one of only two hospitals in Scotland with a PICU (the other being in

Glasgow) and receives intensive care admissions from the whole of the east of Scotland. Admissions to the RHSCE are coded using the International Classification of Diseases-10 (ICD-10) coding system. These data are submitted to the Information and Statistics Division (ISD) of the Scottish Health Service.

The Scotland A MREC stipulated that study subjects should be selected and contacted in batches according to year of TBI admission, chiefly targeting admissions within the last few years. Committee members were concerned that contacting subjects admitted many years previously might cause distress. In order to identify subjects for the study, I compiled a list of ICD-10 diagnostic codes that might be associated with moderate or severe TBI (Table 3.ii), overleaf). The manager of the *4D Patient* Hospital Information System at the RHSCE provided a list of all patients admitted under any of these codes between 1st January 2001 and 31st May 2007 aged less than 16 years. *4D Patient* is the computer system used to record all admissions, discharges and accident and emergency or outpatient attendances at the RHSCE. Details of subjects admitted between 1st June and 31st August 2007 were recorded prospectively. Subjects admitted after this period were eligible for a different study.

From the admissions dataset provided, I compiled an initial shortlist of individuals who might be eligible to take part in the study. This included all emergency admissions to the neurology ward, high dependency or intensive care units, or to any other ward under a neurologist or neurosurgeon. Emergency admissions under a general surgeon (typically minor head injuries) were only short-listed if of greater than 48 hours' duration. Day case and other elective admissions were discounted.

I examined *4D Patient* computerised admission details for each short-listed subject. These usually incorporate a short summary of diagnosis and management. In the majority of cases, this information was sufficient to determine whether or not a subject would be eligible to take part in the study. In a few ambiguous cases, I checked the patient's hospital case record and consulted the consultant neurologist on the study team before deciding eligibility status. This precaution was taken to reduce the risk of any individuals being invited to take part in the study inappropriately.

Table 3.ii: ICD-10 codes that may indicate moderate or severe head injury

S02	**Fracture of skull and facial bones**
S02.0	Fracture vault of skull
S02.1	Fracture base of skull
S02.2	Fracture of nasal bones
S02.3	Fracture of orbital floor
S02.4	Fracture of malar and maxillary bones
S02.6	Fracture of mandible
S02.7	Multiple fractures of skull / facial bones
S02.8	Fractures of other skull and facial bones
S02.9	Unspecified fracture of skull / facial bones
S06	**Intracranial injury**
S06.0	Concussion
S06.1	Traumatic cerebral oedema
S06.2	Diffuse brain injury
S06.3	Focal brain injury
S06.4	Extradural haemorrhage
S06.5	Subdural haemorrhage
S06.6	Subarachnoid haemorrhage
S06.7	Intracranial injury and coma
S06.8	Other intracranial haemorrhage unspecified
S06.9	Other intracranial injury unspecified
S07	**Crush injuries of the head**
S07.0	Crushing injury of face
S07.1	Crushing injury of skull
S07.8	Crushing injury of other parts of head
S07.9	Crushing injury of head, part unspecified
S09	**Other and unspecified injuries of head**
S09.7	Multiple injuries of the head
S09.9	Unspecified injuries of the head
T90	**Sequelae of injury of the head**
T90.2	Skull fracture
T90.5	Intracranial injury
T90.8	Other specified head injury
T90.9	Unspecified head injury

3.2.2 Adult hospitals

Some 13 to 15 year-old TBI patients are admitted to the Royal Infirmary of Edinburgh, or to the Western General Hospital (WGH), where adult neurology and neurosurgery services for Edinburgh are based. For this reason, I requested a list of all patients admitted to either of these hospitals at less than 16 years of age under any of the aforementioned ICD-10 codes. The Information and Statistics Division of the Scottish Health Service provided data for the period running from 1st January 2001 to 31st August 2007.

Short-listing was carried out in the same way as for RHSCE patients. Similarly, computerised hospital admission details were checked to confirm short-listed subjects' eligibility. Frequently, little was recorded for WGH patients. Thus, the eligibility of several short-listed subjects admitted to the high dependency or neurology wards could not be confirmed, precluding their involvement in the study. The Scotland A MREC's preference was that patient records were not accessed to determine eligibility where possible. However, the WGH intensive care unit has a supplementary in-house computer database that was interrogated to confirm the eligibility of any short-listed intensive care admissions.

3.3 Contacting subjects

Subjects were eligible for recruitment if at least 12 months had elapsed since their TBI. As stated previously, the Scotland A MREC requested that subjects should be contacted in batches according to year of TBI admission, with a focus on more recent admissions. Committee members had concerns about the possibility of causing unnecessary anxiety in subjects admitted many years previously. Advertising was not permitted, including posters in hospitals or GP surgeries.

I sought permission to contact subjects from the lead consultant responsible for them during their TBI admission. Permission was also requested to examine subjects' medical records, with their consent. Potential participants were invited to take part in the study via their general practitioner (GP). Current GP details were identified from the hospital computer system or through the Department of Public Health and Health Policy.

GPs were sent packs containing a letter about the study, the patient information leaflet and summary study protocol, and two envelopes addressed to each subject or their parents/guardian. GPs were asked to forward the first envelope to the subject or inform the study team if they felt it would be inappropriate to contact the subject or their family.

Envelopes to subjects contained a letter inviting them to take part in the study and a patient information leaflet. Subjects were invited to register their interest by telephone, email or post. I contacted interested subjects by telephone to arrange a date for them to come to Edinburgh for assessment. If there was no response within two weeks of the first invitation being sent, I telephoned the GP to ensure that the first invitation had been sent. Once

confirmed, the GP was asked to send out a second invitation. If there was no response to the second invitation, no further attempts were made to contact the individual.

3.4 Assessment

Study participants underwent a single assessment. Subjects aged less than 16 years were evaluated at the RHSCE. Older subjects were assessed at the WGH. Subjects were asked to fast from midnight the night before their assessment. They were asked to bring an early morning urine sample collected on the day of assessment and I sent out specimen pots for this purpose. Local accommodation was arranged for the night before and/or after assessment for subjects coming from a distance.

Informed consent was obtained on the day of assessment, prior to entry of each subject into the study. In additional to parental consent, I sought the agreement of children aged less than 16 years to take part. Young people aged 16 years and above gave informed consent independently. No young adults without capacity to consent took part in the study.

3.4.1 History and Examination

A medical history was taken for each subject with emphasis on psychosocial functioning and symptoms associated with endocrine disorders. Height and weight were recorded in centimetres and kilograms respectively. Parent-reported parental heights were also recorded. A basic examination was performed including measurement of blood pressure in millimetres of mercury (mmHg).

An MREC stipulation was that pubertal staging should only be performed if clinically indicated and with the subject's consent. Where applicable, pubertal stage was determined on the day of assessment using the Tanner staging system (Marshall & Tanner 1969, Marshall & Tanner 1970). Findings were grouped together into three categories: pre-puberty, early/mid-puberty and late/post-puberty. Pre-puberty was defined as Stage B1 in girls, or G1 in boys with testicular volumes less than 4 mLs. Stages B2-3 in girls or G2-3 in boys were categorised as early/mid-puberty. Late/post-puberty was defined as Stage B4-5 in girls or G4-5 in boys. Females within this category had reached menarche and boys had started shaving.

3.4.2 Endocrine assessment

3.4.2.1 Endocrine testing

Allergy status and past medical history were ascertained and baseline observations were recorded prior to testing. Anaesthetic cream was offered prior to insertion of a venous cannula, or applied automatically in the case of young children. A basal blood sample was obtained between 08:00 and 10:00 to measure early morning plasma cortisol, IGF1, TSH, free T4, prolactin, urea, electrolyte and creatinine concentrations, and plasma osmolality. The early morning urine sample was sent for urinary osmolality. The plasma electrolytes and paired osmolalties were used to screen subjects for posterior pituitary dysfunction in the form of ADH deficiency.

GH and cortisol responses to stress were measured using the ITT. The ITT is the reference standard for joint evaluation of the GH and HPA axes and is used routinely at the RHSCE. It provides a stimulus at the level of the hypothalamus, thus enabling evaluation of the entire HPA axis (Inder & Hunt 2002, Agwu et al 1999). The cortisol response to insulin-induced hypoglycaemia has been validated against surgical stress (Plumpton & Besser 1969). The glucagon test was used as a second line investigation in those with a history of seizures. It also permits simultaneous evaluation of the GH and HPA axes; however, glucagon does not elicit an adequate cortisol response in approximately 10% of healthy individuals (Agha et al 2004a, Rao & Spathis 1987). Both these tests are discussed in more detail in sections 5.2.4.2, 5.2.4.3, 5.2.5.4 and 5.2.5.5).

Potential risks have been identified with both the ITT and the glucagon test. In particular, there are dangers associated with over-treatment of hypoglycaemia during the ITT and rebound hypoglycaemia following the glucagon test (Shah et al 1992). Both are considered safe when conducted according to departmental protocols, under proper supervision by experienced medical and nursing staff and with appropriate safety measures in place. Clinicians at the Royal Hospital for Sick Children in Glasgow have reviewed the safety profile of the ITT (Galloway et al 2002). They examined 550 cases where the ITT had been employed, spanning a ten-year period. There were no cases of hypoglycaemia requiring intravenous glucose and no cases of hypoglycaemic seizure. No published data are available for the RHSCE but no significant adverse events have occurred in the last 25 years.

The insulin tolerance or glucagon tests were performed after obtaining baseline samples for serum GH, cortisol and glucose concentrations at -30 minutes and time 0. Two baseline

samples were taken in case a spontaneous GH peak occurred at or prior to the start of the test. Peri-pubertal subjects were not primed with sex steroids before the tests. This is discussed in section 5.2.5.3.

For the ITT, short acting insulin was administered intravenously at time 0. The dose was determined by the baseline near patient testing glucose meter reading: 3.5-4.0 mmol/L, 0.075 u/kg; 4.0- 4.5 mmol/L, 0.1 u/kg; >4.5 mmol/L, 0.15 u/kg. No insulin was administered if the baseline glucose meter reading was less than 3.5 mmol/L. Further glucose meter readings and blood samples for GH, cortisol and glucose concentrations were taken at 20, 30, 60 and 90 minutes post-injection. A sugary drink containing 40 grams of glucose monohydrate was given after achievement of adequate hypoglycaemia (at between 20 and 30 minutes in all cases). This was defined as a glucose meter reading $\leq$2.2 mmol/L as a stimulus for GH and cortisol secretion or a 50% reduction in baseline glucose meter reading for GH secretion alone.

For the glucagon test, an intramuscular (IM) injection of 20 μg/kg of glucagon was administered at time 0 (up to a maximum of 1 mg). Glucose meter readings and blood samples for GH, cortisol and glucose concentrations were taken at 20, 60, 90, 120, 150 and 180 minutes. A sugary drink containing 40 g of glucose monohydrate was given if a glucose meter reading of less than 3.5 mmol/L was recorded.

Subjects were provided with a meal at the end of the insulin tolerance or glucagon tests. Those who underwent an ITT were observed until four hours post-insulin injection. Subjects who underwent a glucagon test were observed for two hours after completion of a meal.

The low-dose GnRH test (Zevenhuijzen et al 2004) was performed alongside the ITT or glucagon test to assess FSH and LH responses to stimulation. It is well tolerated with infrequent side effects. FSH and LH and either oestradiol or testosterone concentrations (as appropriate) were measured at time 0 prior to intravenous injection of 10 μg of GnRH. Repeat LH and FSH samples were obtained at 20 and 60 minutes post-injection. The rationale for using the low-dose GnRH test is discussed in section 5.2.3.

Stimulation testing of the hypothalamo-pituitary-thyroid axis was not performed. The reasons for this are explained in section 5.2.1.

3.4.2.2 Assays

Urea, creatinine, electrolytes, osmolality and glucose were measured by standard laboratory methods. All hormones were measured by automated chemiluminescent immunoassays. Free T4, TSH and oestradiol concentrations were measured using the Architect analyzer (Abbott Diagnostics, Maidenhead, UK). Coefficients of variation were <6% and <3% respectively for free T4 and TSH and 14% for oestradiol at 70 pmol/L. LH and FSH, prolactin, cortisol and testosterone concentrations were measured using the Centaur analyzer (Siemens Healthcare Diagnostics UK). Coefficients of variation were <3%, <3%, <5%, <6% and <9% respectively. GH was measured using an immunoassay calibrated against International Standard 98/574 on the Immulite 2000 analyzer (Siemens Healthcare Diagnostics UK) with a coefficient of variation of <5%. IGF1 levels were measured by a two-site immunoenzymometric assay (OCTEIA IGF1) supplied by Immunodiagnostic Systems Ltd, Tyne and Wear, UK. Intra assay precision was <5% and inter-assay precision <11% over the assay range.

Further local laboratory information about assay quality control, lower limit of detection and antibody cross-reactivity is provided in Appendix 1. GH reference standards are discussed in section 5.2.5.9, with further information provided in Appendices 1 and 2A.

3.4.2.3 Endocrine results

An adequate GH response to insulin-induced hypoglycaemia or glucagon was defined as a peak GH response greater than 5 μg/L (15 mU/L). The ITT cut-off was established locally using data from Scottish children and the Immulite 2000 GH assay (PM Crofton, personal communication). These data are provided in Appendix 2A. The same cut-off was used for the glucagon test, for reasons that are explained in section 5.2.5.5.

Locally determined age-dependent cut-offs were used to evaluate peak cortisol response to the ITT: >470 nmol/L in subjects ≥10 years; >550 nmol/L in subjects <10 years (Crofton et al 2004, see Appendix 2B). A cut-off of 450 nmol/L was used to define an adequate peak cortisol response to glucagon, based on responses amongst 31 healthy adults in a study by Agha et al (2004a). There appear to be no published paediatric reference range data for peak cortisol response to glucagon.

Peak FSH and LH responses to the low-dose GnRH test were evaluated using reference range data established by Zevenhuijzen et al (2004). IGF1 concentrations were interpreted

in relation to age- and sex-related reference ranges determined for the Nichols Advantage assay (Nichols Institute Diagnostics, San Clemente, California, USA) (Brabant et al 2003). Data obtained using the Nichols Advantage and OCTEIA IGF1 assays showed good agreement when directly compared in patient samples (M. Wallace, personal communication). Other basal results were interpreted using established local laboratory paediatric reference ranges.

3.4.3 Anthropometry and body composition assessment

3.4.3.1 Anthropometry and bioelectrical impedance analysis

Height and weight were measured to the nearest 0.1 cm and 0.1 kg respectively. Height, weight and BMI SDS were determined using the KIGS Auxology Calculator (Pfizer Endocrine Care™). This program was also used to calculate SDS for target height and parental-adjusted height. Comparisons were made between subjects with normal and abnormal GH responses to stimulation.

Bioelectrical impedance analysis (BIA) was performed using the Quantum II analyzer (RJL Systems). This bedside test is rapid, painless and non-invasive. Subjects were asked to lie flat on the bed with their legs slightly apart and their arms at their sides not touching the rest of the body. Electrodes were attached to the right hand and wrist and the right foot and ankle. A low energy, high frequency electrical signal (50 kHz, 800 μamps) was passed through the body and a measurement of body resistance (ohms) to the flow of the electrical current taken using the Quantum II analyzer. For further detail, see section 1.12.2.

The Resistance Index was calculated as follows:

$$\text{Resistance Index} = \text{Height (cm)}^2 / \text{Resistance (ohms)}$$
$$= HT^2 / R$$

3.4.3.2 Calculation of body composition variables

I used anthropometric and Resistance Index data to calculate body composition variables using a series of age- and gender-specific equations from the literature (Wells et al 2009). Using the aggregate prediction approach (section 5.3.1.2), the body composition values derived from these equations were combined and averaged. The original study by Wells et al (2009) used 12 equations, including three that incorporated data from skinfold thickness measurements. As I did not measure skinfold thickness during this study, I used only nine of

the 12 equations to inform aggregate body composition variables. These are described in Table iii below.

Table 3.iii: Body composition equations used in aggregate prediction

Raw data	Predicted variable	Age (years)	Equation	Groupings
Equations using height (cm) and weight (kg) data				
Morgenstern et al 2002	TBW	0.25-13	$0.0846 \times 0.95(\text{if F}) \times (HT \times WT)^{0.65}$	-
	TBW	>13	$0.0758 \times 0.84(\text{if F}) \times (HT \times WT)^{0.69}$	-
Mellits & Cheek 1970	TBW	-	$-1.927 + (0.465 \times WT) + (0.045 \times HT)$	Males, HT <132.7 cm
	TBW	-	$-21.933 + (0.406 \times WT) + (0.209 \times HT)$	Males, HT >132.7 cm
	TBW	-	$0.076 + (0.507 \times WT) + (0.013 \times HT)$	Females, HT <110.8 cm
	TBW	-	$-10.313 + (0.252 \times WT) + (0.154 \times HT)$	Females, HT >110.8 cm
Equations using BMI (kg/m^2) data				
Deurenberg et al 1991a	% Fat	7-15	$1.4 + (1.51 \times BMI) - (0.70 \times age) - 0.36(\text{if M})$	-
Pietrobelli et al 1998	FM	5-19	$-29.91 + (2.06 \times BMI)$	Males
	FM	5-19	$-26.06 + (1.96 \times BMI)$	Females
Equations using Resistance Index (HT^2/R) data				
Horlick et al 2002	TBW	4-18	$0.725 + (0.475 \times HT^2/R) + 0.14 \times WT)$	-
Houtkooper et al 1992	FFM	10-19	$1.31 + (0.61 \times HT^2/R) + (0.25 \times WT)$	-
Schaefer et al 1994	FFM	3-19	$0.15 + (0.65 \times HT^2/R) + (0.68 \times age)$	-
Deurenberg et al 1991b	FFM	7-15	$-6.48 + (0.406 \times HT^2/R) + (0.36 \times WT) + (5.58 \times HT\ [m]) + 0.56(\text{if M})$	-
Cordain et al 1988	FFM	9-14	$6.86 + (0.81 \times HT^2/R)$	-

TBW, total body water (l); FM, fat mass (kg); FFM, fat-free/lean mass (kg); F, female; M, male; WT, Weight (kg); HT, height (cm, except in the equation by Deurenberg et al [1991b] where height is in metres [m]); R, resistance (ohms)

Approximately three quarters of the body's FFM is water. FFM can be derived from total body water (TBW) using a hydration constant as follows:

FFM (kg) = [TBW (litres) / hydration constant (%)] x 100

However, the hydration of FFM is not static and varies with age (section 5.3.1.3). In this study, I used age- and gender-specific hydration constants established by Fomon et al (1982)

for subjects aged between three and ten years. Age- and gender-specific constants established by Lohman et al (1986) were used for subjects over the age of ten years. These constants are tabled below (Tables 3.iv A & B).

Table 3.iv: Age- and gender-specific hydration constants adapted from Fomon et al (1982, A) and Lohman et al (1986, B)

A

Age (years)	Hydration constant (%, Fomon et al 1982)	
	Males	Females
3	77.5	77.9
4	77.0	77.7
5	76.6	77.6
6	76.3	77.5
7	75.9	77.3
8	75.7	77.2
9	75.4	77.1
10	75.1	76.9

B

Age (years)	Hydration constant (%, Lohman et al 1986)	
	Males	Females
7-9	76.8	77.6
9-11	76.2	77.0
11-13	75.4	76.6
13-15	74.7	75.5
15-17	74.2	75.0
17-20	74.0	74.8
20-25	73.8	74.5

N.B. Constants in shaded cells were not used

FM was calculated from FFM as follows:

FM (kg) = Weight (kg) – FFM (kg)

Percentage body fat was calculated as follows:

Body fat (%) = [FM (kg) / Weight] x 100

Comparisons were made between subjects with normal and abnormal GH responses to stimulation.

3.4.4 QoL assessment

3.4.4.1 QoL measures and the Strengths and Difficulties Questionnaire

Subjects were asked to complete age-appropriate health-related QoL questionnaires. Children aged less than 16 years completed the KIDSCREEN-52 measure (eight to 18 years; Ravens-Sieberer et al 2005). One subject aged 16, reported by his parents and GP to be relatively immature (which was verified subjectively), also completed the KIDSCREEN-52 measure. All other subjects aged 16 years and over completed the QLS-H (Blum et al 2003) and QoL-AGHDA questionnaires (McKenna et al 1999). Both measures were developed and have been validated for use in adults with hypopituitarism. I also planned to use the

Short Form-36 Health Survey (SF-36) questionnaire (Ware & Sherbourne 1992, McHorney et al 1993) amongst older subjects. However, the cost of the licence and analysis software precluded the use of this tool.

Children aged less than 16 years also completed the Strengths and Difficulties Questionnaire (SDQ, 11 to 16 years; Goodman et al 1998). Parents/guardians of children aged four to 16 years completed the parent-report version of the SDQ (Goodman R 1997). I used the SDQ to evaluate behaviour and as a screening tool for mental health difficulties.

3.4.4.2 Scoring

SDQ: Answers to items in the SDQ contributed to scores for five different sub-scales: Emotional Symptoms, Conduct Problems, Hyperactivity, Peer Problems and Pro-social Behaviour. The latter sub-scale reflects positive attributes; the others reflect areas of difficulty. Options for responses to each item include Not true, Certainly true or Somewhat true. The latter is always scored 1; whether Not true or Certainly true are scored 0 or 2 depends on whether the question is framed as a strength or a difficulty.

A maximum score of 10 is possible for each sub-scale of the SDQ. Combining difficulties scales gives a total difficulties score out of 40. Normal, 'borderline abnormal' and abnormal bandings exist for individual subscale and total difficulties scores. These were selected on the basis that 80% of children from a community sample would score within the normal range, 10% within the borderline abnormal range and 10% within the abnormal range. To simplify comparisons between subject groups for this study, borderline abnormal and abnormal scores were amalgamated.

KIDSCREEN-52: Answers to items in the KIDSCREEN-52 questionnaire were assigned scores of 1 to 5. Negatively formulated questions were recoded so that higher values indicated higher quality of life. The KIDSCREEN-52 item responses inform raw scores for ten sub-scales. Any incomplete responses invalidate the score for the sub-scale concerned.

Reference tables in the KIDSCREEN manual (2006) were used to convert raw scores into gender-specific percentile and T-scores for 8-11 year olds or 12-18 year olds as appropriate. The mean T-score is approximately 50 for each scale, with a standard deviation (SD) of 10. Percentile and T-scores were compared between normal and abnormal subject groups.

QoL-AGHDA: The QoL-AGHDA measures the impact of GHD on QoL in one overall dimension. Each of the 25 items represents a problem and is scored dichotomously (yes or no). Every question answered in the affirmative is assigned a score of one. Individual scores were added to produce a total score out of 25, where the lower the score, the better the QoL.

McKenna et al (1999) did not establish cut-offs in terms of what constitutes impaired QoL in a GH-deficient adult. However, a score greater than 11 is required to qualify for adult GH therapy in the UK (NICE 2003). Koltowska-Haggstrom et al (2005) reported mean-weighted QoL-AGHDA scores of 6.2 and 7.1 for men and women from the general population, compared with 13.6 and 15.7 for GH-deficient adults. These findings were used as a guide to interpretation of QoL-AGHDA scores.

QLS-H: For the QLS-H questionnaire, subjects gave each of nine health-related areas a satisfaction score of 1 to 5, to produce a total score ranging from 9 to 45. The nine health-related areas are tabled in section 4.1.13.3 (Table xxiii). Subjects were also asked to score each health-related area from 1 to 5 on the basis of how important it was to them. In the analysis, the satisfaction score for each health-related area was 'weighted' according to the level of importance attached. The weighted satisfaction score was derived as follows (Blum et al 2003):

Weighted satisfaction = (importance - 1) x ([2x satisfaction] - 5)

The weighted satisfaction score ranges from -9 to +12 for each health-related area. The sum of weighted satisfaction scores across all nine areas ranges from -108 to +180. Any incomplete responses invalidate the sum score. The sum score can be converted into a Z-score using software that compares the sum score with gender- and country-specific normal population reference ranges. However, as the QLS-H questionnaire has only been standardised for use in those ≥18 years, it was not possible to generate Z-scores for four of seven subjects who completed the questionnaire. For this reason, comparisons were made based on sum scores alone.

3.5 Retrospective case record review

3.5.1 TBI characteristics and severity of injury

Subjects' medical case records were reviewed and head injury details were extracted. I recorded details of age at injury, mode of injury, post-resuscitation pupil response (normal vs. unilaterally or bilaterally dilated and unresponsive) and GCS score. The latter was used to grade the severity of TBI. Severity of injury was also evaluated using several other anatomical and physiological scoring systems:

ISS: The ISS was calculated for each subject (section 3.1.1).

Marshall Score: CT scan findings were categorised using the Marshall CT score (Table 3.v), which provides a framework for grading diffuse TBI (Marshall et al, 1991). Although this system was developed in adults, there are no equivalent paediatric scoring systems.

Table 3.v: Marshall CT scoring system, adapted from Marshall et al (1991)

Score	Marshall Score Category	Features
1	Diffuse Injury I	No visible intracranial pathology on CT scan
2	Diffuse Injury II	Cisterns present with midline shift 0-5 mm No high- or mixed-density lesion >25 cm^3
3	Diffuse Injury III (swelling)	Cisterns compressed/absent with midline shift 0-5 mm No high- or mixed-density lesion >25 cm^3
4	Diffuse Injury IV (shift)	Midline shift > 5 mm
5	Evacuated Mass Lesion	Any lesion surgically evacuated
6	Non-Evacuated Mass Lesion	High- or mixed-density lesion >25 cm^3

Revised Trauma Score (RTS): Where possible, the RTS was calculated (Champion et al 1989, Champion et al 1981). This is a physiological scoring system used to predict mortality from trauma in adults. It is scored from the first set of data obtained on the patient (Table 3.vi, overleaf). It is less useful in young children, in whom normal systolic blood pressure and respiratory rate differ significantly from adult values.

Table 3.vi: Revised Trauma Score scoring system

GCS score	SBP (mmHg)	RR / minute	Coded value
13-15	>89	10-29	4
9-12	76-89	>29	3
6-8	50-75	6-9	2
4-5	1-49	1-5	1
3	0	0	0

SBP, systolic blood pressure; RR, respiratory rate

RTS = 0.9368 GCS + 0.7326 SBP + 0.2908 RR

A RTS calculator is available at http://www.trauma.org/index.php/main/article/387/

Trauma – Injury Severity Score (TRISS): The TRISS was determined from the RTS and the ISS (Boyd et al 1987). It is a measure of probability of survival after blunt or penetrating trauma. Scores are assigned differently for subjects aged 0-54 years or 55 years and over. Subjects aged less than 15 years are automatically presumed to have sustained blunt trauma. A TRISS calculator is available at http://www.trauma.org/index.php/main/article/387/

Pediatric Trauma Score (PTS): The PTS was also calculated from the first set of data obtained on the patient as a guide to severity of injury (Tepas et al 1985). The total score ranges from -6 to +12 (Table 3.vii). A score greater than 8 predicts a mortality of less than 1%. A score of 4 predicts 50% mortality and a score less than 1 predicts over 98% mortality.

Table 3.vii: Pediatric Trauma Score scoring system

PTS Category	+2 points	+1 point	-1 point
Size of child	> 20 kg	10-20 kg	<10 kg
Airway	Normal	Maintainable	Not maintainable
SBP	> 90 mmHg	50-90 mmHg	< 50 mmHg
Conscious level	Awake (GCS 15), no loss of consciousness	Obtunded (GCS 9-14), any (even brief) loss of consciousness	Comatose (GCS <9)
Open wound	None	Minor	Major or penetrating
Fractures	None	Closed	Open or multiple

SBP, systolic blood pressure

3.5.2 Primary brain injury

I reviewed CT scan findings to identify any injuries that might be directly associated with pituitary dysfunction. These included evidence of hypothalamo-pituitary injury, damage to the third ventricle and basal skull fracture. Focal injuries were documented, including extra-axial bleeds and intra-parenchymal clots or contusions. Evidence of diffuse brain injury (and in particular, DAI) was also documented.

3.5.3 Secondary brain insult

I documented evidence of intra-cerebral oedema (with or without midline shift) on CT scan. Any history of post-traumatic seizures was also noted. The burden of secondary insult was measured as described below (section 3.5.3.1).

3.5.3.1 Measurement of the burden of secondary brain insult

Where available, computerised records of physiological monitoring during intensive care management were analysed by neurology research colleagues to quantify secondary brain insult. These data, previously downloaded from the bedside monitors, were available in time series format, at a one-minute resolution, for heart rate, temperature, arterial blood pressure, ICP, CPP and oxygen saturation. Data were collected for the duration of subjects' stay in intensive care.

Using the Edinburgh Browser software programme, all data were screened for artefact (caused by events such as turning, physiotherapy or transfers to CT or theatre). Such data were excluded from the analysis. Values were considered abnormal if outside the normal age-specific range for each variable (Jones et al 2003).

The Pressure Time Index (PTI) was used to evaluate the severity of secondary brain insult in study subjects (Chambers et al 2006). This is a cumulative, two-dimensional measure of the amplitude and duration of deranged ICP and CPP for children in age bands of 2-6, 7-10 and 11-15 years (normal age-adjusted cut-off thresholds). Chambers et al (2006) used PTI values derived from ICP (PTIicp) and CPP (PTIcpp) measurements (given in mmHg.mins) to identify the secondary brain insult threshold in relation to outcome. The PTIcpp had a very high predictive value for outcome: areas under the curve were 0.957 and 0.890 respectively for mortality and favourable outcome. The insult thresholds with the best predictive values for outcome were identical to the age-specific physiological variables, and

were as follows: 48, 54 and 58 mmHg for children aged 2-6, 7-10 and 11-15 years respectively.

3.5.4 Outcome

King's Outcome Scale for Childhood Head Injury (KOSCHI) rating was scored at study assessment to give an indication of functioning across the study sample.

KOSCHI score six months post-injury was also documented, if it could be determined from the medical case record (Crouchman al 2001). The KOSCHI was adapted from the adult Glasgow Outcome Scale (GOS, Jennett & Bond, 1975). It expands the five categories of the GOS to provide a more detailed description of children at the milder end of the disability range (Table 3.viii).

Table 3.viii: King's Outcome Scale for Childhood Heady Injury scoring system

Score	Description	Definition
1	Death	Death
2	Vegetative state	Breathing spontaneously; may have non-purposeful or reflex movements of limbs or eyes; no evidence of ability to communicate verbally or nonverbally or to respond to commands
3	Severe Disability	a) Some purposeful movement of body/eyes to command or spontaneously; may be conscious and able to communicate; unable to carry out self-care activities b) Exhibits high level of dependency but can assist with own care; fully conscious but with post-traumatic amnesia
4	Moderate disability	a) Mostly independent but requires supervision or help; has overt problems e.g. moderate hemiplegia and dyspraxia b) Age-appropriately independent but with residual learning/behaviour problems or neurological sequelae
5	Good recovery	a) Head injury resulted in a new condition that does not affect well being or functioning b) Complete recovery, no detectable sequelae

3.6 Data Analysis

Baseline characteristics were compared between recruited subjects and those who did not respond to the study invitation. I examined differences in male:female ratio, severity of TBI, age at injury and age at study invitation or assessment between groups. This was done to evaluate whether the sample recruited was representative of the entire at-risk cohort.

Baseline and TBI characteristics, and endocrine, body composition and QoL results were summarised for the study cohort. Comparisons were made between groups with normal and abnormal pituitary hormone results in terms of anthropometric, body composition and QoL data, and TBI characteristics.

3.6.1 Statistics

Results were expressed as mean (SD) if Gaussian. For non-Gaussian data, results were expressed as median (inter-quartile interval).

The parametric two-sample t-test was used to compare Gaussian data. The non-parametric Mann Whitney test was employed for non-Gaussian data or where numbers were too small to ascertain the likelihood of a Gaussian distribution. The Chi-squared or Fisher's exact tests were used to compare categorical data. Ninety-five percent confidence intervals (CI) are provided for p-values less than 0.1.

3.7 Follow-up

Appropriate follow-up arrangements were made with a local endocrinologist for subjects with abnormal pituitary hormone results.

Chapter 4: Results: Table of Contents

Chapter 4: Results

I investigated 35 survivors of childhood TBI in this retrospective observational pilot study. The majority (n=33) were survivors of accidental TBI. Two children had backgrounds of suspected NAHI at less than two years of age.

Results for these two groups of children were analysed separately for several reasons. The mechanism of injury is more consistent in suspected NAHI, more frequently involving rotational injury (Hymel et al 2007, Lo et al 2003). Typically, extensive subdural haemorrhages feature (Lo et al 2003). The brain injury sustained tends to be more severe and children may be more acutely unwell at presentation to medical services (Hymel et al 2007). Children with inflicted TBI are younger and the spectrum of ages at which injury occurs is narrower than for accidental TBI. The majority of NAHIs occur in infants aged less than one year; however, outcome studies may involve survivors of NAHI occurring at up to three years of age (Hymel et al 2007, Barlow et al 2005). NAHI has been associated with worse outcomes than accidental TBI (Goldstein et al 1993, Keenan et al 2006a; section 1.10).

Recruitment was more successful amongst survivors of accidental TBI than suspected NAHI: 33 of 102 invited subjects (32%) compared with two of 12 (17%). This and other differences between groups are discussed in more detail in sections 5.1.1 and 5.5.

4.1 Accidental TBI group

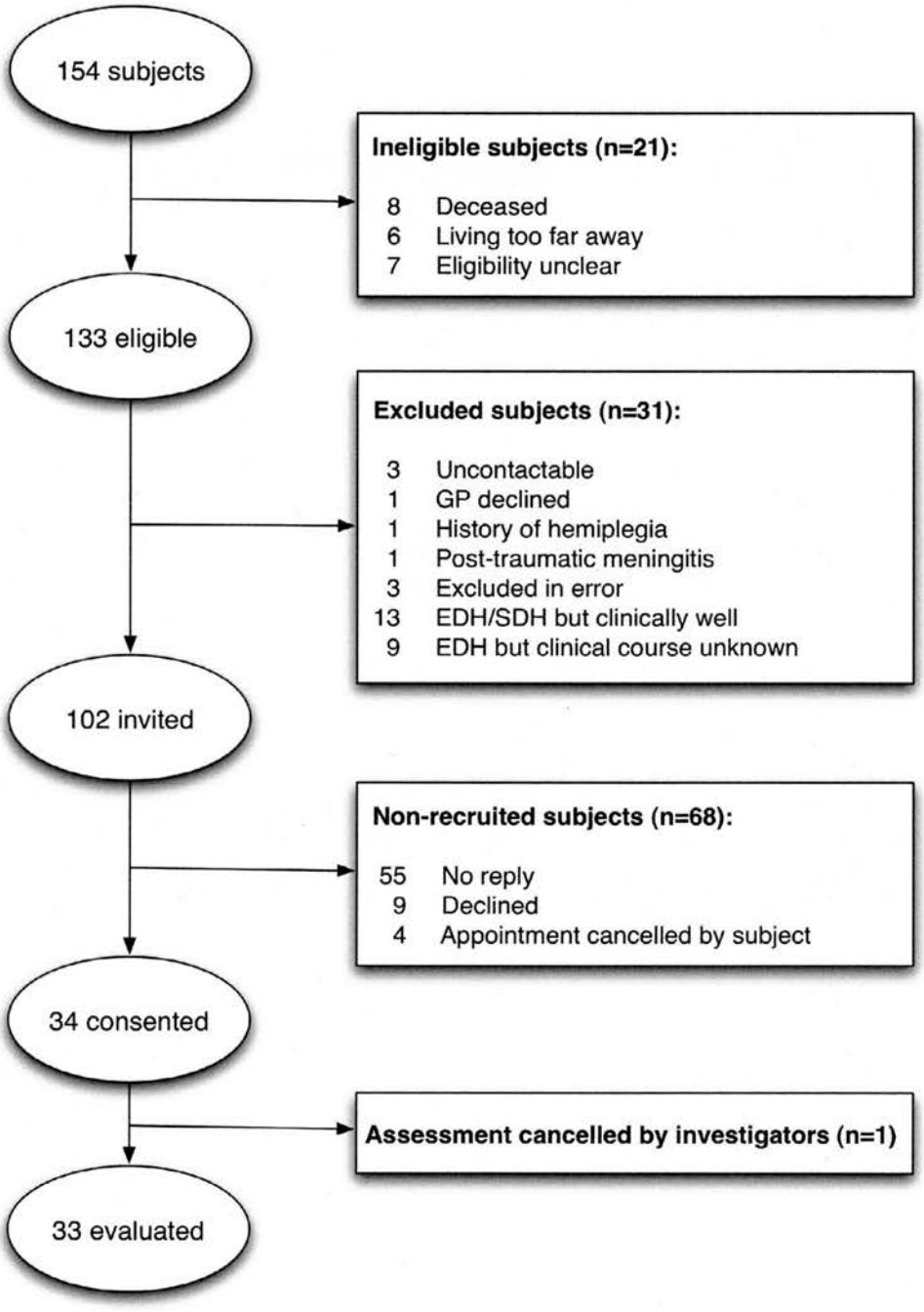
154 children aged less than 16 years were admitted to hospital in Edinburgh between 1st January 2001 and 31st August 2007 with moderate or severe TBI, or minor TBI with an ISS ≥16. Of these, eight were deceased, six were no longer living in Scotland or northeast England, and eligibility could not be clarified in seven cases. Thus, 133 individuals were eligible to take part in the study.

Invitations were sent to 102 of 133 eligible subjects (77%); 31 others were excluded. Contact addresses could not be ascertained in three cases and in a fourth, the GP declined to forward an invitation pack to the family concerned (no reason given). A fifth child had a history of cerebral palsy with hemiplegia preceding their TBI. Another contracted post-traumatic meningitis, which might have affected outcome in terms of pituitary function.

Three children were excluded in error and their eligibility only ascertained at the end of the study when reviewing excluded cases. Thirteen others were excluded in spite of sustaining small extradural or subdural haemorrhages incurring ISS scores of 16. These children had remained clinically well throughout their respective admissions with GCS scores of 13 to 15 and no requirement for ventilation or neurosurgery. It was concluded that the benign clinical course did not justify invasive testing. Nine subjects admitted to the WGH with extradural haemorrhages were also excluded. In these cases, too little information was available from computer admission records to be sure of GCS score at presentation and clinical course during admission.

Thirty-eight individuals/families expressed an interest in taking part in the study. Four of these cancelled their study appointments. Thirty-four subjects consented to take part but investigations were only carried out in 33. Tests were cancelled in one case because secure venous access could not be established. Results are therefore available for 33 subjects, constituting 32% (33/102) of those invited to take part in the study and 25% (33/133) of eligible subjects. This information is summarised in figure 4a, overleaf.

Figure 4a: Flow diagram of subject recruitment, accidental TBI group



EDH, extradural haemorrhage; SDH, subdural haemorrhage

4.1.1 Comparison of subject groups

In order to determine whether the sample investigated was representative of the cohort as a whole, characteristics such as gender, age at injury, age at invitation and severity of TBI were compared between groups.

Proportions of males were almost identical between subject groups, precluding the need for statistical analyses (Table 4.i).

Table 4.i: Proportions of male subjects within subject groups

Sample	**N=**	**Males (%)**
Whole cohort	154	114 (74%)
Eligible	133	101 (76%)
Invited	102	75 (74%)
Evaluated	33	25 (76%)

Mean age at injury was compared between subjects invited to take part in the study and subjects not invited to take part. No significant difference was detected between groups using the two-sample T-test (Table 4.ii).

Table 4.ii: Age at injury: invited vs. uninvited subjects

Characteristic	**Invited (N=102)**	**Uninvited (N=52)**	**p-value**
Mean (SD) age at injury (y)	9.5 (3.8)	9.7 (4.8)	0.8

y, years

I also compared mean age at injury and mean age at study invitation between recruited and non-recruited subjects. During these comparisons, the one subject who consented to take part in the study but was not evaluated was included in the non-recruited group. No significant differences were detected between groups using the two-sample T-test (Table 4.iii).

Table 4.iii: Ages at injury and at study invitation: recruited vs. non-recruited subjects

Characteristic	**Recruited (N=33)**	**Not recruited (N=69)**	**p-value**
Mean (SD) age at injury (y)	9.2 (3.4)	9.6 (4.0)	0.6
Mean (SD) age at invite (y)	13.4 (3.7)	13.5 (4.7)	0.9

y, years

Uncertainty regarding TBI severity amongst some subjects who were not invited to enter the study precluded comparisons being made with invited subjects in this respect. However, TBI severity was compared between recruited and non-recruited subjects. No significant difference was detected between groups using a Chi-square test ($p=0.2$). However, comparatively more subjects with minor TBI were recruited to the study and fewer individuals with severe TBI (Table 4.iv).

Table 4.iv: Severity of head injury: recruited vs. non-recruited subjects

Severity of TBI	**Recruited (%)**	**Not recruited (%)**	**Total**
Minor (ISS $\geq$ 16)	6 (18%)	5 (7%)	11
Moderate	15 (45%)	33 (48%)	48
Severe	12 (36%)	31 (45%)	43
Total	33 (100%)	69 (100%)	102

4.1.2 Subject characteristics

Baseline characteristics for the 33 subjects evaluated are shown in Table 4.v.

Table 4.v: Subject characteristics

No.	Sex	TBI Severity	Age (y) at study	Age (y) at injury	Years since injury	HT SDS	WT SDS	BMI SDS	Pubertal stage	KOSCHI at study
1	M	SEV	10.3	6.3	4.0	1.89	2.65	2.75	Pre-	4b
2	M	SEV	18.0	11.4	6.7	1.49	0.88	0.39	Late/post-	5a
3	M	SEV	16.4	12.7	3.7	0.64	-0.41	-0.84	Late/post-	4b
4	M	SEV	12.2	8.9	3.3	-0.81	0.81	1.69	Pre-	4b
5	M	SEV	13.1	8.5	4.6	-0.02	0.61	1.00	Early/Mid-	4b
6	M	SEV	17.2	12.2	5.0	0.56	0.57	0.56	Late/post-	5b
7	M	SEV	13.2	10.9	2.3	1.96	1.85	1.41	Late/post-	4b
8	M	SEV	17.0	11.6	5.4	-0.93	-1.28	-0.76	Late/post-	3b
9	M	MOD	15.1	10.0	5.1	-0.21	0.12	0.52	Late/post-	5b
10	M	MOD	9.8	7.5	2.4	1.38	1.20	0.96	Pre-	5b
11	M	MOD	16.3	10.0	6.2	0.67	-0.19	-0.55	Late/post-	4b
12	M	MOD	9.9	5.1	4.8	-1.57	-0.98	0.17	Pre-	4b
13	M	MOD	9.0	2.9	6.1	0.10	0.89	1.34	Pre-	4b
14	M	MOD	12.0	9.2	2.8	1.30	2.23	2.31	Early/Mid-	4a
15	M	MOD	8.4	4.7	3.7	-0.60	-0.20	0.44	Pre-	4b
16	M	MOD	12.6	6.3	6.3	0.02	-0.65	-1.01	Early/Mid-	5b
17	M	MOD	11.1	8.6	2.5	0.75	1.29	1.44	Pre-	4b
18	M	MOD	15.1	12.8	2.3	0.90	1.10	1.06	Late/post-	5a
19	M	MOD	14.8	10.5	4.3	-1.52	0.12	1.45	Early/Mid-	5b
20	M	MOD	12.9	7.0	5.9	1.00	1.35	1.30	Early/Mid-	4b
21	M	MIN	18.0	13.4	4.6	1.40	-0.20	-0.93	Late/post-	5a
22	M	MIN	12.9	8.2	4.7	0.23	0.05	-0.03	Early/Mid-	4b
23	M	MIN	5.4	0.6	4.8	-0.37	-0.18	0.42	Pre-	5b
24	M	MIN	8.5	6.3	2.3	-0.31	0.41	1.01	Pre-	5b
25	M	MIN	6.5	5.1	1.4	1.01	-0.04	-0.96	Pre-	5b
26	F	SEV	15.6	12.1	3.5	-1.53	-0.23	0.81	Late/post-	4b
27	F	SEV	18.9	14.1	4.8	3.00	2.20	1.16	Late/post-	3a
28	F	SEV	11.7	9.4	2.3	2.16	0.61	-0.75	Early/Mid-	5b
29	F	SEV	15.0	13.3	1.7	-0.77	0.17	0.77	Late/post-	4a
30	F	MOD	15.3	11.8	3.5	0.66	1.31	1.25	Late/post-	5b
31	F	MOD	13.0	7.5	5.4	0.49	1.44	1.46	Late/post-	5a
32	F	MOD	14.6	11.8	2.8	-0.20	2.86	2.92	Late/post-	4b
33	F	MIN	21.7	13.9	7.8	2.33	1.33	0.30	Late/post-	5b

M, male; F, female; MIN, minor; MOD, moderate; SEV, severe; y, years; HT, height; WT, weight

The system used to categorise pubertal stage is described in the Methods chapter (section 3.4.1). KOSCHI scores at study were recorded to give an indication of functioning across the cohort, between 1.4 and 7.8 years following TBI.

Baseline characteristics are summarised in Tables 4.vi and 4.vii.

Table 4.vi: Summary of subject characteristics part I

Variable	**N = 33**					
Gender	Males	25	Females	8		
Severity of head injury	Minor (ISS >16)	6	Moderate	15	Severe	12
Pubertal status at study	Pre-	10	Early/Mid-	7	Late/post-	16
KOSCHI score at study	Good outcome	15	Moderate disability	16	Severe disability	2

Table 4.vii: Summary of subject characteristics part II

Variable	**Mean (SD), N=33**		**Variable**	**Mean (SD), N=32**
Age at study (y)	13.4 (3.7)		Height (SDS)	0.50 (1.15)
Age at injury (y)	9.2 (3.4)		Weight (SDS)	0.65 (1.03)
Years since injury	4.1 (1.6)		BMI (SDS)	0.67 (1.04)

y, years

Summary height, weight and BMI statistics for N=32 subjects exclude data for one subject (no. 4) who was on long-term (supra-physiological) corticosteroid therapy for an unrelated medical condition. Mean height, weight and BMI SDS for the sample were comparable and within population norms.

No subject had clinical evidence of poor growth/short stature, with height SDS ranging from -1.6 to +3.0. Target height and parental-adjusted height values were within ± 2.0 SD in all but one subject. Parental-adjusted height SDS was +2.2 in one case.

4.1.3 Baseline endocrine investigation results

Summary statistics for baseline endocrine tests, together with paediatric reference ranges are shown in Table 4.viii and results for each subject are tabled overleaf (Table 4.ix). Paired early morning urine and plasma osmolalities were available for all but one subject. There was no evidence of DI from these or plasma sodium concentrations. Thyroid function tests were essentially normal. TSH concentrations were mildly raised in one child (5.2 mU/L; subject no. 12), excluding central hypothyroidism but raising the possibility of an unrelated thyroid disorder. His GP was advised to repeat thyroid function tests in six months' time. One male subject (no. 13) was prolactin deficient (<50 mU/L). Two subjects (nos. 16 & 19) had mildly reduced IGF1 concentrations for age and pubertal stage: 146 μg/L at 12.6 years

and 179 μg/L at 14.8 years. The former had a normal GH response to insulin-induced hypoglycaemia (section 4.1.6, Table 4.xiii); the other had a sub-optimal GH response to the ITT and is now on GH therapy (sections 4.1.6, 5.2.5.1). Baseline early morning cortisol results are described in section 4.1.5; basal gonadotrophin and oestradiol/testosterone results are described in section 4.1.4.

Table 4.viii: Summary statistics for baseline endocrine test results

Variable	**N=**	**Mean (SD)**	**Reference range**
Urine osmolality mmol/kg	32[a]	880 (211)	50-1200
Plasma osmolality mmol/kg	33	290 (5)	275-295
Plasma sodium mmol/L	33	140 (1.5)	132-144
Free T4 pmol/L	33	12.4 (1.3)	10-17
TSH mU/L	33	1.91 (1.03)	0.5-4.2
Prolactin mU/L	33	140 (81)	60-500
IGF-1 μg/L	33	246 (99)	2-5 y 13-180 5-8 y 26-200 8-11 y 20-460 11-14 y 150-600 14-17 y 200-650 17-26 y 100-580

y, years; [a] missing data for one subject

Table 4.ix: Baseline endocrine test results by subject

No.	Sex	Age (y) at study	Urine osmolality mmol/kg	Plasma osmolality mmol/kg	Plasma sodium mmol/L	Free T4 pmol/L	TSH mU/L	Prolactin mU/L	IGF1 µg/L
1	M	10.3	705	289	140	13	1.40	87	170
2	M	18.0	803	290	142	12	3.10	144	185
3	M	16.4	1157	291	140	11	1.70	98	388
4	M	12.2	680	288	139	13	2.50	235	270
5	M	13.1	843	295	141	11	1.30	95	357
6	M	17.2	886	276	141	12	1.10	87	337
7	M	13.2	1174	296	140	12	0.84	136	375
8	M	17.0	859	293	137	12	1.70	141	207
9	M	15.1	868	290	142	13	3.80	86	228
10	M	9.8	475	288	141	13	1.50	77	139
11	M	16.3	773	285	139	12	3.20	456	225
12	M	9.9	876	292	140	14	5.40[a]	77	81
13	M	9.0	813	286	140	13	1.60	<50[b]	153
14	M	12.0	968	289	140	10	1.60	114	432
15	M	8.4	580	292	141	12	3.30	171	99
16	M	12.6	952	284	142	15	1.50	120	146[c]
17	M	11.1	1132	299	142	11	1.50	68	179
18	M	15.1	1076	293	140	12	1.20	169	412
19	M	14.8	1073	288	139	13	0.73	80	179[c]
20	M	12.9	1044	293	141	11	1.10	137	389
21	M	18.0	438	284	141	11	1.30	115	332
22	M	12.9	811	291	139	13	1.50	70	244
23	M	5.4	1078	283	138	12	2.80	98	122
24	M	8.5	535	279	139	15	3.00	86	132
25	M	6.5	723	290	138	16	1.50	148	103
26	F	15.6	1215	293	143	12	0.68	192	276
27	F	18.9	No sample	296	139	11	0.88	112	309
28	F	11.7	884	296	141	13	2.80	86	283
29	F	15.0	1210	290	140	11	1.90	120	251
30	F	15.3	735	289	142	12	2.50	102	332
31	F	13.0	1121	295	142	13	1.50	231	258
32	F	14.6	847	296	141	13	1.20	265	313
33	F	21.7	832	296	144	12	1.40	289	218

M, male; F, female; y, year; [a] elevated TSH; [b] prolactin deficiency; [c] mildly reduced IGF-1 level for age and pubertal stage. See Table 4.viii for reference ranges.

4.1.4 HPG axis

Basal oestradiol or testosterone concentrations were appropriate for age, sex and pubertal stage in all subjects. All basal testosterone samples were obtained between 08:20 and 10:10 hours. Baseline FSH and LH concentrations and peak response to GnRH were appropriate for sex and pubertal stage (Table 4.x). Peak responses could not be measured in one subject

(no. 27) whose cannula came out during dynamic testing. She was, in any case, post-pubertal with appropriate baseline LH, FSH and oestradiol concentrations. Baseline LH was <0.5 U/L in one early/mid-pubertal male (no. 19) at 14.8 years of age but basal testosterone concentrations and gonadotrophin response to GnRH were appropriate for pubertal stage. Testicular volumes in this subject were 6 and 8 mLs. This boy probably had constitutional delay of puberty that was resolving spontaneously. GnRH test results are presented in full in Appendix 3A. See Appendix 3B for complete stimulation test results by subject.

Table 4.x: Baseline and stimulation test results for the HPG axis by subject

No.	Sex	Age (y) at study	Pubertal stage	Basal FSH U/L	Peak FSH U/L	Basal LH U/L	Peak LH U/L	Testo-sterone nmol/L	Oestra-diol pmol/L
1	M	10.3	Pre-	<0.5	3.1	1.9	5.9	0.5	–
2	M	18.0	Late/post-	1.1	2.8	1.6	25.1	15.5	–
3	M	16.4	Late/post-	3.1	4.7	1.4	10.7	12.9	–
4	M	12.2	Pre-	3.1	6.1	<0.5	2.7	<0.4	–
5	M	13.1	Early/Mid-	1.4	1.7	1.5	6.8	9.6	–
6	M	17.2	Late/post-	1.7	2.3	2.1	10.6	19.5	–
7	M	13.2	Late/post-	2.7	4.0	2.7	14.6	18.3	–
8	M	17.0	Late/post-	4.3	11.2	2.8	35.3	17.0	–
9	M	15.1	Late/post-	1.6	2.4	2.1	11.8	18.2	–
10	M	9.8	Pre-	0.5	3.6	<0.5	1.9	0.5	–
11	M	16.3	Late/post-	7.2	9.8	3.2	14.0	12.4	–
12	M	9.9	Pre-	<0.5	1.9	<0.5	1.0	1.0	–
13	M	9.0	Pre-	<0.5	2.4	<0.5	1.0	0.6	–
14	M	12.0	Early/Mid-	4.5	9.6	0.9	11.5	5.0	–
15	M	8.4	Pre-	<0.5	3.0	<0.5	1.3	0.6	–
16	M	12.6	Early/Mid-	0.7	1.5	<0.5	3.7	1.0	–
17	M	11.1	Pre-	1.3	2.8	<0.5	4.7	0.4	–
18	M	15.1	Late/post-	1.8	2.5	1.9	6.8	24.9	–
19	M	14.8	Early/Mid-	1.5	2.1	<0.5[a]	4.2	2.7	–
20	M	12.9	Early/Mid-	1.5	2.5	1.6	10.1	6.9	–
21	M	18.0	Late/post-	2.2	3.3	1.3	9.2	16.6	–
22	M	12.9	Early/Mid-	2.4	4.3	0.5	10.7	3.0	–
23	M	5.4	Pre-	0.5	3.2	<0.5	2.0	<0.4	–
24	M	8.5	Pre-	0.5	3.0	<0.5	1.1	<0.4	–
25	M	6.5	Pre-	1.1	4.4	<0.5	0.7	<0.4	–
26	F	15.6	Late/post-	4.2	13.8	12.2	106.0	–	1183
27	F	18.9	Late/post-	6.1	No sample	10.2	No sample	–	507
28	F	11.7	Early/Mid-	6.9	14.6	4.2	25.8	–	78
29	F	15.0	Late/post-	3.4	7.5	2.4	20.9	–	59
30	F	15.3	Late/post-	2.3	2.5	1.0	15.6	–	411
31	F	13.0	Late/post-	5.8	7.9	7.7	20.4	–	240
32	F	14.6	Late/post-	3.5	5.9	9.5	38.9	–	379
33	F	21.7	Late/post-	3.3	8.2	6.3	65.8	–	281

M, male; F, female; y, years; [a] probable constitutional delay of puberty, resolving spontaneously

4.1.5 HPA axis

Baseline early morning cortisol samples were obtained between 08:20 and 10:10 hours. Median baseline cortisol concentration was 298 nmol/L (inter-quartile range 216 to 443 [06:00-10:00 reference range 150-600] nmol/L). In two subjects (nos. 19 and 30), baseline cortisol concentrations were below the reference range for 06:00-10:00 hours (110 and 146 nmol/L). Both subjects had suboptimal cortisol responses to the ITT (Table 4.xii).

HPA response to stimulation was assessed using the ITT in 25 subjects and the glucagon test in eight (all with previous seizures). Plasma glucose fell to ≤2.2 mmol/L in all subjects undergoing an ITT (Table 4.xii). One subject's cannula came out during the glucagon test (no. 27). Attempts at re-cannulation failed but a venous blood sample obtained 140 minutes after glucagon administration demonstrated an adequate cortisol response. Summary statistics for basal cortisol concentrations and peak responses to stimulation are displayed below (Table 4.xi). Individual baseline and peak cortisol results for each subject are displayed overleaf (Table 4.xii).

Table 4.xi: Baseline early morning cortisol and peak responses to stimulation

Variable	N=	Q1	Median	Q3
Baseline early morning cortisol nmol/L	33	216	298	443
Peak cortisol response to ITT nmol/L	25	460	538	611
Peak cortisol response to glucagon nmol/L	8	–	562	–

1st quartile; Q3, 3rd quartile; too few subjects had a glucagon test to provide Q1 & Q3

Peak cortisol responses to insulin-induced hypoglycaemia were suboptimal in nine of 25 subjects (<470 nmol/L ≥10 years; <550 nmol/L <10 years [Crofton et al 2004]). One child had a high baseline early morning cortisol level (624 nmol/L) so their suboptimal response to the ITT (459 nmol/L) was regarded as having no clinical significance. In five of eight others, peak cortisol response was only borderline low (within 50 nmol/L below the cut-off), requiring no treatment. In the remaining three, the peak cortisol response was between 50 and 100 nmol/L below the cut-off. For these subjects, steroid cover was recommended during moderate or severe illness or injury, with a view to reassessing the HPA axis in one to two years' time.

The peak cortisol cut-off used for the glucagon test was 450 nmol/L (Agha et al 2004a). Two of eight subjects had flat cortisol responses to glucagon, a recognised phenomenon in approximately 10% of healthy individuals (Agha et al 2004a, Rao & Spathis 1987). The sub-optimal response was discounted in one case, as baseline early morning cortisol

concentrations were high (722 nmol/L). The other subject (no. 18) was offered an appointment to discuss options for further assessment of the HPA axis but did not attend. No treatment was recommended for this subject. ITT and glucagon test results are presented in full in Appendix 3A. See Appendix 3B for complete stimulation test results by subject.

Table 4.xii: Baseline and stimulation test results for the HPA axis by subject

No.	Sex	TBI Severity	Age (y) at study	Basal cortisol nmol/L	Time at basal sample	Test used	Peak cortisol nmol/L	Glucose nadir mmol/L
1	M	SEV	10.3	166	09:00	Glucagon	517	4.3
2	M	SEV	18.0	471	08:35	ITT	717	1.4
3	M	SEV	16.4	341	09:20	ITT	462[c]	1.0
4	M	SEV	12.2	218	09:05	ITT	538	1.2
5	M	SEV	13.1	248	09:25	ITT	564	1.5
6	M	SEV	17.2	281	08:45	ITT	443[c]	0.8
7	M	SEV	13.2	202	09:39	ITT	488	1.0
8	M	SEV	17.0	494	09:15	ITT	598	1.5
9	M	MOD	15.1	487	09:25	Glucagon	466	3.0
10	M	MOD	9.8	150	09:10	Glucagon	496	3.4
11	M	MOD	16.3	722[b]	08:20	Glucagon	No peak[b]	3.7
12	M	MOD	9.9	320	10:00	ITT	593	1.4
13	M	MOD	9.0	356	09:40	ITT	546[c]	0.9
14	M	MOD	12.0	402	09:35	ITT	367[d]	1.5
15	M	MOD	8.4	624[b]	09:35	ITT	459[b]	1.5
16	M	MOD	12.6	214	09:40	ITT	522	1.5
17	M	MOD	11.1	261	09:45	ITT	545	1.1
18	M	MOD	15.1	210	09:10	Glucagon	289[f]	3.3
19	M	MOD	14.8	110[a]	09:20	ITT	458[c]	1.2
20	M	MOD	12.9	262	09:05	ITT	385[d]	1.8
21	M	MIN	18.0	342	09:20	ITT	507	1.2
22	M	MIN	12.9	290	09:45	ITT	458[c]	1.4
23	M	MIN	5.4	294	09:45	ITT	716	2.0
24	M	MIN	8.5	373	09:45	ITT	645	1.0
25	M	MIN	6.5	415	09:30	Glucagon	729	3.1
26	F	SEV	15.6	152	10:10	Glucagon	606	3.2
27	F	SEV	18.9	316	09:55	Glucagon	624	4.4
28	F	SEV	11.7	236	09:40	ITT	528	1.1
29	F	SEV	15.0	622	09:15	ITT	526	1.8
30	F	MOD	15.3	146[a]	10:00	ITT	414[d]	1.2
31	F	MOD	13.0	298	09:20	ITT	542	1.0
32	F	MOD	14.6	562	09:20	ITT	660	1.3
33	F	MIN	21.7	589	08:55	ITT	707	1.4

M, male; F, female; y, years; MIN, minor; MOD, moderate; SEV, severe; [a] low baseline early morning cortisol concentrations; [b] baseline early morning cortisol value high so inadequate peak response discounted; [c] sub-optimal response to ITT, peak cortisol within 50 mmol/L below cut-off; [d] sub-optimal response to ITT, peak cortisol 50-100 mmol/L below cut-off; [f] sub-optimal cortisol response to glucagon. See text for reference ranges.

4.1.6 GH axis

The GH axis was assessed using the ITT in 25 subjects and the glucagon test in the remaining eight (with previous seizures). Peri-pubertal subjects were not primed with oestradiol or testosterone beforehand (discussed in section 5.2.5.3). A plasma glucose nadir of $\leq$ 2.2 mmol/L was reached in all subjects undergoing an ITT. One subject's cannula came out during the glucagon test (no. 27). Attempts at re-cannulation failed but a venous blood sample obtained 140 minutes after glucagon administration demonstrated an adequate GH response.

Median peak GH response to the insulin tolerance or glucagon tests was 7.9 μg/L (inter-quartile range 5.2 to 13.6 μg/L). Individual GH results for each subject are displayed overleaf (Table 4.xiii). Seven males had sub-optimal GH responses (<5 μg/L) to insulin-induced hypoglycaemia (in six cases) or glucagon. Six were in late pre-puberty or early/mid-puberty and their low responses could reflect physiological blunting of GH secretion (section 5.2.5.3). Height SDS at assessment were within ± 2 in all six (range -1.5 to 1.4 SD). Four had normal growth velocities in relation to pubertal stage at follow-up (>-0.8 SD in all cases); in the fifth (no. 8), growth velocity was inappropriately slow for pubertal stage and GH was prescribed. All remain under review. The sixth failed to attend two follow-up appointments (subject no. 20).

The seventh subject (no. 8) was post-pubertal, with a borderline low GH response to insulin-induced hypoglycaemia (3.2 μg/L, height SDS -0.93). European Society of Paediatric Endocrinology (ESPE) guidelines define GHD in the transition period between childhood and adulthood by a peak GH response less than 5 μg/L during the ITT (Clayton et al 2005). However the cut-off recommended by NICE for this group is 3 μg/L (NICE 2003). Management options are discussed in more detail in section 5.2.6.

Three subjects had suboptimal GH responses to stimulation following spontaneous GH peaks at baseline. This has been reported previously (Leong et al 2001) and may be due to a refractory period (section 5.2.5.5). The sub-optimal responses were discounted and the peak GH values listed in Table xiii refer to the peaks at baseline. ITT and glucagon test results are presented in full in Appendix 3A. See Appendix 3B for complete stimulation test results by subject.

Table 4.xiii: GH stimulation test results by subject

No.	Sex	TBI Severity	Age (y) at study	HT SDS	Pubertal stage	Test used	Peak GH µg/L	Glucose nadir mmol/L
1	M	SEV	10.3	1.89	Pre-	Glucagon	5.9	4.3
2	M	SEV	18.0	1.49	Late/post-	ITT	15.1	1.4
3	M	SEV	16.4	0.64	Late/post-	ITT	11.0	1.0
4	M	SEV	12.2	-0.81	Pre-	ITT	7.9	1.2
5	M	SEV	13.1	-0.02	Early/Mid-	ITT	5.2	1.5
6	M	SEV	17.2	0.56	Late/post-	ITT	14.8	0.8
7	M	SEV	13.2	1.96	Late/post-	ITT	7.0	1.0
8	M	SEV	17.0	-0.93	Late/post-	ITT	3.2 [a]	1.5
9	M	MOD	15.1	-0.21	Late/post-	Glucagon	14.2	3.0
10	M	MOD	9.8	1.38	Pre-	Glucagon	2.5 [a]	3.4
11	M	MOD	16.3	0.67	Late/post-	Glucagon	9.5 [b]	3.7
12	M	MOD	9.9	-1.57	Pre-	ITT	5.6 [b]	1.4
13	M	MOD	9.0	0.10	Pre-	ITT	5.1	0.9
14	M	MOD	12.0	1.30	Early/Mid-	ITT	2.9 [a]	1.5
15	M	MOD	8.4	-0.60	Pre-	ITT	6.1	1.5
16	M	MOD	12.6	0.02	Early/Mid-	ITT	7.1	1.5
17	M	MOD	11.1	0.75	Pre-	ITT	3.5 [a]	1.1
18	M	MOD	15.1	0.90	Late/post-	Glucagon	15.3	3.3
19	M	MOD	14.8	-1.52	Early/Mid-	ITT	2.8 [a]	1.2
20	M	MOD	12.9	1.00	Early/Mid-	ITT	4.4 [a]	1.8
21	M	MIN	18.0	1.40	Late/post-	ITT	20.6 [b]	1.2
22	M	MIN	12.9	0.23	Early/Mid-	ITT	15.8	1.4
23	M	MIN	5.4	-0.37	Pre-	ITT	7.5	2.0
24	M	MIN	8.5	-0.31	Pre-	ITT	3.9 [a]	1.0
25	M	MIN	6.5	1.01	Pre-	Glucagon	14.0	3.1
26	F	SEV	15.6	-1.53	Late/post-	Glucagon	9.4	3.2
27	F	SEV	18.9	3.00	Late/post-	Glucagon	10.9	4.4
28	F	SEV	11.7	2.16	Early/Mid-	ITT	13.0	1.1
29	F	SEV	15.0	-0.77	Late/post-	ITT	25.4	1.8
30	F	MOD	15.3	0.66	Late/post-	ITT	10.6	1.2
31	F	MOD	13.0	0.49	Late/post-	ITT	5.7	1.0
32	F	MOD	14.6	-0.20	Late/post-	ITT	13.2	1.3
33	F	MIN	21.7	2.33	Late/post-	ITT	8.6	1.4

M, male; F, female; y, years; MIN, minor; MOD, moderate; SEV, severe; HT, height; [a]GH peak <5 µg/L; [b] value refers to spontaneous GH peak at baseline

4.1.7 Summary of endocrine results

Endocrine abnormalities were identified in 13 of 33 accidental TBI subjects (39%). Suboptimal GH responses to stimulation were seen in seven subjects, abnormalities of the HPA axis in nine and prolactin deficiency in one. Multiple abnormalities were seen in four of 33 subjects (12%). In each case, two hormone axes were affected: GH and HPA axes in three, and prolactin and HPA axes in the fourth.

Over the following pages, comparisons of reported symptoms, QoL data, TBI characteristics and functional outcome after TBI are made between normal (n=20) and abnormal (n=13) pituitary hormone groups. Comparisons of anthropometric and body composition data are made between GH-sufficient subjects (n=25) and those with suboptimal responses (peak GH <5 μg/L, n=7). This is due to the particular impact of GH on growth and body composition (section 1.12.1).

4.1.8 Age at study and anthropometric data comparisons

Mean (SD) age at study amongst subjects with normal pituitary function (n=20) was 13.5 (4.1) years, compared with 13.2 (3.0) years amongst subjects with abnormal results (n=13). No significant age difference was detected between groups using the 2-sample T-test (p=0.9). These data are displayed on a boxplot in section 4.1.13 (Figure 4j), alongside data comparing mean age at TBI and mean time interval since TBI between groups.

Anthropometric data comparisons exclude results for one subject (no. 4) who was on long-term (supra-physiological) corticosteroid therapy for an unrelated medical condition. Comparisons were made between subjects with normal (n=25) and suboptimal (n=7) GH responses to stimulation testing (peak GH <5 μg/L). No significant differences were seen between groups in terms of median height, weight or BMI SDS using the Mann-Whitney test (Table 4.xiv). Similarly, no significant differences were seen in median target height SDS or parental-adjusted height SDS.

Table 4.xiv: Comparison of auxology data, GH-normal vs. GH-abnormal groups

Variable	**GH-normal group (n=25)**	**GH-abnormal group (n=7)**	**p-value**
Median height SDS	0.6	0.8	0.7
Median weight SDS	0.6	1.2	0.5
Median BMI SDS	0.5	1.3	0.1
Median target height SDS	0.3	0.5	0.6
Median parental-adjusted height SDS	0.1	-0.2	0.2

Comparisons are shown on two individual value plots (Figures 4b & 4c). There was a trend towards lower parental-adjusted height SDS in the GH-abnormal group. Subjects' height, weight and BMI SDS are plotted against age at study in Figs. 4d-f. The results were proportional, regardless of GH status.

Figure 4b: Individual value plot of anthropometric data, GH-normal vs. -abnormal groups

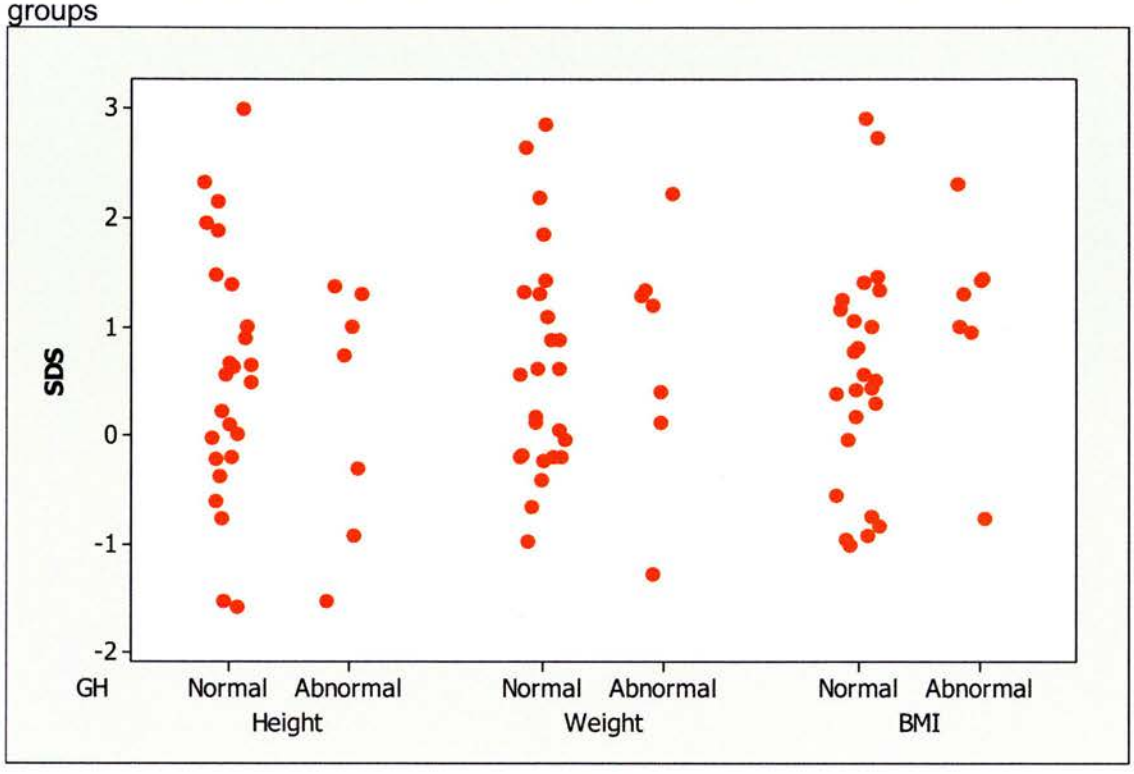


Figure 4c: Individual value plot: target height and parental-adjusted height SDS, GH-normal vs. -abnormal groups

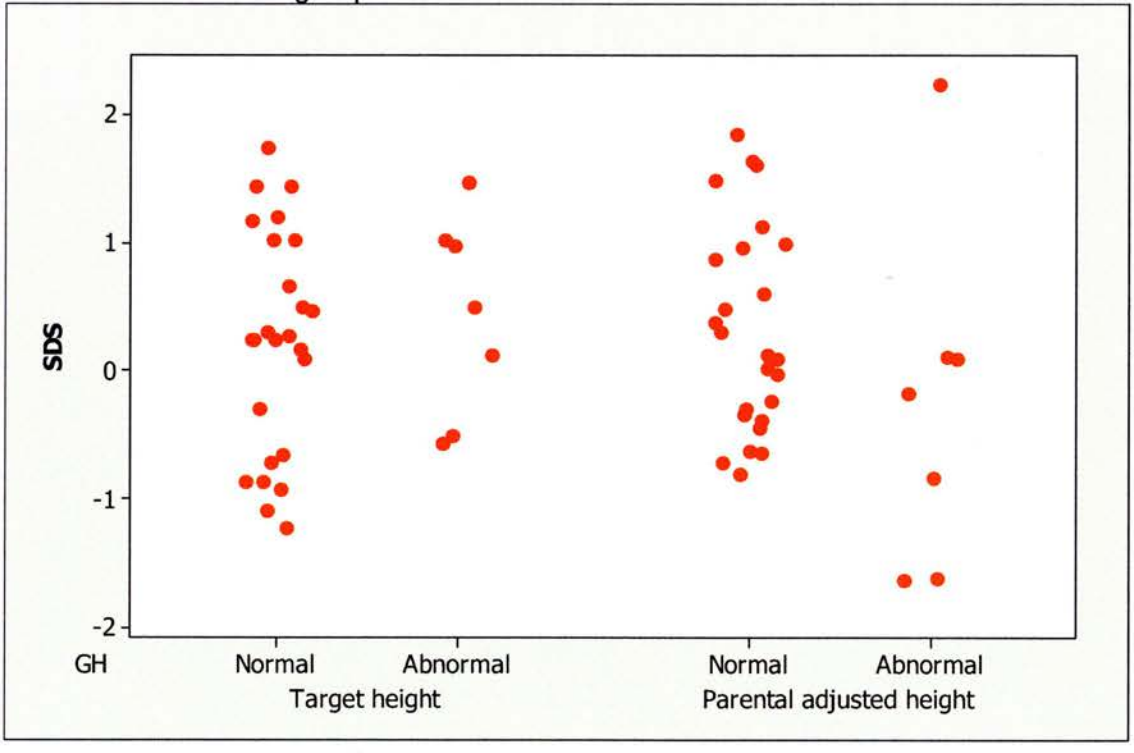


Figure 4d: Scatter plot of height SDS against age, GH-normal and -abnormal groups

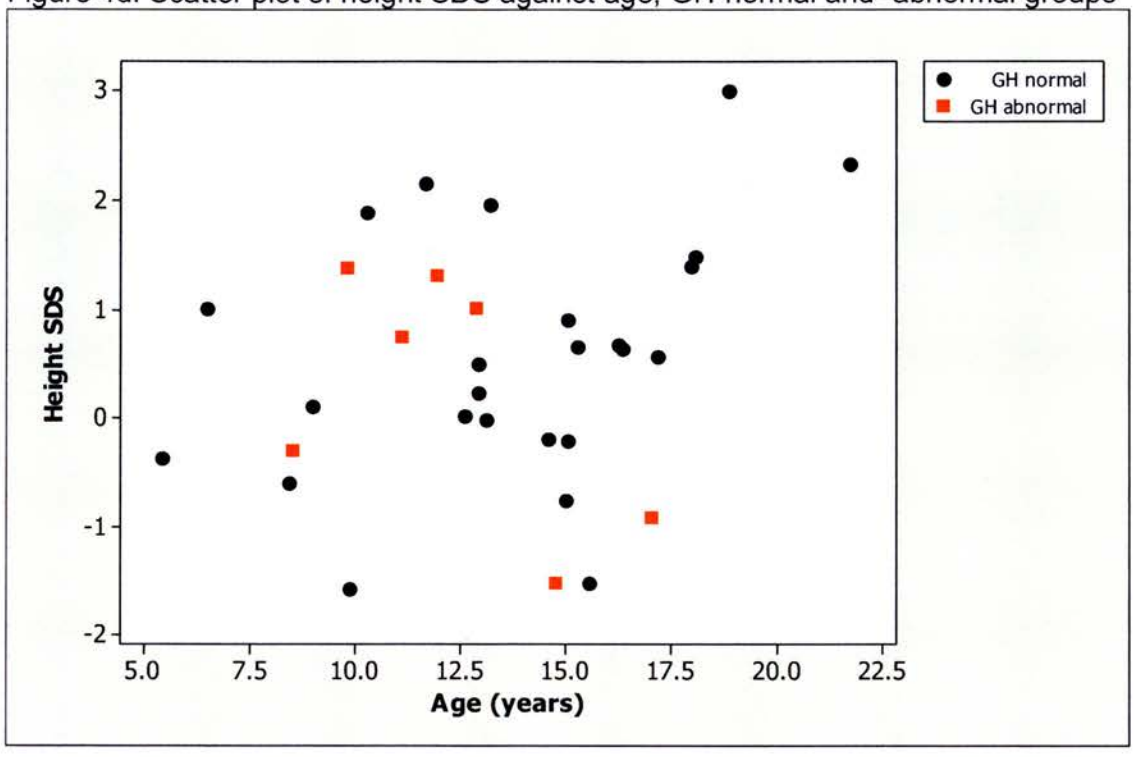


Figure 4e: Scatter plot of weight SDS against age, GH-normal and -abnormal groups

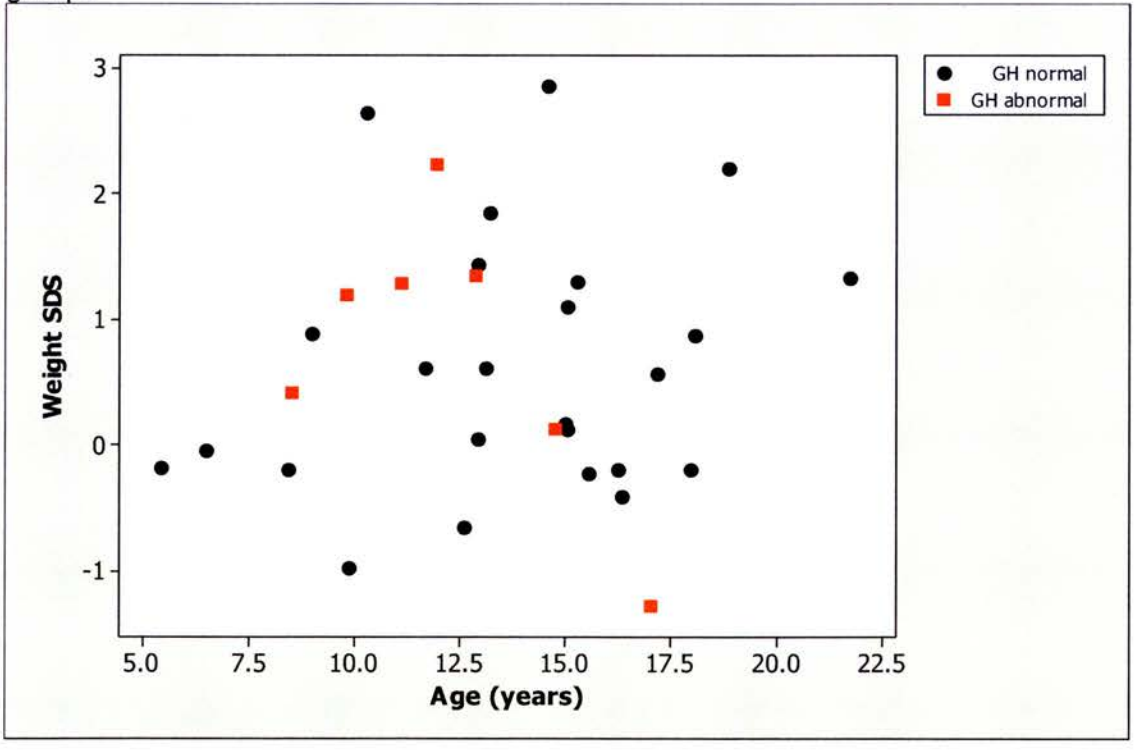
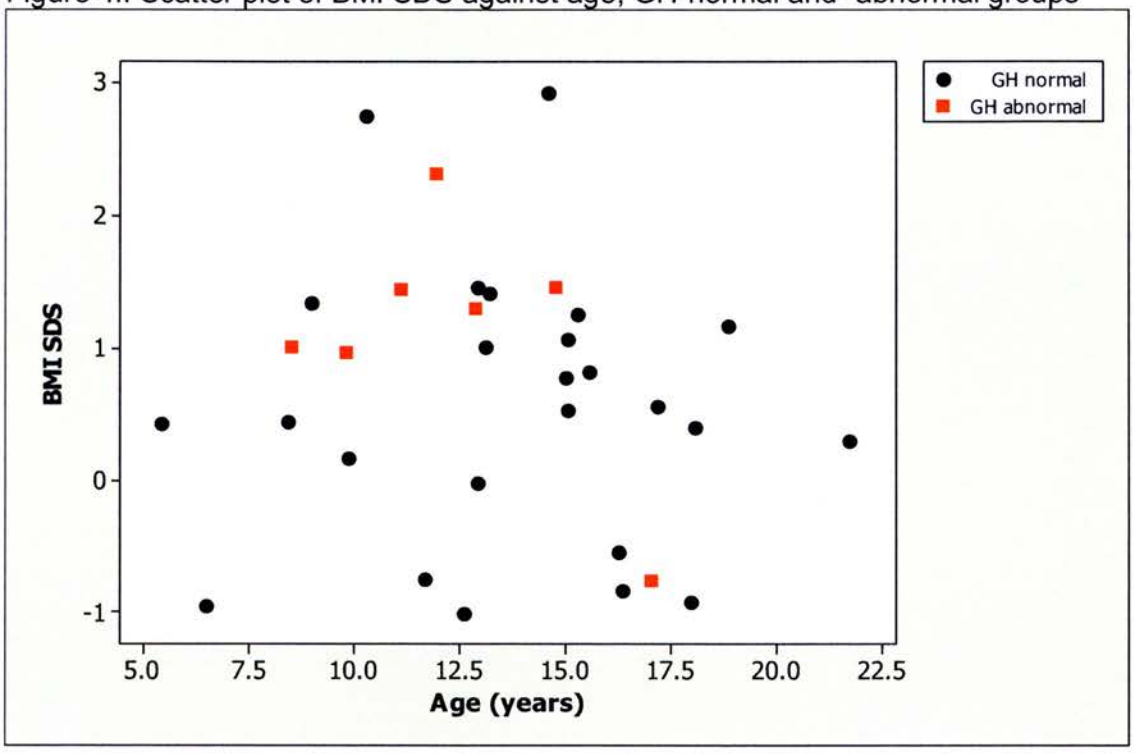


Figure 4f: Scatter plot of BMI SDS against age, GH-normal and -abnormal groups



4.1.9 Body composition data comparisons

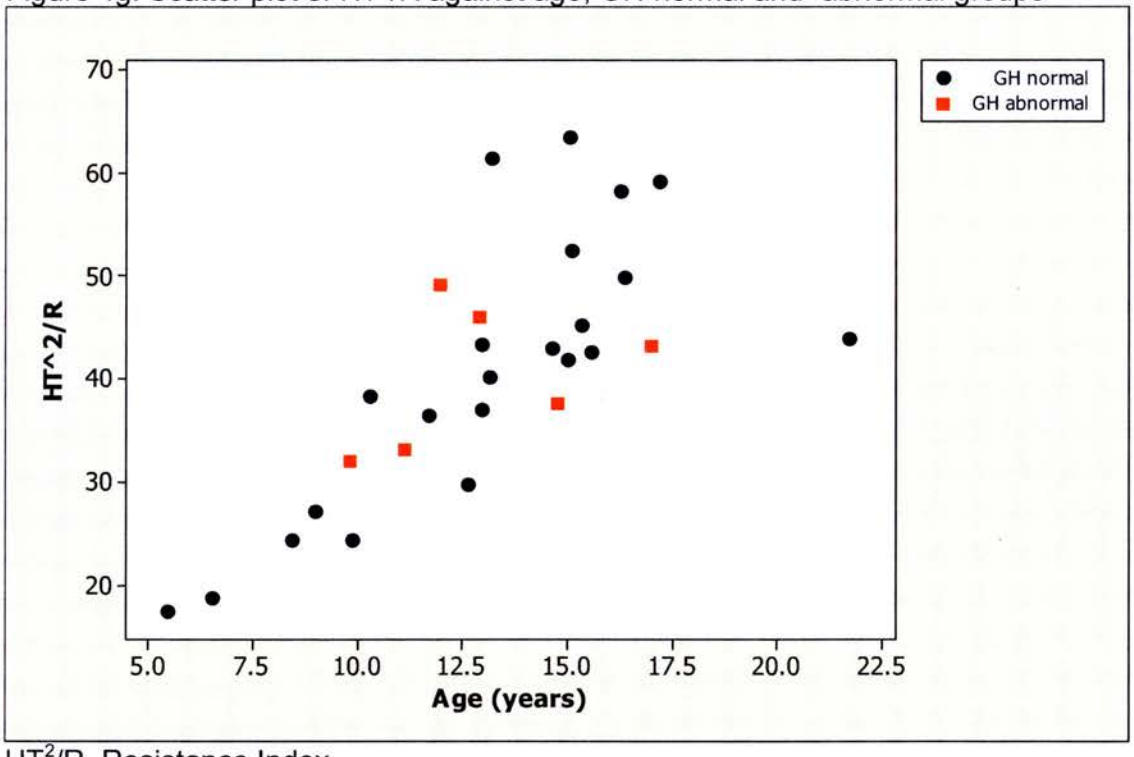
Body composition was evaluated in 32 of 33 subjects (excluding one subject [no. 4] on long-term supra-physiological corticosteroid therapy). The aggregate prediction method was used to derive estimates of fat-free mass (FFM), fat mass (FM) and percentage body fat (Wells et al 2009, section 5.3.1.2). I used nine body composition equations compiled by Wells et al (2009). Each was specific to a particular age range, with few applicable to young children or young adults. Thus aggregate body composition variables for the oldest and youngest subjects were derived from fewer equations than those for subjects in mid-childhood. Individual body composition results for each subject are shown in Table 4.xv.

Standardised UK reference ranges for FFM, FM and percentage body fat (taking into account age and sex) have yet to be established. This precluded statistical comparisons being made between GH-normal and -abnormal groups in terms of body composition results. However, there was no obvious discrepancy between groups when Resistance Index (HT^2/R) was plotted against age (Figure 4g). The Resistance Index is related to FFM and has a linear relationship with increasing age.

To evaluate percentage body fat status, I used UK age- and gender-specific reference curves established by McCarthy et al (2006b) using the Tanita Body Composition Analyser BC-418MA. Three subjects in the group with normal GH responses had percentage fat values greater than the 91st percentile, compared with four children in the group with suboptimal GH responses.

Median percentage body fat values were higher in female than male subjects (29% compared with 18%). However, female subjects were also older than male subjects (median age 15.2 years compared with 12.9 years). Adolescent girls lay down proportionally more body fat than boys during puberty in response to rising oestrogen levels (McCarthy et al 2006b), therefore this difference was to be expected on physiological grounds.

Figure 4g: Scatter plot of HT^2/R against age, GH-normal and -abnormal groups



HT^2/R, Resistance Index

Table 4.xv: Body composition results by subject

No.	Sex	Age (y) at study	Weight SDS	HT^2/R	No. of equations used	Fat-free mass (kg)	Fat mass (kg)	Body fat (%)
1	M	10.3	2.65	38.4	9	40.0	18.4	32 [a]
2	M	18.0	0.88	–	3	65.8	9.4	12
3	M	16.4	-0.41	49.9	6	49.2	8.4	15
4	M	12.2	0.81	–	–	–	–	–
5	M	13.1	0.61	40.3	9	38.8	10.0	21
6	M	17.2	0.57	59.2	6	56.6	13.3	19
7	M	13.2	1.85	61.5	9	53.0	10.7	17
8	M	17.0	-1.28	43.1	6	44.3	9.0	18
9	M	15.1	0.12	52.5	8	47.2	9.5	17
10	M	9.8	1.20	31.9	8	30.1	8.0	21
11	M	16.3	-0.19	58.2	6	52.4	6.8	12
12	M	9.9	-0.98	24.3	8	21.8	4.7	18
13	M	9.0	0.89	27.1	8	25.4	7.4	23
14	M	12.0	2.23	49.1	9	44.0	17.0	28 [a]
15	M	8.4	-0.20	24.3	7	21.2	4.6	18
16	M	12.6	-0.65	29.7	9	30.5	5.8	16
17	M	11.1	1.29	33.2	9	32.9	11.8	27 [a]
18	M	15.1	1.10	63.4	8	55.6	12.1	18
19	M	14.8	0.12	37.6	9	41.7	15.3	28 [a]
20	M	12.9	1.35	45.9	9	42.0	12.9	24
21	M	18.0	-0.20	–	3	59.7	4.7	7
22	M	12.9	0.05	43.3	9	36.9	5.7	14
23	M	5.4	-0.18	17.4	5	15.6	3.4	18
24	M	8.5	0.41	–	4	22.9	6.0	21
25	M	6.5	-0.04	18.7	5	18.3	3.2	16
26	F	15.6	-0.23	42.6	6	38.1	14.4	28
27	F	18.9	2.20	–	3	57.4	23.6	29
28	F	11.7	0.61	36.5	9	34.3	9.3	19
29	F	15.0	0.17	41.8	9	39.6	14.9	28
30	F	15.3	1.31	45.3	6	44.6	21.3	33 [a]
31	F	13.0	1.44	36.9	9	37.8	20.6	36 [a]
32	F	14.6	2.86	43.0	9	47.1	36.5	44 [a]
33	F	21.7	1.33	44.0	2	51.4	19.0	27

M, male; F, female; y, years; HT^2/R, Resistance Index; [a] % fat > 91st centile (using UK age- and gender-specific charts by McCarthy et al 2006b)

4.1.10 Self- or parent/guardian-reported symptoms

The prevalence of self- or parent/guardian-reported symptoms and indicators of impaired psychosocial functioning were compared between normal (n=20) and abnormal (n=13) pituitary hormone groups. Comparisons were made using the Fisher's exact test.

There was a high prevalence of neuropsychological and attentional difficulties across the whole sample and this is discussed in section 5.4.1. There was no evidence that any of the following symptoms were more prevalent amongst the group with pituitary hormone abnormalities: tiredness, disturbed sleep, emotional difficulties, reduced strength, poor concentration, altered energy levels (lethargy or hyperactivity) or poor short term memory. Contrary to expectation, irritability and low mood were more prevalent amongst subjects with normal pituitary function. Neuropsychological symptoms are common in survivors of TBI (section 1.10.1) and these findings may reflect small sample bias. The results of these analyses are displayed in full below (Table 4.xvi).

Table 4.xvi: Prevalence of self- or parent-/guardian-reported symptoms

Symptom	**Prevalence, normal hormone group (N=20)**	**Prevalence, abnormal hormone group (N=13)**	**p-value, Fisher's exact test**
Tiredness	6	2	0.4
Disturbed sleep	3	3	0.7
Irritability	9	1	0.05
Emotional difficulties	11	6	0.7
Reduced strength	2	1	1.0
Low mood	4	0	0.1
Poor concentration	10	8	0.7
Lethargy or hyperactivity	4	3	1.0
Poor short term memory	6	5	0.7

4.1.11 SDQ Results

4.1.11.1 Parent-report version

Parents/guardians of subjects aged less than 16 years (n=25) and one immature 16 year-old completed parent-report versions of the SDQ (total n=26). As previously described (section 3.4.4.2), normal, borderline abnormal and abnormal bandings exist for individual subscale and total difficulties scores. To simplify comparisons between subject groups for this study, borderline abnormal and abnormal scores were amalgamated.

There was a high prevalence of difficulties across the whole sample and this is discussed in section 5.4.2. However, few parents rated their children poorly in the pro-social behaviour sub-scale, highlighting positive aspects of subjects' behaviour as well as problem areas. The table overleaf (4.xvii) summarises these results and compares the prevalence of difficulties between normal and abnormal pituitary hormone groups. Comparisons were made using the Fisher's Exact test. There was no evidence of a difference between groups in terms of parent-reported emotional difficulties, conduct problems, hyperactivity, peer relationship

difficulties or overall level of difficulties. There was no evidence of a difference in parent-reported strengths between groups.

Table 4.xvii: SDQ results, parent-report version

Subscale	Prevalence of *abnormal* SDQ result[a]		N*	p-value
	Normal hormone group (N=15)	Abnormal hormone group (N=11)		
Difficulties subscales (high score indicates abnormal SDQ result)				
Emotional symptoms	6	2	1	0.4
Conduct problems	8	4	0	0.5
Hyper-activity	7	7	0	0.5
Peer problems	4	3	0	1.0
Sumscore, difficulties subscales	7	4	0	0.7
Strengths subscale (low score indicates abnormal SDQ result)				
Pro-social behaviour	1	3	1	0.3

N*, missing data; [a] Abnormal and borderline abnormal SDQ results amalgamated

4.1.11.2 Self-report version

Subjects aged 11 to 15 years (n=18) and one 16 year-old subject completed the self-report version of the SDQ (total n=19). The prevalence of difficulties across the whole sample was high, correlating with parent-report scores. The table below (Table 4.xviii) summarises the self-report SDQ results and compares the prevalence of difficulties between normal and abnormal pituitary hormone groups. Comparisons were made using the Fisher's exact test. There was no evidence of a difference between groups in terms of self-reported emotional difficulties, conduct problems, hyperactivity, peer relationship difficulties or overall level of difficulties. There was no evidence of a difference in self-reported strengths between groups.

Table 4.xviii: SDQ results, self-report version

Subscale	Prevalence of *abnormal* SDQ result[a]		N*	p-value
	Normal hormone group (N=11)	Abnormal hormone group (N=8)		
Difficulties subscales (high score indicates abnormal SDQ result)				
Emotional symptoms	3	0	0	0.2
Conduct problems	6	3	0	0.7
Hyper-activity	4	5	1	0.6
Peer problems	2	1	0	1.0
Sumscore, difficulties subscales	4	3	0	1.0
Strengths subscale (low score indicates abnormal SDQ result)				
Pro-social behaviour	0	2	0	0.2

N*, missing data; [a] Abnormal and borderline abnormal SDQ results amalgamated

4.1.12 QoL data comparisons

QoL may be impaired in survivors of childhood TBI (section 1.11.1). I evaluated the results from standardised QoL questionnaires to determine any differences in perceived QoL status normal and abnormal pituitary hormone groups.

Subjects aged eight to 15 years (n=23) and one 16-year old subject completed the self-report version of the KIDSCREEN-52 questionnaire (total n=24).

All other subjects aged 16 years and over (n=7 in total, five males) completed QLS-H and QoL-AGHDA questionnaires. Both questionnaires were developed and have been validated for use in adults with hypopituitarism.

4.1.12.1 KIDSCREEN-52 data

Item responses informed the raw scores for ten sub-scales. Raw scores were converted into gender- and age-specific percentile and T-scores as described in section 3.4.4.2. Any incomplete responses invalidated the score for the sub-scale concerned. Questionnaire responses for one child had to be discarded because duplicate answers were given to most questions.

Median percentile and T-scores were compared between normal and abnormal subject groups using the Mann Whitney test (Tables 4.xix and 4.xx). There were no significant differences for individual sub-scales between groups. However, there was a trend towards lower psychological wellbeing in the group with *normal* pituitary function, correlating with a higher prevalence of self- or parent/guardian-reported low mood (section 4.1.10). There was also a trend towards lower satisfaction at school amongst this group.

Table 4.xix: KIDSCREEN-52 results, sub-scale centile scores

Subscale	N*	Median centile score (inter-quartile range)		p-value
		Normal hormone group (N=13)	Abnormal hormone group (N=11)	
Physical Wellbeing	1	72 (21-97)	79 (65-100)	0.4
Psychological Wellbeing	1	43 (11-70)	64 (36-55)	0.1
Moods & Emotions	3	44 (13-69)	51 (42-67)	0.5
Social Support & Peers	2	47 (19-80)	51 (24-78)	0.9
Relationship with Parents & Home Life	1	46 (27-76)	66 (46-86)	0.3
Self-perception	3	54 (21-78)	73 (67-100)	0.2
Autonomy	1	56 (12-97)	62 (35-100)	0.5
School Environment	1	22 (6-97)	77 (43-97)	0.1
Social Acceptance (Bullying)	1	80 (6-100)	50 (29-100)	0.9
Financial Resources	1	56 (22-100)	67 (38-100)	0.7

N*, missing data

Table 4.xx: KIDSCREEN-52 results, sub-scale T-scores

Subscale	N*	Median T-score (inter-quartile range)		p-value
		Normal hormone group (N=13)	Abnormal hormone group (N=11)	
Physical Wellbeing	1	55.1 (40.9-65.2)	56.9 (53.5-63.1)	0.5
Psychological Wellbeing	1	48.7 (35.6-55.3)	53.5 (50.8-56.4)	0.1
Moods & Emotions	3	49.6 (38.1-55.1)	51.2 (48.9-54.6)	0.6
Social Support & Peers	2	49.8 (40.8-57.8)	50.9 (42.6-57.2)	0.9
Relationship with Parents & Home Life	1	49.9 (44.2-56.7)	54.9 (50.0-59.4)	0.3
Self-perception	3	50.9 (41.5-57.4)	55.9 (54.3-60.3)	0.2
Autonomy	1	52.0 (35.4-62.5)	53.2 (46.4-62.3)	0.8
School Environment	1	41.3 (30.3-64.0)	57.6 (48.2-63.6)	0.1
Social Acceptance (Bullying)	1	54.8 (29.5-56.5)	51.2 (45.9-56.5)	0.7
Financial Resources	1	52.1 (40.4-60.4)	55.2 (47.3-55.2)	0.4

N*, missing data

There are no centile or T-scores for the total KIDSCREEN-52 score across the ten sub-scales. To estimate overall impairment of QoL status for comparison between groups, I recorded the number of subjects who had centile scores below the 15th percentile or T-scores more than 1 SD below the mean in three or more subscales. No significant differences were observed comparing normal and abnormal pituitary hormone groups using Fisher's exact test (Table 4.xxi).

Table 4.xxi: Prevalence of low percentile or T-scores across KIDSCREEN-52 subscales

Measure of low score	**Normal hormone group (N=13)**	**Abnormal hormone group (N=11)**	**Total (N=24)**	**p-value**
Percentile score <15th centile in ≥ 3 subscales	4	2	6	0.3
T-score ≤ -1 SD in ≥ 3 subscales	5	1	6	0.2

4.1.12.2 QoL-AGHDA data

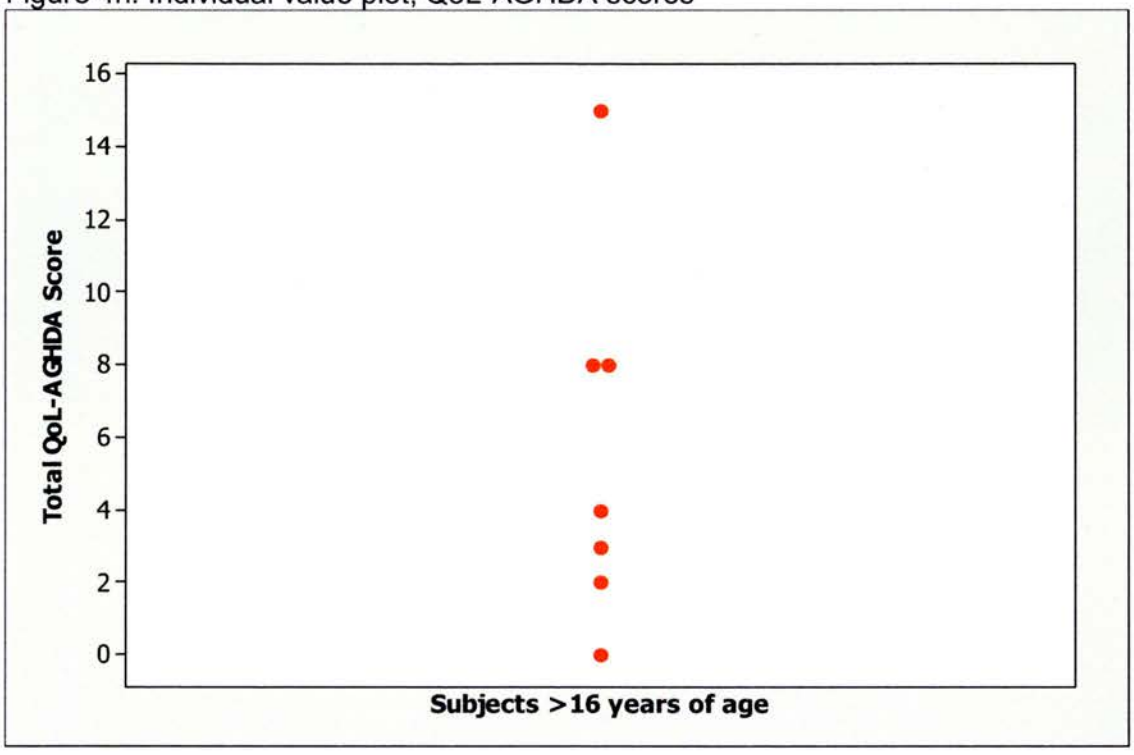
Subjects' QoL-AGHDA scores are tabled below (Table 4.xxii). It should be noted that three subjects were aged less than 18 years and the QoL-AGHDA has only been validated for use in adults ≥18 years. A score of ≥11 out of 25 is required to qualify for adult GH therapy in the UK (NICE 2003). Koltowska-Haggstrom et al (2005) reported mean-weighted QoL-AGHDA scores of 6.2 and 7.1 for men and women from the general population, compared with 13.6 and 15.7 for GH-deficient adults. In my study, QoL-AGHDA score was noticeably higher in one male (no. 8) who had a low peak GH response (3.2 μg/L) during the ITT than for other subjects (Table 4.xxii and Figure 4h).

Table 4.xxii: Subject responses to QoL-AGHDA questionnaire

Subject No.	**2**	**6[a]**	**8[b]**	**11**	**21**	**27**	**33**
Age (years)	18.0	17.2	17.0	16.3	18.0	18.9	21.7
Severity of TBI	SEV	SEV	SEV	MOD	MIN	SEV	MIN
QoL-AGHDA score out of 25	**2**	**3[a]**	**15[b]**	**4**	**8**	**8**	**0**

MIN, minor; MOD, moderate; SEV, severe; [a] Borderline low cortisol response to ITT (443 nmol/L; cut-off 470 nmol/L); [b] low peak GH response to ITT (3.2 μg/L; cut-off 5 μg/L)

Figure 4h: Individual value plot, QoL-AGHDA scores



4.1.12.3 QLS-H data

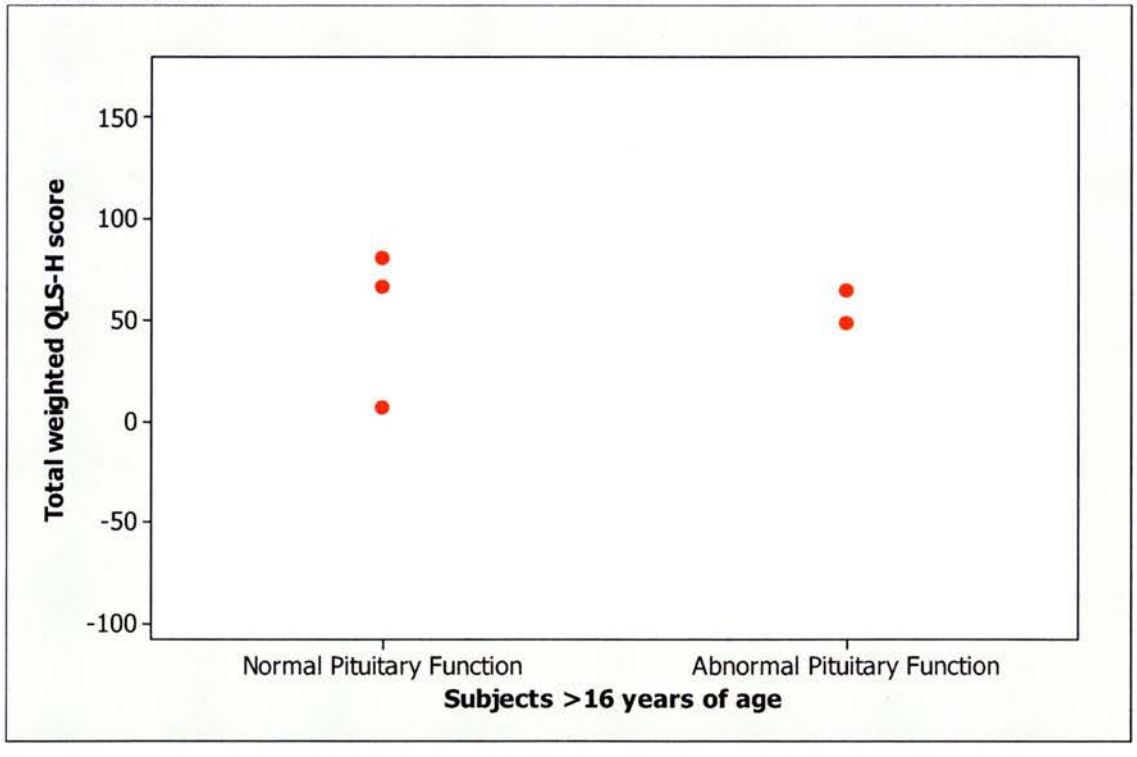
Weighted satisfaction scores for the QLS-H are tabled overleaf (Table 4.xxiii). The method used to calculate these scores is described in section 3.4.4.2. Weighted satisfaction scores may range from -9 to +12 for each of nine health-related areas. The sum of weighted satisfaction scores across all nine areas may range from -108 to +180. In two cases, it was not possible to calculate a sum score due to missing data. Scores were no lower in subjects with abnormal pituitary hormone results than in normal subjects (Figure 4i). Comparisons used raw scores alone because software to convert raw scores into Z-scores has only been standardised for use in subjects aged 18 years and over.

Table 4.xxiii: Weighted-satisfaction scores, QLS-H questionnaire

QLS-H health-related area	Weighted satisfaction score						
	2	**6[a]**	**8[b]**	**11**	**21**	**27**	**33**
Age (years)	18.0	17.2	17.0	16.3	18.0	18.9	21.7
Severity of TBI	SEV	SEV	SEV	MOD	MIN	SEV	MIN
Ability to handle stress	9	15	9	6	6	3	9
Body shape / appearance	6	6	6	0	12	-4	6
Self-confidence	9	4	9	0	-3	12	12
Ability to become sexually aroused	6	6	6	0	3	3	9
Ability to concentrate	12	12	3	-8	9	20	3
Physical endurance	9	3	2	0	2	1	9
Initiative / drive	9	2	12	6	2	-4	9
Ability to deal with anger	12	-2	9	3	6	-	6
Being able to stand the disturbances and noise of everyday life	9	3	9	0	-	4	4
Total weighted satisfaction score (-108 to +180)	**81**	**49[a]**	**65[b]**	**7**	**-**	**-**	**67**

MIN, minor; MOD, moderate; SEV, severe; [a] Borderline low cortisol response to ITT (443 nmol/L; cut-off 470 nmol/L); [b] low peak GH response to ITT (3.2 µg/L; cut-off 5 µg/L)

Figure 4i: Weighted-satisfaction QLS-H scores in subjects with normal vs. abnormal hormone results



4.1.13 Retrospective review of head injury characteristics

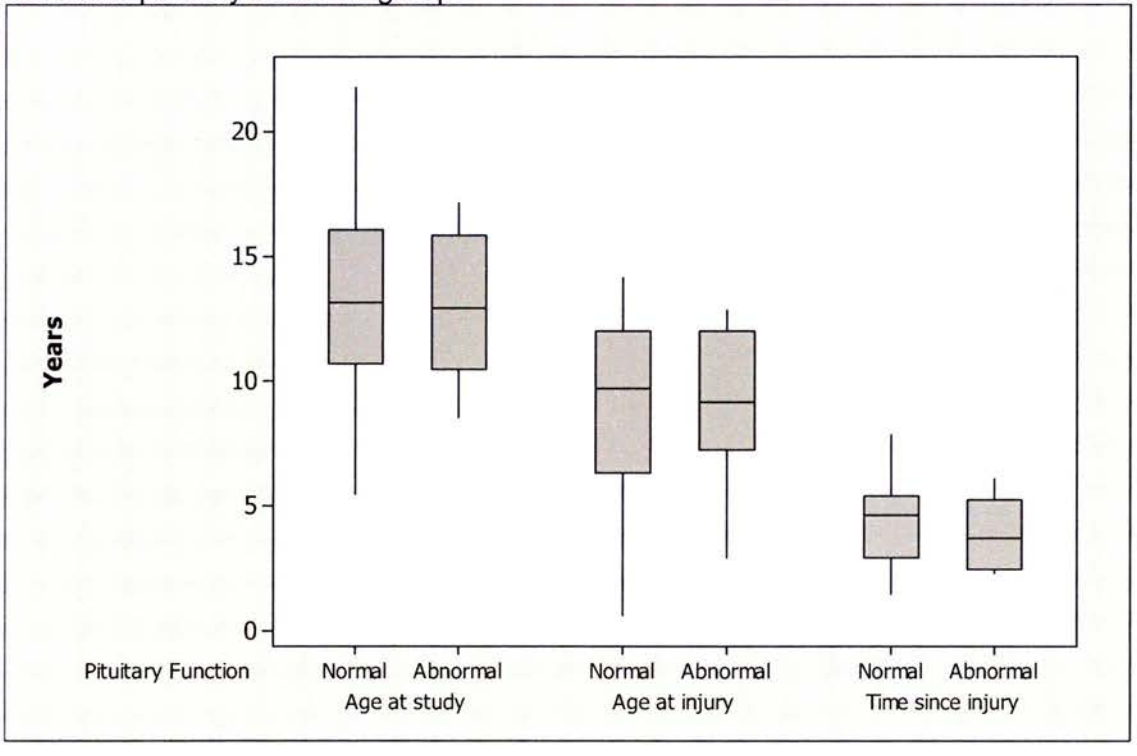
Subjects' case records were reviewed to determine whether there were any associations between TBI characteristics and emergence of pituitary hormone abnormalities.

Mean age at TBI, age at study and interval since TBI were compared between normal (n=20) and abnormal (n=13) pituitary hormone groups. No significant differences were observed between groups using the two-sample T-test. These data are displayed in Table 4.xxiv and in Figure 4j that follows.

Table 4.xxiv: Age at study, age at injury, time since injury: normal vs. abnormal hormone groups

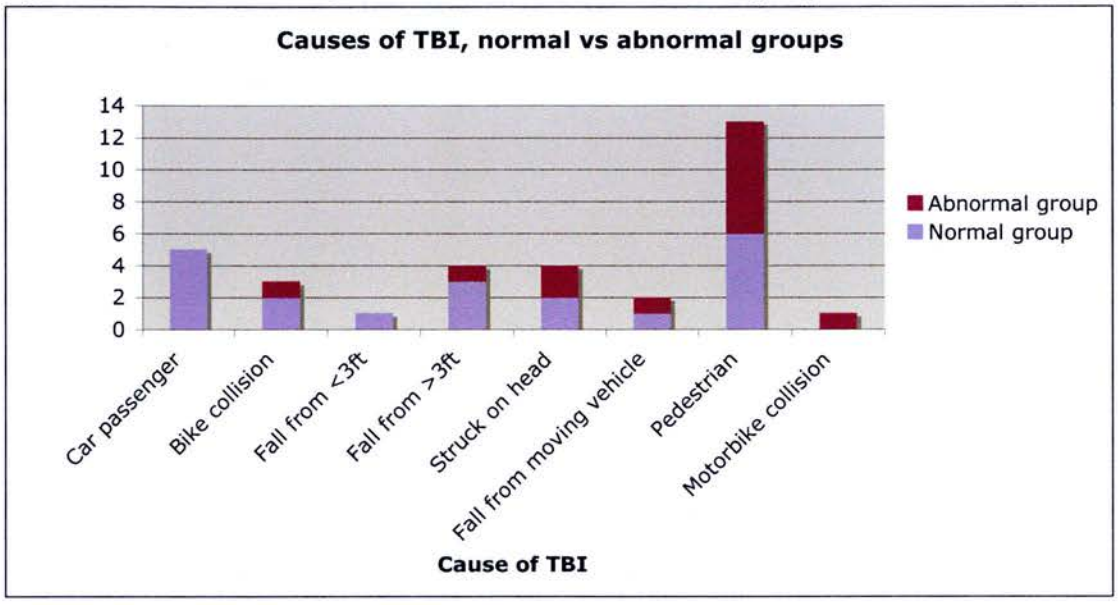
Variable	Mean (SD), normal pituitary hormone group (N=20)	Mean (SD), abnormal pituitary hormone group (N=13)	p-value
Age at study (years)	13.5 (4.1)	13.2 (3.0)	0.9
Age at injury (years)	9.2 (3.7)	9.3 (3.0)	0.9
Years since injury	4.3 (1.7)	3.9 (1.4)	0.5

Figure 4j: Boxplot of age at study, age at injury and time since injury; normal vs. abnormal pituitary hormone groups



The causes of TBI amongst subjects are displayed in the bar chart below (Figure 4k).

Figure 4k: Causes of TBI, normal vs. abnormal hormone groups



4.1.13.1 TBI severity

Severity of TBI was defined according to GCS classification. Nine of 12 survivors of severe TBI had normal pituitary function (75%). No significant difference in TBI severity was observed between normal and abnormal pituitary hormone groups using Pearson's Chi-Square test (p=0.3, Table 4.xxv). Two of 28 subjects had abnormal pupillary responses at the time of injury: a single dilated, unresponsive pupil in both cases. One had normal pituitary function; the other (subject no. 8) had a borderline low GH response during the ITT aged 17 years (3.2 μg/L). Information regarding pupillary responses was missing in five cases.

Table: 4.xxv: Severity of TBI, normal vs. abnormal groups

Severity of TBI	Normal Group	Abnormal Group	Total
Minor (ISS >16)	4	2	6
Moderate	7	8	15
Severe	9	3	12
All	20	13	33

ISS, PTS, RTS and TRISS scores were calculated as described in section 3.5.1. These scoring systems are used to predict morbidity and/or mortality after TBI. Median scores were compared between normal and abnormal pituitary hormone groups using the Mann Whitney test. There was no evidence of any significant differences between groups (Table 4.xxvi). Scores could not be calculated in several cases where relevant observations had not been recorded in subjects' records.

Table: 4.xxvi: Trauma scores, normal vs. abnormal hormone groups

Subscale	**N***	**Median score (inter-quartile range)**		**p-value**
		Normal hormone group (N=20)	**Abnormal hormone group (N=13)**	
ISS	0	19 (16-28)	19 (13-25)	0.4
PTS	3	6 (5-9)	8 (6-9)	0.4
RTS	6	6.0 (5.0-6.9)	6.8 (5.4-6.9)	0.4
TRISS (%)	6	95 (79-97)	98 (82-99)	0.4

N*, missing data

Two subjects with abnormal endocrine results and three with normal pituitary function had PTS scores ≤ 4 at injury (associated with 50% mortality, p=1.0).

Marshall CT scores are tabled below (Table 4.xxvii). Eight subjects had no visible intracranial pathology on CT scan, five in the group with pituitary hormone abnormalities. Only two subjects had significant diffuse brain swelling, both with normal pituitary function. There was no evidence of an association between a Marshall score greater than 1 and later pituitary hormone abnormalities using the Fisher's exact test (p=0.2).

Table: 4.xxvii: Marshall CT scores, normal vs. abnormal hormone groups

Score	**Marshall Score Category**	**Prevalence, normal group (n=20)**	**Prevalence, abnormal group (n=13)**	**Total**
1	Diffuse Injury I	3	5	8
2	Diffuse Injury II	8	6	14
3	Diffuse Injury III (swelling)	2	0	2
4	Diffuse Injury IV (shift)	0	0	0
5	Evacuated Mass Lesion	7	2	9
6	Non-Evacuated Mass Lesion	0	0	0

4.1.13.2 Primary brain injury

Findings are tabled below (Table xxviii). No subject had evidence of hypothalamo-pituitary injury on CT scan. There was no evidence that subjects with pituitary hormone abnormalities were more likely to have sustained a basal skull fracture. A suboptimal peak GH response (3.2 μg/L) was seen in the only subject to have sustained a brain stem injury (no. 8). This subject had a thalamic haemorrhage. There was no evidence of an association between frontal lobe injury, extra-axial haemorrhage, intra-parenchymal clot or contusion, or DAI and pituitary dysfunction. Two of the extra-axial bleeds were subarachnoid haemorrhages, one in each group.

Table 4.xxviii: Primary brain injuries, normal vs. abnormal hormone groups

Head injury characteristic	**Prevalence, normal group (n=20)**	**Prevalence, abnormal group (n=13)**	**p-value, Fisher's exact test**
Hypothalamo-pituitary injury	0	0	-
Basal skull fracture	6	5	0.7
Brain stem injury	0	1	-
Frontal lobe injury	7	3	0.7
Intra-parenchymal clot/contusion	9	5	1.0
Extra-axial bleed	9	7	0.7
DAI	2	0	-

4.1.13.3 Secondary brain injury

There was no association between the presence of intra-cerebral oedema ± midline shift on CT scan and emergence of pituitary hormone abnormalities. Small areas of focal oedema were not included in the comparison. Midline shift was usually associated with extradural or subdural haemorrhages that were evacuated. There was no evidence of an association between early post-traumatic seizures and later pituitary dysfunction (Table 4.xxix).

Table 4.xxix: Indicators of secondary brain injury, normal vs. abnormal hormone groups

Head injury characteristic	**Prevalence, normal group (n=20)**	**Prevalence, abnormal group (n=13)**	**p-value, Fisher's exact test**
Oedema/midline shift on CT scan	12	5	0.3
Post-traumatic seizure	4	3	1.0

Our intention had been to compare the duration and intensity of deranged ICP and CPP between normal and abnormal groups, using the PTI to quantify the burden of secondary brain insult. However, only 16 subjects had ICP monitoring (six with endocrine

abnormalities). Physiological monitoring data were only retrievable in 12 of these (four with endocrine abnormalities), precluding any statistical comparisons. No trends were identifiable between groups.

4.1.13.4 Outcome

KOSCHI score at six months post-TBI could only be determined from the case records of 19 of 33 subjects. The rest had been followed up in other hospitals or received no follow-up post-TBI. Thus, I could not evaluate whether there were differences in outcome at six months post-injury in groups with normal as compared with abnormal pituitary function.

I documented KOSCHI score at study assessment. In view of the fact that subjects were not assessed at a comparable time point post-injury, no statistical analyses were carried out. However, the raw data provide an indication of the prevalence of significant disability across normal and abnormal hormone groups, which appeared similar (Table 4.xxx).

Table 4.xxx: KOSCHI score at study, normal vs. abnormal hormone groups

KOSCHI score	**Normal group (N=20)**	**Abnormal group (N=13)**
3 (Severe disability)	1	1
4 (Moderate disability)	10	6
5 (Good outcome)	9	6

Moderate or severe disability was observed in 18 subjects (55%). Behavioural, emotional and cognitive sequelae were more common than deficits of a physical nature (sections 4.10, 4.11). Two subjects were severely physically disabled (one with pituitary dysfunction): both had spastic quadriplegia in addition to other difficulties. Dysarthria was seen in three subjects (one who was anarthric) and visual impairment in two. Two subjects were hemiplegic.

4.2 Suspected NAHI group

Twenty-two children were admitted to the RHSCE between 1st January 2001 and 31st August 2007 at less than two years of age with suspected NAHIs meeting eligibility criteria. Characteristics of the cohort are listed in Table 4.xxxi overleaf. Three children were deceased and one no longer living in Scotland or northeast England, leaving 18 eligible to take part.

Twelve of the 18 eligible children were invited to take part in the study (67%). Of six who were not invited, one was uncontactable (address unknown) and in two cases, the GP felt it would be inappropriate to contact the family due to the history of suspected inflicted injury. Three children were not invited because they remained clinically well throughout their respective admissions with GCS scores of 13 to 15 and no requirement for ventilation or neurosurgery, in spite of TBIs incurring ISS ≥16.

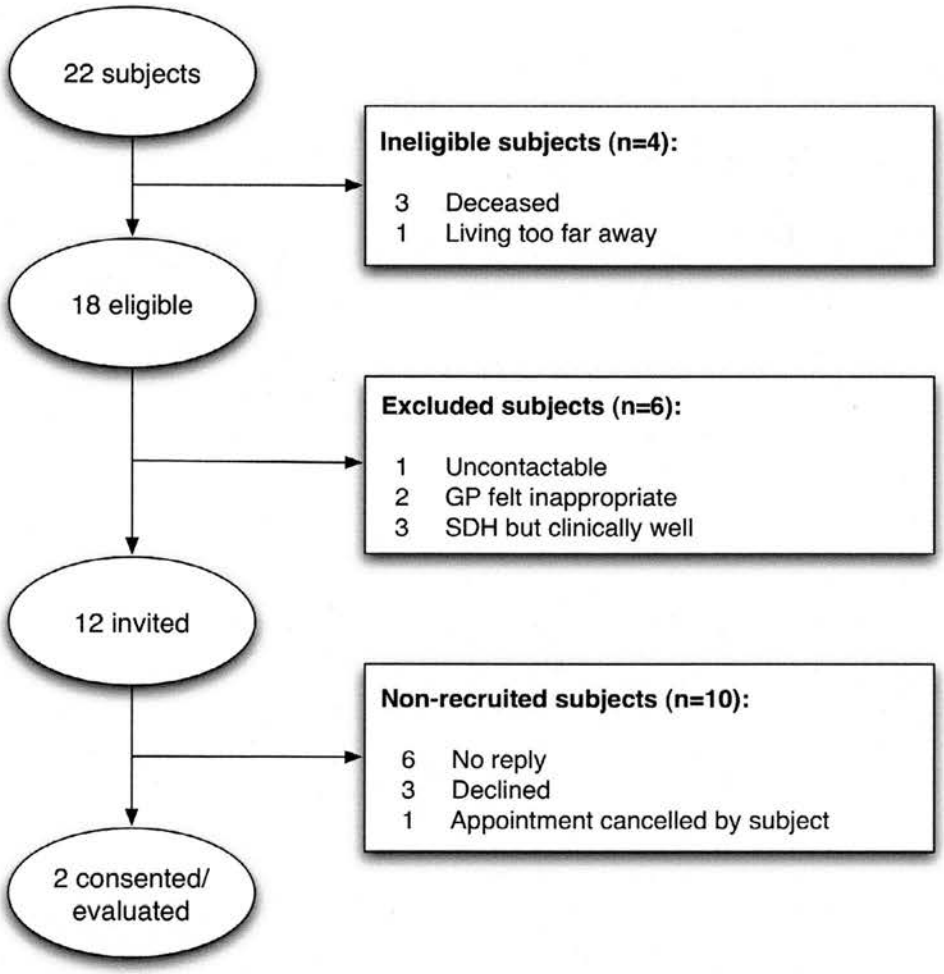
There were responses from three families, of whom two took part in the study. The parents of the third child cancelled their study appointment after deciding they did not want their daughter to undergo venepuncture. This information is summarised in Figure 4l overleaf.

Table 4.xxxi: Eligibility status & invited vs. uninvited subjects, suspected NAHI group

Eligibility status	Sex	Age (years) at injury	Age (years) at invite	Severity of TBI
Ineligible				
Deceased	F	0.7	n/a	SEV
Deceased	F	0.5	n/a	SEV
Deceased	F	0.4	n/a	SEV
Living too far away	M	0.2	n/a	SEV
Eligible but not invited				
Uncontactable	M	0.5	n/a	MOD
GP felt inappropriate	F	1.6	n/a	SEV
GP felt inappropriate	F	0.3	n/a	MOD
SDH, clinically well	M	0.1	n/a	MIN (ISS ≥ 16)
SDH, clinically well	M	0.1	n/a	MIN (ISS ≥ 16)
SDH, clinically well	M	0.5	n/a	MIN (ISS ≥ 16)
Eligible, invited				
	M	0.3	2	SEV
	M	0.4	3	SEV
	M	0.3	3	MOD
	M	0.4	3	MOD
	M	0.3	4	MOD
	M	0.2	4	MOD
	F	0.7	4	SEV
	M	0.1	5	MOD
	F	0.3	5	SEV
	M	0.3	6	SEV
	M	0.2	6	SEV
	M	0.6	6	MOD

SDH, subdural haemorrhage; M, male; F, female; MIN, minor; MOD, moderate; SEV, severe

Figure 4l: Flow diagram of subject recruitment, NAHI group



SDH, subdural haemorrhage

4.2.1 Subject characteristics

SDS for height, weight and BMI were all within ± 2 (Table 4.xxxii).

Table 4.xxxii : Subject characteristics, suspected NAHI group

No.	Sex	TBI Severity	Age (y) at study	Age (y) at injury	Years since injury	HT SDS	WT SDS	BMI SDS	Pubertal stage	KOSCHI at study
A	M	MOD	5.0	0.1	4.9	-0.69	-1.07	-0.53	Pre-	4a
B	M	MOD	3.7	0.4	3.3	+0.02	-1.03	-1.30	Pre-	5a

M, male; MOD, moderate; y, years; HT, height; WT, weight

4.2.2 Endocrine data

Basal endocrine investigations demonstrated normal thyroid function, prolactin and IGF-1 concentrations in both children (Table 4.4.xxxiii). Paired plasma and urine osmolalities and plasma sodium concentrations did not suggest underlying DI. Reference ranges can be found in Table 4.viii (section 4.1.3).

Table 4xxxiii. : Baseline endocrine results, suspected NAHI < 2 years group

	Sex	Age (y) at study	Urine osmolality mmol/kg	Plasma osmolality mmol/kg	Plasma sodium mmol/L	Free T4 pmol/L	TSH mU/L	Prolactin mU/L	IGF-1 ug/L
A	M	5.0	1034	290	140	13	1.4	126	38
B	M	3.7	846	286	139	16	4.1	93	119

M, male; y, years

Both subjects were pre-pubertal on examination with appropriate basal concentrations of FSH, LH and testosterone. FSH response to stimulation with GnRH was a little brisk in one subject (B), but LH response was appropriately pre-pubertal (Table 4.xxxiv).

4.xxxiv: Baseline and stimulation test results for the HPG axis, NAHI group

	Sex	Age (y) at study	Pubertal Stage	Baseline FSH U/L	Peak FSH U/L	Baseline LH U/L	Peak LH U/L	Testosterone nmol/L
A	M	5.0	Pre-	<0.5	3.5	<0.5	1.4	<0.4
B	M	3.7	Pre-	0.5	7.4	<0.5	1.6	<0.4

M, male; y, years

Baseline early morning (08:00-10:00) cortisol concentrations were within normal range (06:00-10:00 reference range 150-600 nmol/L, Table 4.xxxv). Subject A underwent a glucagon test due to a history of seizures. Subject B underwent an ITT and achieved a glucose nadir of 2.0 mmol/L. Cortisol and GH responses to stimulation were adequate in both children.

4.xxxv: Baseline and stimulation test results for the HPA and GH axes, NAHI group

	Sex	Age (y) at study	HT SDS	Basal cortisol nmol/L	Time at basal sample	Test used	Peak cortisol nmol/L	Peak GH µg/L	Pubertal stage
A	M	5.0	-0.69	266	09:45	Glucagon	830	8.7	Pre-
B	M	3.7	+0.02	309	09:30	ITT	688	8.0	Pre-

M, male; y, years, HT, height

4.2.3 Body composition data

Body composition data are shown in Table 4.xxxvi. Few of the equations compiled by Wells et al (2009) were applicable to young children (see also 4.1.9). Percentage body fat values were evaluated using UK age- and gender-specific reference curves established by McCarthy et al (2006b) using the Tanita Body Composition Analyser BC-418MA. Both subjects had percentage fat values below the 91st percentile for age and gender.

Table 4.xxxvi: Body composition results by subject

No.	Sex	Age (y) at study	WT SDS	HT^2/R	No. of equations used	FFM (kg)	FM (kg)	Body fat (%)
A	M	5.0	-1.07	13.0	5	13.5	2.9	18
B	M	3.7	-1.03	10.2	3	11.1	3.1	22

M, male; WT, weight; y, year; HT^2/R, Resistance Index

4.2.4 QoL

Both subjects were too young to complete self-report QoL questionnaires. Parent-report versions of the SDQ were completed for both subjects and gave an indication of functioning (section 3.4.4.2). There was no evidence of significant psychosocial difficulties. The results for emotional difficulties, conduct problems, hyperactivity and pro-social sub-scales were within normal range in both cases. One child's score in the peer relationships sub-scale was borderline abnormal. However, the sum score across all four difficulties sub-scales was within normal range for both children.

Table 4.xxxvii: SDQ results, parent-report version, NAHI group

	SDQ Sub-scales					Sum of sub-scales 1-4 (0-40)
	1 Emotion (0-10)	2 Conduct (0-10)	3 Hyperactivity (0-10)	4 Friendships (0-10)	5 Pro-social (0-10)	
A	1	0	3	2	8	6
B	2	0	4	3	7	9

4.2.5 Retrospective review of head injury characteristics

Both children were admitted with moderate TBIs according to GCS classification. There were no pupil abnormalities. Trauma scores including Marshall scores are tabled below

(Table xxxviii). Scores were derived as described in section 3.5.1. Subject A did not have a CT scan on admission (he had a cranial ultrasound scan followed by a cranial MRI scan).

Table 4.xxxviii: Trauma scores, NAHI group

No.	Age (y) at injury	Years since injury	TBI Severity	ISS	PTS	RTS	TRISS (%)	Marshall CT score
1	0.1	4.9	MOD	26	7	*	*	-
2	0.4	3.3	MOD	25	7	6.6	94	5

*Missing data

Both children were admitted with bilateral subdural haemorrhages as part of features suggestive of NAHI. In subject A, left fronto-parietal lacerations were also demonstrated on MRI. Subject B had a contusion of the left caudate nucleus. Subject A had early post-traumatic seizures at presentation. At study, he had moderate cerebral visual impairment and a unilateral peripheral visual field defect as a consequence of his TBI. Subject B required insertion of a ventriculoperitoneal shunt during his TBI admission but was felt to be developing normally on follow-up. King's Outcome Scale for Head Injury Scores were 4a (moderate disability) and 5a (good outcome) respectively.

Due to the small sample size and lack of abnormal endocrine findings, it was not possible to identify any correlations between TBI characteristics and risk of pituitary function in the NAHI group.

Chapter 5: Discussion: Table of Contents

Chapter 5: Discussion

Thirty-five survivors of childhood TBI were investigated in this retrospective observational pilot study. The majority (n=33) were survivors of accidental TBI. Mean (SD) age at injury amongst the accidental injury sub-group was 9.2 (0.6) years. Mean (SD) age at study 13.4 (0.6) years. TBI severity had been mild in six subjects, moderate in 15 and severe in 12, according to GCS classification. KOSCHI score at study demonstrated a good outcome in 15 subjects, moderate disability in 16 and severe disability in two, at a mean (SD) interval of 4.1 (1.6) years since injury (range 1.4 to 7.8 years).

Pituitary hormone abnormalities were observed in 13 survivors of accidental TBI (39%), affecting two hormone axes in four cases. The significance of most of these abnormal findings is questionable and this will be discussed in more detail in due course. The commonest abnormalities were of the GH (n=7) and HPA axes (n=9). Prolactin deficiency was identified in one male. No significant differences were seen between subjects with normal compared with abnormal hormone profiles in terms of age at injury, age at study, anthropometric measurements or head injury characteristics. I was unable to standardise body composition results to enable statistical comparisons between groups. Contrary to expectation, irritability (p=0.05) and mood disturbance (p=0.1) were reported more frequently amongst subjects with normal pituitary function. There were trends towards differences in QoL between groups but no statistically significant findings. QoL-AGHDA score was high in one post-pubertal subject with a sub-optimal GH response to stimulation (3.2 μg/L).

Two children had a history of suspected NAHI at less than two years of age. TBI was of moderate severity in these subjects and KOSCHI scores at study were 5a (good outcome) and 4a (moderate disability) at 4.9 and 3.3 years post-injury respectively. Pituitary function was normal in both children.

5.1 Evaluation of study design weaknesses

5.1.1 Recruitment and selection bias

The primary aim of this cross-sectional study was to establish whether post-traumatic hypopituitarism was a common occurrence after childhood TBI in the UK. I identified 176 subjects who had been admitted to hospital in Edinburgh with moderate or severe TBI, or

minor TBI with an ISS ≥16, between 1st January 2001 and 31st August 2007. One hundred and fifty-one met eligibility criteria to take part in the study: 133 survivors of accidental TBI and 18 with a background of suspected NAHI. One hundred and two (77%) and 12 (67%) subjects were invited to take part from each of these groups respectively.

The purpose of including 'minor' TBI subjects an ISS of ≥16 was to enable recruitment of subjects who were intubated and ventilated for neurosurgery before their GCS score fell below 13. Children who had sustained small extradural or subdural haemorrhages without impairment of consciousness or requirement for neurosurgery or ventilation were excluded. Whilst they fulfilled eligibility criteria, it was concluded that the benign clinical course did not justify subjecting these individuals to invasive testing. Clearly this introduced an element of selection bias but ethical considerations were paramount. Similarly, several WGH patients with known extradural haemorrhages were excluded because their condition at presentation and subsequent clinical course could not be clarified from computerised records.

Twenty-three percent of all eligible subjects underwent a comprehensive assessment of pituitary function (35 of 151 children). This is comparable with other retrospective paediatric studies. Poomthavorn et al (2008) investigated 29 subjects from a potential cohort of 113 (26%). Ten subjects declined to participate, 53 were not contactable and 21 were felt not to require endocrine evaluation on the basis of responses to two QoL questionnaires. Dynamic function tests were only performed in eight subjects. Einaudi et al (2006) evaluated 22 subjects from a total cohort of 98 (22%). Thirty-eight individuals were not contactable or considered too ill to participate, 18 declined and 17 had moved away from the region. One patient had died. Niederland et al (2007) investigated 26 survivors of TBI but it is not clear how subjects were selected to take part in their study.

In any retrospective study, there are significant risks of selection bias in terms of how cohorts are defined, which subjects are approached and the characteristics of those who choose to take part. In contrast with other studies (Poomvathorn et al 2008, Einaudi et al 2006), I was able to ascertain up-to-date contact details for all but four of 151 eligible subjects. However, as previously discussed, I elected to exclude 25 subjects with extradural or subdural haemorrhages whose condition at presentation and clinical course were mild or about whom too few clinical details were known.

Recruitment was more successful amongst survivors of accidental TBI than suspected NAHI: 33 of 102 invited subjects (32%) versus two of 12 (17%). Parents of children admitted with suspected NAHI may not wish to revisit the past and may have a complex relationship with health services. The GPs of two families felt that it would be inappropriate for us to contact their patients, perhaps for these reasons. To my knowledge, no previous study has evaluated neuroendocrine sequelae in survivors of suspected NAHI, although Miller et al (1980) reported three cases of pituitary dysfunction in survivors of NAHI.

It is difficult to comment on how representative the two NAHI subjects recruited were of other children with inflicted brain injuries, due to the extremely low recruitment rate. However, the sample of accidental TBI subjects recruited was reasonably representative of the wider cohort. No significant differences were seen between recruited subjects and non-responders in terms of gender, age at injury or age at study. There were proportionally more minor TBI subjects and fewer survivors of severe TBI in the recruited sample, but these differences were not statistically significant ($p=0.2$).

Parents responding to the study invitation may have been more anxious, more motivated or had more concerns about their children than non-responders. McCullagh and Feinstein (2003) found that survivors of minor TBI were more likely to participate in research if injuries had been more significant and healthcare utilisation was higher after injury. Emotional difficulties were reported in 17 of 33 accidental TBI subjects (52%) in my study and concentration problems in 18 (55%). Both are observed frequently after moderate or severe TBI (Schwartz et al 2003, Anderson et al 2005b, Lehnung 2001, Verger et al 2000; Yeates et al 2005) but may have been more prevalent amongst recruited subjects than non-responders. On the other hand, parents of children with significant physical or learning disability as a consequence of TBI may have been reluctant to subject them to invasive tests. The absence of information regarding outcome from TBI in non-responders precluded any comparison being made with recruited subjects.

5.1.2 Ethics committee constraints

The Scotland A MREC stipulated that this should be a pilot study because of little evidence at the study's inception that children were at the same (or greater) risk of post-traumatic hypopituitarism as adults. For this reason, and because the Committee was concerned about raising anxiety among TBI survivors not under hospital follow-up, the number of subjects that could be recruited was capped. Advertising of any sort was not permitted. The MREC

also requested that subjects should be contacted according to when they sustained their head injuries, starting with those injured more recently and going back no further than required to recruit the number permitted. For this reason, subjects were contacted in batches according to year of TBI admission. It was also the MREC's preference that patient records were not accessed prior to recruitment where possible. This prevented us from inviting some WGH patients to participate in the study, as their eligibility could not be verified.

These constraints undoubtedly limited the study's recruiting potential. However, recruitment rates did go down as the length of time between TBI and invitation to enter the study increased. Many subjects who had been admitted in 2001 were aged between 18 and 21 years when invited to take part and might have been in new jobs or living away at university. Others might have perceived the risk of having undiagnosed neuroendocrine problems so long after their head injuries took place to be low.

5.1.3 Limitations of cross-sectional study design

Subjects underwent a single evaluation of pituitary function at a non-uniform time point post-TBI. For this reason, it was not possible to comment on the natural history of post-traumatic pituitary dysfunction or the likelihood of abnormalities detected being permanent. To do this, it would be necessary to conduct a prospective longitudinal study with baseline assessment of pituitary function at the time of injury. It is difficult to be certain that pituitary hormone abnormalities detected in this study did not predate TBI. However, this is unlikely considering the low prevalence of pituitary dysfunction in the general population (section 1.2). In any case, the majority of abnormalities observed were of debatable significance. The retrospective study design and single assessment point also limited evaluation of anthropometric, body composition and QoL data. All are optimally evaluated over time (sections 5.2.5.1, 5.3, 5.4.5).

5.1.4 Lack of control subjects

The other significant weakness of the study design was lack of inclusion of control subjects without a history of TBI. As discussed in the Introduction chapter, physical, intellectual and emotional deficits are common after TBI (section 1.10.1). These factors might, in themselves, be sufficient to influence growth and body composition, potentially obscuring differences between groups with normal versus abnormal pituitary function. Growth may be impaired in children with adverse psychosocial circumstances (Powell et al 1967a, Powel et al 1967b) and in children and young people with chronic illness.

It would have been difficult to justify subjecting healthy control children to an ITT because of the potential risks and unpleasant symptoms associated with hypoglycaemia. Furthermore, local age-related paediatric reference ranges have been established for the ITT in Edinburgh (Crofton et al 2004; Crofton PM, personal communication). Glucagon test cut-offs are largely based on adult studies (Agha et al 2004a, Rao & Spathis 1987, Spathis 1974, Rahim et al 1996). Data from control subjects could have been used to validate these cut-offs for the purposes of this and future paediatric studies. However, the glucagon test carries the risk of late (rebound) hypoglycaemia and may be associated with nausea and vomiting (5.2.4.3). The origins and reliability of glucagon test cut-offs are discussed in more detail in sections 5.2.4.3 and 5.2.5.5.

5.1.5 Limitations of statistical analysis

The study was likely to have been underpowered due to small numbers and this made it difficult to identify significant differences between normal and abnormal pituitary hormone groups in terms of QoL, anthropometry and head injury characteristics. The relatively small sample size also reduced the scope for performing exploratory statistical analyses (for example, linear or logistic regression), which would have been particularly useful for comparing head injury characteristics between groups. This was further hindered by irretrievable or missing data in several areas (see section 5.5). I intended to use the study results to inform a power calculation for a larger study, however the questionable significance of many of the endocrine abnormalities observed make it difficult to extrapolate from our results to enable a meaningful calculation to be made.

5.2 Evaluation of endocrine results and methods of testing

Results of basal and dynamic endocrine tests are discussed in this section. I evaluated anterior and posterior pituitary gland function in 35 survivors of childhood TBI. There were no abnormal endocrine findings in two subjects with a history of suspected NAHI in infancy. Thus, discussion centres on results pertaining to 33 survivors of accidental TBI. In terms of posterior pituitary function, no subject had clinical signs or symptoms consistent with cranial DI. Plasma osmolality and sodium concentrations were all within the normal range and early morning urine osmolalities indicated appropriate overnight urine concentration. The results of anterior pituitary hormone testing are discussed in due course.

5.2.1 Hypothalamo-pituitary-thyroid (HPT) axis

TSH concentrations were mildly elevated in one pre-pubertal male subject in the context of normal free T4 levels. This could be consistent with evolving primary hypothyroidism rather than a central cause. Repeat thyroid function tests were advised in six months. I elected not to use the TRH test in the assessment of HPT function. In this test, TSH concentrations are measured at baseline and at 20 and 60 minutes after administration of 7 μg/kg of TRH (up to a maximum of 200 μg). The TSH response is expected to peak at 20 minutes, followed by a decrease at 60 minutes (Mehta et al 2003). Side-effects of the TRH test include nausea, flushing, chest tightness, a peculiar taste and a desire to micturate (Crofton et al 2004), all of which are short-lived.

An inadequate TSH response is believed to reflect pituitary disease (Mehta et al 2003). An exaggerated or delayed rise in TSH may be suggestive of hypothalamic dysfunction or a compressed pituitary stalk (Crofton et al 2008). However, Crofton et al (2008) demonstrated that although exaggerated or delayed TSH responses were frequently associated with hypothalamic lesions, they also occurred in patients with pituitary lesions or apparently normal pituitary function. Thus, the TRH test did not reliably discriminate between hypothalamic and pituitary disorders. Clinical decisions were based on clinical follow-up and regular free T4 measurements during follow-up rather than the TSH response to TRH (Crofton et al 2008). Patients with subnormal TSH responses but normal free T4 concentrations did not receive treatment. Mehta et al (2003) also concluded that the TRH test alone was not enough to differentiate between hypothalamic and pituitary disease. They also agreed that a normal TSH response to TRH was not sufficient to exclude abnormalities of the HPT axis. In their retrospective review of 232 cases, Pavord et al (1992) found that the TRH test produced normal TSH responses in 50% of subjects with low free T4 concentrations and only 10% of subjects with a suboptimal TSH response had low T4 levels.

Central hypothyroidism has been observed in between 0% and 22% of subjects in adult TBI studies (Intro section 1.6.2). Lieberman et al (2001) reported TSH deficiency in 22% of TBI survivors but only 12% had low or inappropriately normal TSH concentrations in the presence of low thyroxine levels. Post-traumatic TSH deficiency is less common than other anterior pituitary hormone abnormalities. The ventral location of thyrotrophic cells within the anterior pituitary gland may protect them from the effects of trauma to the hypophysial portal venous system (Daniel et al 1959, Kelly et al 2000, section 1.4).

Einaudi et al (2006) reported long-term TSH deficiency in one of 48 childhood TBI survivors (26 evaluated prospectively and 22 retrospectively). This child had multiple anterior pituitary hormone deficiencies. TSH was within normal range (3.4 mU/L, reference range 0.4-4.4 mU/L) in the context of low free T3 and free T4 levels (<3 pg/mL and <6.5 pg/mL respectively). Poomthavorn et al (2008) evaluated basal endocrine function in 29 survivors of severe TBI, detecting one case of possible central hypothyroidism. TSH was low normal (0.76 mU/L, reference range 0.5-4.5 mU/L) in the presence of reduced free T4 concentrations (8.4 pmol/L, reference range 10-25 pmol/L). The results of four previously investigated TBI survivors were also included in the study findings: two of these had TSH deficiency in association with other pituitary hormone abnormalities. MR scans revealed a pituitary stalk transection in one and an absent pituitary stalk and ectopic posterior pituitary in the other. Findings in the latter child (aged three weeks at the time of TBI) were almost certainly related to a congenital rather than acquired cause of hypopituitarism.

5.2.2 Prolactin deficiency

I observed prolactin deficiency (<50 mU/L) in one pre-pubertal male. There were no other abnormal endocrine findings in this subject, apart from a borderline low peak cortisol response to ITT (546 nmol/L in the context of a 550 nmol/L cut-off). Prolactin deficiency is of uncertain clinical significance in males. In females, it is required for puerperal lactation and may have a role in ovarian function (Kaupilla et al 1987, Douchi et al 2001, Falk 1992). Prolactin deficiency is usually seen in conjunction with other pituitary hormone abnormalities (Mukherjee et al 2003). Case reports of isolated prolactin deficiency are few (Falk 1992, Douchi et al 2001, Kauppila et al 1987, Zargar et al 1997) and there may be a genetic link (Zargar et al 1997).

Possibly because of the association with multiple pituitary hormone deficiencies, the presence of prolactin deficiency in adults is considered to indicate severe hypopituitarism (Mukherjee et al 2003, Toledano et al 2007). Mukherjee et al (2003) detected hypoprolactinaemia (<50 mU/L) in 22 of 369 patients with hypothalamo-pituitary disease (6%). All were GH- and gonadotrophin-deficient and many also had deficiencies of ACTH and/or TSH. Thirteen (59%) had been surgically treated for Cushing's disease. The prevalence of hypoprolactinaemia amongst subjects surgically treated for Cushing's disease (n=62) was 21%. Toledano et al (2007) reviewed the records of 100 adults with diseases of the hypothalamo-pituitary axis. Severe hypoprolactinaemia (defined as <3 ng/mL [<60

mU/L]) was observed in 14 cases. The incidence was highest amongst patients with other pituitary hormone deficits.

Bondanelli et al (2004) detected hypoprolactinaemia in four survivors of adult TBI. All had sustained severe TBI. Cut-offs of 2 μg/L (40 mU/L) and 4 μg/L (80 mU/L) were used for males and females respectively. Prolactin deficiency was isolated in one subject and associated with multiple hormone abnormalities (but not GHD) in another. In two subjects, prolactin deficiency was associated with partial GHD (defined as a peak GH response to the GHRH-arginine test between 9 μg/L and 16 μg/L). Hypoprolactinaemia has not, thus far, been detected in any other TBI studies.

5.2.3 HPG axis

There were clinical and biochemical findings consistent with resolving constitutional delay of growth and puberty in one male subject aged 14.8 years. Basal LH concentrations were low (<0.05 U/L) but LH and FSH responses to the low dose GnRH test and basal testosterone concentrations (2.7 nmol/L) were appropriate for pubertal stage (see below). Testicular volumes were 6 mL and 8 mL. Height SDS was -1.52. Peak GH response to ITT was low (2.8 μg/L), possibly reflecting physiological blunting of GH secretion in (delayed) early to mid-puberty. Similarly, IGF-1 concentrations were slightly low for age (179 μg/L, reference range for 14-17 year olds 200-650 μg/L) but appropriate for pubertal stage. The rationale for priming before GH stimulation testing in late pre-puberty and early puberty is discussed in section 5.2.5.3.

In normal pubertal development, pulsatile secretion of GnRH by the hypothalamus stimulates high amplitude pulsatile gonadotrophin secretion from the anterior pituitary gland (Stanhope & Preece 1988). In males, LH stimulates testosterone production by the Leydig cells and FSH supports maturation of the spermatozoa after spermarche (Blondell et al 1999). Delayed puberty is defined as testicular volumes less than 4 mLs and the absence of secondary sexual characteristics at 14 years of age (Kauschansky et al 2002). Constitutional delay of growth and puberty is commoner in males than females (Stanhope & Preece 1988), affecting 3% of boys and accounting for 95% of pubertal delay (Al-Shaikh et al 2001). Differential diagnoses include hypogonadotrophic hypogonadism (HH) and hypergonadotrophic hypogonadism. Several studies only advocate the use of stimulation tests to evaluate delayed puberty when the basal testosterone is less than 1.75 nmol/L (Degros et al 2003, Martin et al 2005).

5.2.3.1 Evaluation of the low dose GnRH test

I used the low dose (10 μg) GnRH test to evaluate HPG function. The basis for using this test rather than the standard 100 μg GnRH test was to assess pituitary responsiveness to GnRH rather than maximal secretory capacity (Zevenhuijzen et al 2004). It has been established that very small doses of endogenous GnRH are required to achieve physiological bursts of LH secretion. The LH response to low dose GnRH correlates well with measures of nocturnal LH secretion in pre-pubertal and pubertal boys (Wu et al 1990). Zevenhuijzen et al (2004) observed a similarity between peak LH responses in boys with constitutional delay of growth and puberty and responses in normal pre-pubertal boys older than 12 years. Boys with HH showed a complete lack of LH response to low dose GnRH. An LH peak less than 1.2 U/L diagnosed HH with 100% sensitivity and 96% specificity in pre-pubertal boys older than 12 years in the population studied. An FSH peak less than 2.2 U/L diagnosed HH with 100% sensitivity and 86% specificity. Using these cut-offs, peak FSH response in my subject with delayed puberty was borderline low (2.1 U/L) but peak LH response was appropriate (4.2 U/L).

Alternative stimulation tests include the use of human chorionic gonadotrophin (hCG), GnRH agonists or the standard GnRH test (100 μg) to evaluate HPG function. Nocturnal serial sampling for LH and FSH is another option (Ghai et al 1995) but this is impractical and expensive (Zevenhuijzen et al 2004). Studies have suggested that the hCG test is more useful than GnRH (Martin et al 2005) or GnRH agonists (Degros et al 2003) in differentiating between constitutional delay of growth and puberty and HH. GnRH agonists are also expensive (Zevenhuijzen et al 2004). Using ROC analysis, Segal et al (2009) demonstrated that using specific test cut-offs, a combination of the leuteinising-releasing hormone (LHRH), 3-day and 19-day HCG tests gave 100% sensitivity and specificity for HH. In another study, the 100 μg GnRH test was 100% sensitive in the diagnosis of HH but combining the GnRH test with the 3-day hCG test was necessary to be 100% confident of excluding HH (Al-Shaikh et al 2001). There was no diagnostic dilemma in my study, as examination and biochemical findings were consistent with a case of resolving constitutional delay of growth and puberty.

In adult TBI studies, persistent gonadotrophin deficiency has featured in between 0% and 29% of subjects (see section 1.6.2). Like GH-secreting cells, the gonadotrophs are situated laterally in the anterior pituitary gland and are vulnerable to ischaemia caused by rupture of

the hypophysial portal veins (Kelly et al 2000, section 1.4). In terms of paediatric study findings, Einaudi et al (2006) reported central hypogonadism in two male survivors of childhood TBI, one with multiple pituitary hormone deficiencies. In the other, no signs of puberty were seen at three consecutive assessments (the first two performed between 13 and 14 years of age) and response to the LHRH test was pre-pubertal. However these findings could also be consistent with constitutional delay of growth and puberty. Precocious puberty was diagnosed in retrospect in one male subject. Poomvathorn et al (2008) reported precocious puberty in a girl aged 7.7 years, 4.4 years following TBI. Clinically, there were signs of breast development on examination. Details of biochemical evidence to support the diagnosis were not provided. Niederland et al (2007) did not evaluate the HPG axis in their study on the basis that normal hormone ranges were hard to define in pre-pubertal or pubertal children.

5.2.4 HPA axis

I detected abnormalities of the HPA axis in a third of accidental TBI subjects (11 of 33 children). Baseline early morning cortisol concentrations were sub-optimal in two children at 110 and 146 nmol/L (06:00-10:00 reference range 150-600 nmol/L). Nine of 25 subjects, including the aforementioned two, had suboptimal peak cortisol responses to the ITT (<470 nmol/L in subjects ≥10 years; <550 nmol/L in subjects <10 years [Crofton et al 2004]). Two of eight children had inadequate peak cortisol responses to glucagon (<450 nmol/L [Agha et al 2004]). In two cases (one ITT and one glucagon test), low cortisol responses were discounted due to high baseline early morning cortisol levels (624 nmol/L and 722 nmol/L respectively). This phenomenon has been reported by Leong et al (2001), who postulated that the stress of attending for investigations might exceed that of the stimulation test for some subjects.

In other studies in children, Niederland et al (2007) reported low basal cortisol concentrations in 34% of subjects, although the reference range used was not provided. Peak cortisol responses to the ITT were neither disclosed nor evaluated. Poomthavorn et al (2008) measured baseline early morning cortisol in 15 of 29 TBI survivors, detecting a low cortisol level in one subject (96 nmol/L). This child had a normal peak cortisol response to the low-dose (1 μg) Synacthen test (668 nmol/L). Three of eight other children who underwent stimulation testing had low peak cortisol responses (<450 nmol/L) to the glucagon test. The HPA axis was not evaluated in the remainder of the cohort. Einaudi et al (2006) reported HPA insufficiency in the context of multiple pituitary hormone deficiencies in one of 22

subjects evaluated retrospectively. They also diagnosed HPA insufficiency in one of 26 prospective subjects six months post-TBI on the basis of a sub-optimal peak cortisol response to glucagon (<500 nmol/L). This abnormality persisted on repeat testing at 12 months. Only children with low basal cortisol concentrations underwent glucagon stimulation testing (two of 26 subjects); the cut-off used to define inadequate basal levels was not defined. HPA insufficiency has been reported in between 0% and 19% of subjects in adult TBI studies (section 1.6.2).

5.2.4.1 Utility of early morning cortisol concentrations

Several studies have evaluated the utility of early morning cortisol values in assessment of the HPA axis. In a review of glucocorticoid replacement in adults following pituitary surgery, Inder and Hunt (2002) concluded that the early morning cortisol concentration could be used to gauge the need for further investigation of the HPA axis. They established that less than 4% of patients with an early morning cortisol level greater than 350 nmol/L in the early post-operative phase would fail an ITT (based on studies using peak cortisol cut-offs between 500 and 600 nmol/L). Pavord et al (1992) reviewed 232 adult cases and suggested that baseline early morning cortisol concentrations less than 100 nmol/L almost always indicated ITT failure, and levels greater than 400 nmol/L, a pass. The findings of Gleeson et al (2003) were similar. In this study, the sensitivity and specificity of baseline early morning cortisol concentrations greater than 400 nmol/L were 91% and 85% respectively, compared with the standard Synacthen test.

Schmidt et al (2003) used ROC analysis in their evaluation of the utility of baseline early morning cortisol cut-offs compared with the ITT. They determined an 'optimal' cut-off of 285 nmol/L for the diagnosis of HPA insufficiency (100% sensitivity and 65% specificity). A cut-off of 98 nmol/L provided 100% specificity but sensitivity was poor. Stimulation testing was recommended for patients with early morning cortisol levels between 98 and 285 nmol/L. Healthy control data collected in parallel established a lower early morning cortisol cut-off of 267 (mean 493.3 ± 24.9) nmol/L.

Agwu et al (1999) questioned the value of a single baseline early morning cortisol level in one of the only paediatric studies evaluating tests of the HPA axis. The lowest 08:00 cortisol concentration above which subjects achieved a normal response to both standard and low dose synacthen tests was 500 nmol/L. Using this cut-off, the 08:00 cortisol concentration had 100% sensitivity but only 33% specificity compared with the lose dose Synacthen test.

All subjects who failed the low dose Synacthen test had 08:00 cortisol concentrations less than 200 nmol/L. No comparative control data were provided.

In my study, median baseline early morning cortisol concentration amongst accidental TBI subjects was 298 (interquartile range 216-443, reference range 150-600) nmol/L. Without comparative data from healthy controls, it is difficult to evaluate whether or not this value was lower than expected. Samples were obtained between 08:20 and 10:10, most between 09:00 and 10:00. Earlier sampling might have produced higher early morning cortisol levels.

Twenty-three of 33 subjects had baseline early morning cortisol concentrations below the 350 nmol/L cut-off suggested by Inder and Hunt (2002). There were sub-optimal peak cortisol responses to stimulation testing in four subjects with baseline early morning cortisol levels greater than 350 nmol/L. In two cases, as previously discussed, baseline early morning cortisol concentrations were supranormal. I hypothesised that the stress of attending for investigations might have exceeded that of the stimulation test in these subjects. The third and fourth had baseline early morning cortisol concentrations of 356 and 402 nmol/L. Use of three different stimulation test cut-offs within the study (glucagon test versus age-dependent ITT cut-offs) precluded accurate evaluation of the sensitivity and specificity of the 350 nmol/L basal cut-off as a predictive measure.

5.2.4.2 Evaluation of cortisol responses to the ITT

The ITT has been validated against the response to surgical stress and perhaps for this reason remains the reference standard for stimulation tests of the HPA axis (Plumpton & Besser 1969). Furthermore, the ITT is thought to provide a stimulus at the level of the hypothalamus, thus assessing the function of the entire HPA axis (Inder & Hunt 2002, Agwu et al 1999). The original peak cortisol cut-off defined by Plumpton and Besser (1969) was 580 nmol/L, based on a fluorometric assay. However, with improvements in assay specificity, more recent studies have demonstrated a mean 96 nmol/L positive bias using the fluorometric assay which has resulted in many lowering their reference range cut-off to 500 nmol/L (Orme et al 1996).

Peak cortisol cut-offs for the ITT established in recent adult studies range from 500 to 539 nmol/L (Tuchelt et al 2000, Hurel et al 1996, Gonzalbez et al 2000). Results are assay dependent and therefore locally established reference ranges are optimal (Inder & Hunt

2002). I used locally determined age-dependent cut-offs to evaluate peak cortisol response to ITT (Crofton et al 2004). Crofton et al (2004) reviewed the results of 54 children who underwent ITTs over a six-year period, most (76%) under investigation for short stature. Patients receiving glucocorticoids or those with HPA dysfunction were excluded. Peak cortisol response occurred by 90 minutes in all subjects and was inversely correlated with age. Cut-offs of 470 nmol/L and 550 nmol/L were established for children aged ≥ or less than ten years respectively (see Appendix 2B).

Median peak cortisol response to insulin-induced hypoglycaemia was 538 (inter-quartile range 460 to 611) nmol/L in my study. Peak cortisol responses were suboptimal in nine of 25 subjects using the cut-offs described above (Crofton et al 2004). I have presumed these results to be valid. Several studies have shown that results from the ITT are reproducible and representative of underlying HPA status (Inder & Hunt 2002). Yet, this may not always be the case. Cortisol responses to the ITT are reproducible in healthy adult subjects (Pfeifer et al 2001, Vestergaard et al 1997). However, there may be within-subject variability in patients with pituitary disease, particularly in subjects with subtle HPA dysfunction (Pfeifer et al 2001).

Tsatsoulis et al (1988) described four adult cases where there were satisfactory cortisol responses to the ITT in spite of symptoms consistent with HPA insufficiency. Cortisol day curve profiles were also subnormal in these patients. Abdu et al (1999) also reported one such case. In most cases however, the ITT is a reliable measure and in particular, there is little chance of subjects with severe ACTH deficiency being misclassified (Pfeifer et al 2001). The way in which subjects with suboptimal cortisol responses were managed in my study is discussed in section 5.2.4.6.

5.2.4.3 Evaluation of cortisol responses to the glucagon test

The glucagon test was used as an alternative to the ITT in subjects with a history of seizures. Aside from the ITT, it is the only other stimulation test that allows assessment of both the GH and HPA axes simultaneously. Glucagon can be administered subcutaneously or IM. IM administration is thought to be more reliable and to produce higher serum cortisol concentrations and fewer side-effects (Littley et al 1989, Orme et al 1996). The glucagon test is generally well-tolerated, although nausea may feature in between 20% and 30% of patients (Spathis et al 1987, Leong et al 2001) and vomiting in 10% (Spathis et al 1987). One of eight subjects receiving glucagon vomited in my series, towards the end of the test.

The cortisol response to subcutaneous or IM glucagon has been shown to be ACTH dependent (Spathis et al 1974, Littley et al 1989). The mechanism is not clear but ACTH secretion may be related to glucagon-induced catecholamine release (Leong et al 2001). Peak cortisol concentrations are reached between 120 and 180 minutes post-glucagon administration. In an audit of 500 adult cases, Leong et al (2001) demonstrated that 93% with normal cortisol responses to subcutanaeous glucagon (>550 nmol/L) achieved their peak response by 180 minutes (Leong et al 2001).

There appear to be no published reference range data for the peak cortisol response to glucagon in children. I used a cut-off of greater than 450 nmol/L based on responses amongst 31 healthy adults in a study by Agha et al (2004a). An earlier study by Rao and Spathis (1987) established a peak cortisol cut-off of greater than 500 nmol/L (or an increment >250 nmol/L from baseline) based on the response to glucagon in 92% of 97 healthy adult controls. Johnstone & Cheetham (2004) have used a cut-off value of 500 nmol/L in children but the origins of this were not clear. Using a cut-off of 500 nmol/L, there would have been two more borderline fails (peak responses of 466 and 496 nmol/L). Recruitment of healthy control subjects to the study would have provided an opportunity to establish paediatric reference data. However, potential side-effects and the risk of late hypoglycaemia (Shah et al 1992) make it difficult to justify performing the glucagon test in children who are not presumed to be at risk of pituitary dysfunction, as well as the discomfort associated with administration by IM injection.

Some studies report a good correlation between the ITT and glucagon tests in terms of peak cortisol response (Spathis et al 1974, Orme et al 1996). However, glucagon fails to elicit an adequate cortisol response in approximately 10% of healthy individuals (Agha et al 2004a, Rao & Spathis 1987). Two of eight subjects had sub-optimal cortisol responses to glucagon in this study. Baseline early morning cortisol concentrations were high in one (722 nmol/L). The other subject was offered an appointment to discuss options for further assessment of the pituitary-adrenal axis but did not attend.

5.2.4.4 Use of a Synacthen test in place of the glucagon test

The standard or low dose Synacthen tests could have been used in place of glucagon as second line investigations of the HPA axis. The advantages of the glucagon test, as previously described, are that it can be used for simultaneous assessment of the HPA and GH

axes and probably evaluates the entire HPA axis. An alternative combination of stimulation tests may have required subjects to attend on two separate occasions, which might have been less acceptable to them. However, it must be acknowledged that the low dose or standard Synacthen tests are becoming increasingly used as first line or screening tests of HPA function (Davies and Howlett 1996, Reynolds et al 2006, Abdu et al 1999).

5.2.4.4.1 Standard Syncathen test: The standard (250 μg) Synacthen test was originally designed to assess primary adrenal failure (Abdu et al 1999). In subjects with secondary adrenal insufficiency, results reflect the presence or absence of adrenocortical atrophy secondary to ACTH deficiency (Gonzalbez 2000, Gleeson et al 2003). However, false passes have been reported in adult studies (Cunningham et al 1983, Orme et al 1996, Lindholm & Kehlet 1987, Maghnie et al 2005). The synacthen test is not very reliable in the detection of recent onset ACTH deficiency (Inder & Hunt 2002, Lindholm and Kehlet 1987). In spite of evolving adrenal atrophy, the supra-physiological dose of 250 μg may be capable of eliciting a pass level cortisol response (Gleeson et al 2003, Abdu et al 1999). There are also reports of failure to detect even long-standing ACTH deficiency (Soule et al 1996). Orme et al (1996) concluded that the standard Synacthen test displayed poor diagnostic utility when compared with the IM glucagon test.

Other concerns exist about the use of the standard Synacthen test as an alternative to other stimulation tests for assessment of the HPA axis. It has only been validated against stressful illness in very small studies (Davies & Howlett 1996). Several studies report discordant results with the ITT (Davies & Howlett 1996, Stewart et al 1988). Stewart et al (1988) reported discrepant results in ten of 70 subjects, nine of whom failed the standard Synacthen test but passed the ITT.

It has been suggested that the 30-minute cortisol response to Synacthen correlates most closely with the peak cortisol response to ITT (Davies & Howlett 1996, Orme et al 1996). Raising the 30-minute cortisol pass value for the standard Synacthen test to 500, 550 or 600 nmol/L shows progressively less discordance with the ITT (Clayton 1996, Abdu et al 1999). However, arbitrary cut-off values not based on evidence-based reference ranges may give rise to falsely abnormal results that could lead to patients being started on hydrocortisone unnecessarily (Gonzalbez et al 2000, Gleeson et al 2003). Best practice is to establish local reference ranges for tests based on the assays that are available (Gonzalbez et al 2000, Inder & Hunt 2002).

5.2.4.4.2 Low dose Synacthen test: The plasma cortisol response to the low dose (1μg) Synacthen test is equivalent to the response obtained with 250 μg. Abdu et al (1999) observed that most of their adult subjects achieved their peak cortisol response 30 minutes into the test and all by 40 minutes. Several studies have shown that the low dose test may be better suited to detecting mild adrenal insufficiency than the standard Synacthen test (Gonzalbez et al 2000, Abdu et al 1999), including one paediatric study (Agwu et al 1999). Conversely, the low dose test may also misdiagnose individuals as being cortisol deficient (Inder & Hunt 2002).

It has been suggested that the low dose Synacthen test correlates more closely with the ITT than the standard test (Orme et al 1996, Agwu et al 1999), particularly the 30-minute sample (Stewart et al 1988). This association may not be clear-cut (Maghnie et al 2005). Maghnie et al (2005) found little correlation between ITT, CRH test, low dose or standard Synacthen tests, except in subjects with clinically obvious severe HPA insufficiency. Wientrob et al (1998) reported good correlation between both the standard and low dose Synacthen tests and the ITT in children, but all their subjects had multiple pituitary hormone deficiencies and the degree of ACTH deficiency may have been more pronounced.

5.2.4.5 Evaluation of serum ACTH and use of the CRH test

5.2.4.5.1 Measuring basal and stimulated ACTH levels: Cortisol rather than ACTH produces the end-organ counter-regulatory effects that are required of the HPA axis in times of stress; thus I did not feel it was necessary to measure basal or stimulated ACTH concentrations in this study. ACTH is unstable, requires special handling and the assay used is expensive and labour intensive. Different ACTH assays produce a range of results with poor inter-laboratory agreement and no international reference preparation (Talbot et al 2003). There was a theoretical risk of mistaking primary adrenal insufficiency for ACTH deficiency by not measuring ACTH levels. However, primary adrenal insufficiency is rare (Willis et al 1997). There were no other findings to suggest primary adrenal insufficiency in any of my subjects: plasma sodium and potassium concentrations were within normal range and no subject was hyperpigmented on examination. Hyponatraemia is less common in ACTH deficiency than in primary adrenal insufficiency due to preserved aldosterone function (Jostel et al 2005).

Both the ITT and possibly the glucagon test stimulate the entire HPA axis (Tuchelt et al 2000, Littley et al 1989, Spathis et al 1974). Petersen et al (1984) demonstrated that the ACTH response to insulin-induced hypoglycaemia in children is, as with cortisol, age-dependent. There are no local reference ranges for ACTH response to either test in children and it may be prohibitively expensive to establish these. The ACTH response to glucagon may be less pronounced than the response to insulin-induced hypoglycaemia (Littley et al 1989).

Tuchelt et al (2000) measured the ACTH response to the ITT in 109 adult patients with pituitary disease and 25 healthy control subjects. In control subjects, cortisol response to insulin-induced hypoglycaemia was independent of the magnitude of ACTH response. The cut-off for peak ACTH response to insulin-induced hypoglycaemia was 80 ng/L. Thirty-eight of 109 patients had normal serum cortisol responses (>500 nmol/L) during the ITT in spite of subnormal ACTH responses (some as low as 13-22 ng/L). Only 17 of 30 patients with ACTH responses less than 40 ng/L had subnormal cortisol responses. It was concluded that the normal increment of ACTH during the ITT is greater than necessary for stimulating an adequate cortisol response. Although patients with subnormal ACTH responses may have a decreased ACTH secretory reserve, Tuchelt et al did not feel that they were likely to be at risk of developing an adrenal crisis during stressful situations. Their results demonstrated that there is a spectrum of normality in terms of function of the HPA axis with no clear dividing line between the normal state and the abnormal state.

5.2.4.5.2 Use of the CRH test: The CRH test has been proposed as another suitable measure of HPA function. However, unlike the ITT (and possibly, glucagon), CRH only provides a stimulus at the level of the pituitary and adrenal glands, thus potentially overlooking damage to the hypothalamus and/or pituitary stalk. The results of CRH testing have been reported to correlate with those of the ITT in patients with severe ACTH deficiency and glucocorticoid-induced ACTH deficiency (Maghnie et al 2005). However, other studies of patients with pituitary disease have observed significant variability in terms of cortisol response.

Schmidt et al (2003) used ROC analysis to evaluate the diagnostic value of the CRH test, compared with the ITT as the reference standard. The optimal peak cortisol cut-off for diagnosis of pituitary-adrenal insufficiency (<377 nmol/L) had good specificity (96%) but low sensitivity (76%). Dullart et al (1999) determined much higher cut-offs for the CRH

test, with peak cortisol levels of 420 and 615 nmol/L reflecting 100% specificity and sensitivity respectively. Both these studies concluded that there was no significant advantage of the CRH test over early morning cortisol concentrations in the assessment of the HPA axis.

5.2.4.6 Management of sub-optimal cortisol responses

The definition and practical significance of partial HPA insufficiency are debatable. Physiological and pathological cortisol responses may overlap within a biological continuum. Few guidelines exist in relation to evaluation of HPA function and when hormone replacement therapy is indicated for suboptimal cortisol responses to testing.

Inder and Hunt (2002) placed particular significance on early morning cortisol concentrations as a guide to management of adults following pituitary surgery. They recommended hydrocortisone replacement therapy for patients with early morning cortisol levels less than 100 nmol/L. A single morning hydrocortisone dose was suggested for patients with early morning cortisol concentrations between 100 and 250 nmol/L, pending stimulation testing. Steroid cover was recommended during acute illness for early morning cortisol levels between 250 and 450 nmol/L, with stimulation testing if repeat basal levels were below 350 nmol/L.

In more recent studies, Agha et al (2004d) demonstrated that under unstressed conditions, adults with partial ACTH deficiency have a cortisol day curve similar to controls. They concluded that conventional hydrocortisone replacement regimens over-treat such patients under normal (unstressed) physiological conditions. Instead, they advocated either low dose replacement therapy or hydrocortisone cover during illness only. Paisley et al (2009) measured daily cortisol production rate in ten subjects by isotope dilution using gas chromatography-mass spectrometry. All had pituitary disease and peak cortisol responses to ITT between 350 and 500 nmol/L. They too observed that a sub-optimal cortisol response to ITT did not equate to a low cortisol production rate on a day-to-day basis and should not be an indication for life-long glucocorticoid replacement therapy. Data for children are lacking.

Reynolds et al (2006) conducted a survey amongst UK adult endocrinologists to determine methods of assessment of the HPA axis after pituitary surgery. The response rate was low (81 of 598 responsed) but 85% of respondents were consultants. Fifty-five percent measured the 09:00 plasma cortisol level in the immediate post-operative phase. All but two of 81

respondents carried out stimulation testing of the HPA axis routinely. Twenty-four respondents (31%) used the ITT only to evaluate HPA function. Forty-four (57%) used the standard Synacthen test. Use of other tests (e.g. glucagon, CRH tests) was uncommon. Patients with suboptimal cortisol responses to the stimulation test of choice were treated with regular glucocorticoid therapy by 28% of respondents, re-tested prior to commencing treatment by 38%, and prescribed glucocorticoid cover during illness by 24%.

I measured baseline early morning cortisol concentrations and peak cortisol responses to insulin-induced hypoglycaemia or glucagon. No subject had symptoms or a low enough peak cortisol response to suggest severe ACTH deficiency. The evidence base for management of partial ACTH deficiency is small but regular hydrocortisone replacement therapy is probably excessive (see above). I recommended hydrocortisone cover during illness for three subjects. All had peak cortisol responses during the ITT that were between 50 and 100 nmol/L below the 470 nmol/L cut-off for children aged ≥ ten years (range 385-414 nmol/L). Another subject had a sub-optimal cortisol response to glucagon. He was offered an appointment to discuss options for further investigation of the HPA axis but did not attend.

5.2.5 GH axis

GHD is the commonest pituitary hormone abnormality following TBI in adults and has been observed in between 8% and 38% of subjects (section 1.6.2). In children, Neiderland et al (2007) reported GHD in 42% of TBI survivors, defined as a peak GH response of less than 7 μg/L to both L-dopa and the ITT. These data were compared with GH results for 21 age-matched controls but control data do not appear to have been used to establish local cut-offs. Poomthavorn et al (2008) investigated eight of 29 TBI survivors with low IGF1 concentrations and poor growth velocities using the glucagon test. There were no abnormal findings. Einaudi et al (2006) diagnosed two of 22 children evaluated retrospectively and one of 26 prospective study subjects with GHD. In the latter case, GH status was normal six months post-injury but GHD had developed by the 12-month assessment (peak GH response to GHRH-arginine < 20 μg/L [Ghigo et al 1996a]).

Two of my study subjects had slightly low IGF1 concentrations for age and pubertal status: 146 (reference range 150-600) nmol/L and 179 (reference range 200-650) nmol/L. The first passed the ITT; the other had resolving constitutional delay of growth and puberty and a suboptimal GH response to ITT. In the latter subject, growth velocity was inappropriately

slow for mid-puberty on follow-up and GH was prescribed. In all, seven males had inadequate peak GH responses to insulin-induced hypoglycaemia (6/25) or glucagon (1/8). Two other subjects who underwent the ITT and one who received glucagon had suboptimal GH responses following spontaneous peaks (>5 μg/L) at baseline. These children were considered to be GH-sufficient (see section 5.2.5.5).

The cut-off used to define a normal GH response was 5 μg/L. This was derived using local data from healthy children investigated for short stature using the ITT (Crofton PM, personal communication; Appendix 2A). The same cut-off was used for GH response to glucagon; the reasons for doing so are outlined in section 5.2.5.5. Children in late pre-puberty and early puberty were not primed with sex steroids before testing and the significance of this is discussed in section 5.2.5.3. The GH result in one post-pubertal male (aged 17.0 years), whilst below the aforementioned cut-off, was only borderline low according to cut-offs used to define GHD in young adults (Clayton et al 2005, NICE 2003). The definition and management of GHD during the transition from childhood to adulthood are discussed in more detail in section 5.2.6.

5.2.5.1 Growth measurements in evaluation of the GH axis

Height and growth velocity measurements are a key part of the evaluation of growth in children. These provide an objective non-invasive means of screening for GHD. Consensus guidelines from the GH Research Society (2000) recommend evaluation of the GH axis under the following circumstances (provided that other causes of growth failure have been considered, such as hypothyroidism, chronic systemic disease, Turner syndrome or skeletal dysplasia):

1. Height more than 3 SD below the mean for age
2. Height more than 1.5 SD below the mid-parental height
3. Height more than 2 SD below the mean and height velocity over one year more than 1 SD below the mean for chronological age
4. In the absence of short stature, a height velocity more than 2 SD below the mean for one year or more than 1.5 SD below the mean sustained for over two years
5. Signs indicative of an intracranial lesion, signs of multiple pituitary hormone deficiencies, or neonatal signs and symptoms of GHD

Single height measurements, unless extreme, are often unhelpful in the assessment of growth (Drake & Kelnar 2006). Yet, in my study, height was not routinely evaluated in the months before or after assessment, except in those with suboptimal GH responses to stimulation. I could, in retrospect, have requested parents to provide a recent height measurement (taken within the 12 months prior to study assessment). Alternatively, I could have arranged for subjects' height to be measured locally six or 12 months after assessment. However, problems with the accuracy of different stadiometers and between-observer bias may lead to large errors in calculated height velocity. Consecutive height measurements should ideally be taken by the same individual to maximise accuracy (Drake & Kelnar 2006). As my study population came from all over Scotland, this would not have been practicable.

All subjects' height measurements were within ± 2 SD of the mean for their age at assessment. Whilst none would necessarily have warranted investigation of the GH axis in clinical practice (unless growth velocity was poor), they were presumed to be an at-risk population due to the history of TBI. One child's height was more than 1.5 SD below his mid-parental height (-1.63 SD). Although history and examination findings at 14.8 years seemed consistent with resolving constitutional delay of growth and puberty, growth velocity was inappropriately slow for pubertal stage and this boy is now receiving GH therapy. The GH axis will be reassessed off treatment at adult height.

As described above, serial growth measurements were only taken in subjects followed up due to poor GH responses to stimulation. Thus, I was unable to compare rate of growth between TBI survivors with normal or abnormal findings on GH testing. The lack of routine growth monitoring following childhood TBI has been highlighted recently (Moon et al 2009). Moon et al (2009) observed that height and weight were only documented at 33% of follow-up appointments. Only 69% of 123 TBI patients admitted to PICU were followed up at the hospital in question. Serial growth measurements were only available for 22 children (17%).

Many would consider pharmacological tests to be of secondary importance to growth velocity (Davies & Howlett 1996). Indeed, in Australia, children have been selected for GH therapy solely on the basis of auxological criteria (Werther 1996). However, growth velocity measurements over a year cannot always distinguish between the child with GHD and the healthy child with short stature (Voss et al 1991). Voss et al (1991) found no significant correlation in subjects' growth velocities from year to year, suggesting that

growth velocity is not an accurate predictor of future growth. Physiological inter-individual variations in growth rate may make this an unreliable means of assessment: for example, in the child with constitutional delay of growth and puberty. In some circumstances a normal growth velocity can be associated with GHD (Ayling 2004).

GH concentrations may also be reduced in obese children (Sizonenko et al 2001). This may have been a confounding factor in one subject who failed the ITT in my study, whose weight and BMI SDS scores were greater than +2.

5.2.5.2 Markers of GH status: IGF-1 and IGFBP-3

Many paediatric endocrinologists use serum IGF1 and insulin-like growth factor binding protein 3 (IGFBP-3) concentrations as markers of GH secretion. The insulin-like growth factors (IGFs) are believed to mediate many of the anabolic and mitogenic actions of GH (Shalet et al 1998). Serum levels of the major GH-dependent peptide IGF1 show little diurnal variation; thus a single basal sample is adequate for assessment. IGFBP-3 is the major carrier of IGFs in the circulation and is also GH-dependent (Shalet et al 1998). Both IGF1 and IGFBP-3 are influenced by age and pubertal stage and thus require well-constructed population-matched reference ranges (Sizonenko et al 2001). IGF1 levels are low in young children (Ayling 2004). They are also affected by nutritional status, renal and liver function, diabetes mellitus, and thyroid hormone status (Sizonenko et al 2001).

5.2.5.1.1 IGF1: Serum IGF1 concentrations are thought to correlate more reliably with GHD in children than adults. About a third of adults with low GH responses to stimulation have IGF1 levels within the normal range (Murray et al 2000, Hoeck et al 2000). However low IGF1 concentrations correlate well with GH responses in patients with hypothalamo-pituitary disease (Hoeck et al 2000). Serum IGF1 may have better diagnostic sensitivity in adults with childhood-onset GHD rather than adult-onset disease (Kaushal & Shalet 2007). In a review of 1317 GH-deficient adults registered on the KIMS database, Lissett et al (2003) found that 86% with childhood-onset GHD had serum IGF1 concentrations more than 2 SD below the mean compared with only 52% of patients with adult-onset GHD. Mean (SD) IGF1 SDS were significantly lower in the group with childhood onset GHD: -4.69 (2.48) compared with -2.24 (2.11). IGF1 SDS were also negatively correlated with the total number of pituitary hormone deficits.

Even in children, however, there is considerable overlap between IGF1 concentrations in healthy children and children with GHD (Preece 1997). Sizonenko et al (2001) considered the findings of 12 studies and calculated that the mean sensitivity of IGF1 as a diagnostic marker of GHD was 71% and the mean specificity 72%, compared with stimulation tests as the standard. There were large ranges for both indices across the studies evaluated. On average, a significant minority of GH-deficient subjects had normal IGF1 levels (29%) and 28% of short normal children had low IGF1 levels. Recently, Bouhours-Nouet et al (2007) have suggested that IGF1 concentrations may be elevated in obese or tall children, in spite of the fact that GH secretion may be blunted. These findings suggest that IGF1 alone is not an adequate screening test for GHD but may be used in combination with other measures of GH status. As mentioned previously, two subjects had low IGF1 levels for age and pubertal stage in my study. One had a suboptimal GH response during the ITT and is now receiving GH therapy.

5.2.5.1.2 IGFBP-3: Early studies suggested that serum IGFBP-3 concentrations were highly sensitive (0.97) and specific (0.95) for GHD in children (Blum et al 1990). Blum et al (1990) reported that 128 of 132 children with GHD had serum IGFBP-3 concentrations below the fifth centile. Yet, these findings have not been replicated in later studies. The specificity of serum IGFBP-3 levels as a marker of GHD exceeds the sensitivity (Sizonenko et al 2001). Thus, whilst IGFBP-3 concentrations are not often low in a healthy child, they may be within normal range in a child with GHD. Juul and Skakkebaek (1997) reported higher sensitivity and specificity in older subjects (aged between ten and 20 years) than young children. However Hasegawa et al (1994) observed that IGFBP-3 concentrations were a more sensitive marker in children below ten years of age. In this study, the sensitivity was especially poor in subjects with partial GHD. IGFBP-3 concentrations may also be increased in obese children (Park et al 1999). There is little evidence either from published studies or local experience that IGFBP-3 measurement provides any additional value to IGF1 measurement in the diagnosis of GHD in children. For this reason, IGFBP-3 concentrations were not measured in my study.

5.2.5.3 Lack of sex steroid priming

I elected not to 'prime' subjects in late pre-puberty or early puberty with sex steroids prior to GH stimulation testing. Usual clinical practice at the RHSCE is to prime children with a bone age >10 years and no signs of puberty in girls or testicular volumes <8 mL in boys. Girls are prescribed ethinyloestradiol 100 μg daily orally for three days before testing. Boys

are prescribed 0.4 mL of Sustanon 250 (a testosterone blend) by IM injection three to five days before testing. In this study, recruited subjects underwent a single assessment (on the day of endocrine evaluation). To prime the subjects would have necessitated an assessment of pubertal stage several days prior to GH stimulation testing, which, if done at the RHSCE, would have required families to make two trips to Edinburgh. This would have been problematic for families coming from as far away as Shetland and might have deterred would-be subjects from taking part in the study. Pubertal stage could have been evaluated locally but would have required involvement of paediatricians with the necessary expertise. Furthermore, the Scotland A MREC stipulated that pubertal staging should only be performed if clinically indicated and with the subject's consent.

5.2.5.3.1 Rationale for sex steroid priming: The rationale for priming is based on studies demonstrating physiological blunting of GH secretion in children in late pre-puberty and boys with delayed puberty (Stanhope & Preece 1988). GH secretion increases as puberty advances (Delemarre-Van De Waal et al 1991, Veldhuis et al 2000, Marin et al 1994), returning to pre-pubertal levels once puberty is complete (Sizonenko et al 2001). Veldhuis et al (2000) observed a 2.3-fold mean increase in integrated serum GH concentration between pre-puberty and adolescence. Pulsatile GH release showed marked maturational dependence.

Cavallo et al (1992) retrospectively reviewed the responses of 574 short normal children to several different stimulation tests at different stages of puberty and concluded that pubertal stage did not impact on response to stimulation testing. However, the study did not include subjects who were more advanced in puberty (equivalent to Tanner stages 4 and 5). By contrast, Marin et al (1994) studied the effects of treadmill exercise, insulin or arginine on GH secretion in 84 normal children (43 males) at differing stages of puberty. Peak GH response to the tests increased significantly with pubertal stage. With advancing puberty, the percentage of normal children who failed to attain a peak GH response greater than 7 μg/L decreased from 61% in pre-pubertal subjects to 0% in post-pubertal subjects. Peak GH response increased from between 1.9 and 20.3 μg/L to between 7.1 and 40.5 μg/L in pre-pubertal subjects after priming with ethinyl estradiol. Thus priming with exogenous sex steroids can temporarily reverse physiological blunting of GH secretion.

Muller et al (2004) studied 26 males with short stature and delayed puberty who had failed the arginine stimulation test. Twenty of 26 responded to a second arginine test with GH

peaks greater than 10 μg/L following administration of a single dose of testosterone. In a similar study, Molina et al (2008) repeated clonidine tests in 39 short peri-pubertal children (31 males) after steroid priming. Mean (SD) height and growth velocity SDS amongst the cohort were -2.24 (0.95) and -2.12 (1.32). Mean (SD) bone age was 9.58 (2.21) years at a chronological age of 12.37 (2.24) years. Twenty-one of 39 (53.8%) demonstrated GH peaks greater than 10 μg/L after priming, whereas mean (SD) peak GH response during earlier testing had been 4.87 (2.72) μg/L.

By contrast, Wilson et al (1993) found no significant difference in GH response between primed and unprimed children who underwent GH stimulation testing with clonidine. However, significantly more primed than unprimed subjects were pre-pubertal. Thus, a greater proportion of the unprimed group may have had sufficient endogenous sex steroids not to require priming. Primed subjects only received their first dose of oestrogen the night before testing, an interval that may have been too short for the effects of the treatment to be fully realised.

In a placebo controlled trial, Martinez et al (2000) observed that priming with oestradiol before GH stimulation testing helped to discriminate between normal and abnormal GH status in children with short stature. They studied 15 pre-pubertal children with suspected GHD and 44 pre- or early pubertal children with idiopathic short stature. Subjects underwent a sequential arginine-clonidine test after priming or placebo, and then were re-tested four weeks later after receiving the other option. Peak GH responses were significantly greater in children with idiopathic short stature who were primed than those who received placebo. Lower 95% confidence limits were 8.3 μg/L versus 3.7 μg/L respectively. A higher number of unprimed non-responders were aged between eight and 12 years, as might be expected. In GH-deficient subjects, there was no significant stimulatory effect of priming on GH response. Using the 3.7 μg/L cut-off after placebo was 73% sensitive and 95% specific for GHD, whereas use of the 8.3 μg/L cut-off after oestradiol resulted in a sensitivity of 87% and specificity of 98%. Diagnostic efficiency increased from 90% to 95%.

However, priming pre-GH stimulation testing remains controversial. A survey of 250 American paediatric endocrinologists in 1995 found that two thirds did not prime children (Wyatt et al 1995). In a survey of 235 ESPE members in 2001, only half reported priming girls and 41%, boys (Juul et al 2002). Some clinicians assert that temporary augmentation of

GH secretion by priming (that then dwindles to pre-existing low levels) may lead to under-diagnosis of GH insufficiency in peri-pubertal children who could have benefited from GH treatment (Lazar & Phillip 2010).

The decision not to prime may explain the low responses to ITT observed in five of six pre- or early pubertal boys during our study. These children showed no evidence of growth impairment at assessment or follow-up. In the sixth, aged 14.8 years at initial assessment, growth velocity remained inappropriately slow even in the context of constitutional delay of growth and puberty. This boy is now receiving GH therapy and will be reassessed when he reaches adult height.

5.2.5.4 Evaluation of the ITT for GH stimulation testing

GH is released from the anterior pituitary gland under the influence of opposing hypothalamic regulatory hormones: GHRH and somatostatin (Ayling 2004). Random determination of serum GH values is unhelpful, as the pulsatile release and short serum half-life of GH often result in low concentrations in normal children (Drake & Kelnar 2006). GH stimulation tests are quick to perform and can be carried out on a day case basis. However, as discussed below, they are difficult to standardise, may give false positive or false negative results and the individual response is variable. GH responses may be influenced by cortisol excess, hypothyroidism and obesity (Sizonenko et al 2001). Due to the ethical dilemma of conducting potentially unpleasant tests in well children, most normative data have been obtained in short normal children or healthy children with a decrease in growth velocity (Sizonenko et al 2001). My reference data for the ITT are based on GH responses amongst a cohort of 54 such children (Crofton PM, personal communication; Appendix 2A).

5.2.5.4.1 Mechanism of action: I used the ITT as a first line investigation for several reasons. The ITT permits simultaneous evaluation of the GH and HPA axes and is considered the reference standard for both (see also section 5.2.4.2). Insulin-induced hypoglycaemia stimulates GH secretion through suppression of somatostatin tone and stimulation of α-adrenergic receptors (Sizonenko et al 2001). Following adequate hypoglycaemia, GH response exceeds 5 μg/L in 85% of normal children (Drake & Kelnar 2006), usually within 30 to 60 minutes. Local reference data from the RHSC Edinburgh demonstrated a peak GH response greater than 5 μg/L in 95%. The overall accuracy of the ITT (true positives plus true negatives divided by the total number of tests performed) ranges

from 100% at a GH cut-off value of 3 μg/L to 85% at a cut-off of 10 μg/L. The controversy surrounding which GH cut-offs should be used is discussed in a later section (5.2.5.7).

Yeste et al (2007) suggested that giving oral glucose solution during the ITT produced lower cortisol and GH responses to insulin. They assigned 100 children to receive either glucose solution or nothing upon reaching a blood glucose concentration of 1.7 mmol/L. GHD was diagnosed more frequently in patients given oral glucose solution to counteract hypoglycaemia. However, this study had a number of weaknesses. An arbitrary cut-off of 10 μg/L was used to define a normal GH response to ITT without evidence of local validation or comparison with control data. It was not clear whether or not subjects were primed. The RHSC Edinburgh's own locally validated cut-off, using the same assay as in this study, is 5 μg/L. Yeste et al did not obtain -30 minute samples prior to administering insulin and may therefore have missed spontaneous GH peaks at baseline in some subjects. Suboptimal GH responses to stimulation have been reported following baseline GH peaks (Leong et al 2001) and may be due to a refractory period.

Our policy of giving a glucose drink during the ITT is consistent with recommendations by Galloway et al (2002). In an audit of 550 ITTs conducted over ten years in their department, they found that the glucose nadir occurred at 15 minutes in just over half of patients and by 30 minutes in all. They advised giving a 20% glucose drink between 15 and 20 minutes into the test to avoid the effects of more profound hypoglycaemia and/or the need for IV dextrose. We give a glucose drink once the blood glucose has fallen to less than 2.2 mmol/L or to 50% of the baseline blood glucose concentration in accordance with Hindmarsh and Swift's (1995) definition of adequate hypoglycaemia during the ITT.

5.2.5.4.2 Reliability and reproducibility: The reliability and reproducibility of the GH response during the ITT are worthy of discussion. GH response to the ITT in healthy adult subjects can vary considerably (Vestergaard et al 1997, Pfeifer et al 2001). Perhaps unsurprisingly, GH responses are more consistent in subjects with severe GHD (Pfeifer et al 2001). Thus, a single ITT is unlikely to misclassify severely GH-deficient patients but may be less accurate in adults with partial GHD. The most important reason for the difficulty in distinguishing between partial GHD and normal GH status is that these are part of a biological continuum. Evidence of overlap in all biochemical and auxological parameters exists between short normal children and those with partial GHD (Shalet et al 1998).

In a study of intra-subject variability, Hoeck et al (1999) measured GH responses to two ITTs, two clonidine tests, two GHRH tests and two pyridostigmine-GHRH tests in 16 non-obese healthy adults. There were GH responses less than 3 μg/L to ITT in two subjects who had normal responses in their first ITT. Thus, although there was no significant difference in median GH response across the group as a whole, intra-subject variability can occur, and healthy people can 'fail' the ITT. It was concluded that caution is justified in the interpretation of low responses to a single test. In a subsequent study, Hoeck et al (2000) investigated the reproducibility of the ITT and other tests amongst adults with hypothalamo-pituitary disease being tested for GHD. There was significant correlation between GH responses to a first and second ITT in this group.

Persistent severe GHD has been found in between 12.5% and 90% of GH-treated subjects on re-testing after achievement of adult height (Sizonenko et al 2001). Understandably, this calls into question the certainty of the original diagnosis, made using a variety of stimulation and other tests of GH repleteness. For all the above reasons and taking into account assay controversies (section 5.2.5.8), it has been recommended that children with suspected isolated GHD undergo two stimulation tests (NICE 2002, GH Research Society 2000). In practice, this decision is influenced by the likelihood of GHD, presence of any other pituitary hormone abnormalities and the possible underlying aetiology. For the purposes of this study, subjects were presumed to be at increased risk of pituitary dysfunction. I expected GHD, if present, to be clearly evident on testing; hence subjects underwent only one stimulation test.

5.2.5.5 Evaluation of the glucagon test for GH stimulation testing

Like the ITT, the glucagon test enables assessment of both the GH and HPA axes (Rao & Spathis 1987; section 5.2.4.3). This does not apply to other GH stimulation tests such as the GHRH-arginine, clonidine or L-dopa tests (Leong et al 2001). The mechanism of glucagon-induced GH secretion is unclear. It may in part be triggered by falling blood glucose concentrations after an early peak 30 minutes into the test, however the glucose nadir is seldom significantly low: rarely less than 4.5 mmol/L in adults (Leong et al 2001). Peak GH response occurs between 120 and 180 minutes post-glucagon administration in approximately 90% of responders, occasionally later (Leong et al 2001). Intramuscular administration of glucagon may stimulate GH secretion more effectively than subcutaneous administration (Rao & Spathis 1987). In a small sub-set of patients (n=12) in Rao & Spathis' study (1987) who underwent two glucagon tests, GH response was reproducible.

Side effects of the glucagon test, including late (rebound) hypoglycaemia, were outlined in 5.2.4.3.

In this study, I used the same cut-off for peak GH response to insulin-induced hypoglycaemia or glucagon. Previous (adult) studies have reported similar GH peaks in both (Leong et al 2001), with good correlation between the two tests (Spathis 1974). Glucagon has been shown to be as potent as the ITT in stimulating GH release in healthy individuals (Rahim et al 1996, Aimaretti et al 2000). Early studies (Cain et al 1972) suggested that glucagon may be the more potent stimulus but GH responses to the two tests were compared between subjects rather than in the same individuals. Rahim et al (1996) measured GH response to the ITT, glucagon, clonidine, arginine and placebo in 18 healthy young adult males. Each subject underwent all tests. The best GH response followed the ITT: greater than 40 mU/L [13 μg/L] in all but one subject whose GH response was 9 μg/L. All but two subjects produced GH responses greater than 20 mU/L (7 μg/L) to glucagon.

Smyczynska et al (2005) reported a significant false fail rate with the glucagon test in a Polish study of 780 children evaluated using clonidine and glucagon. Two hundred and eighty-three subjects who passed the clonidine test had inadequate GH responses to glucagon. However these findings were presented in abstract form only and many details required to interpret the results were lacking. It is not clear whether or not subjects were primed, how much glucagon was administered or by what route, the duration of sampling post-glucagon administration, or what cut-offs were used to define a normal GH response (or their origin). Basal and -30 minute GH concentrations were also unclear. Poor GH responses to glucagon may be observed after a spontaneous GH peak at baseline (Leong et al 2001). In my study, two subjects who underwent the ITT and one who received glucagon had suboptimal GH responses in the context of spontaneous GH peaks (>5 μg/L) at baseline. These children were considered to be GH-sufficient.

5.2.5.6 Alternative GH stimulation tests

Clonidine, L-dopa, arginine and GHRH can also be used to elicit a GH response. Clonidine may stimulate GH secretion through its action as an α-2 adrenoceptor agonist (Rahim et al 1996), possibly via stimulation of GHRH release (Sizonenko et al 2001). It is associated with unpleasant side effects such as hypotension and drowsiness (Rahim et al 1996, Galloway et al 2002). Its reliability may be comparable with the ITT in children (Sizonenko et al 2001), however clonidine does not reliably stimulate GH secretion in adults. L-dopa

stimulates GH secretion through dopaminergic and α-adrenergic pathways. It is used infrequently due to side effects including nausea, vomiting and vertigo and a high false-fail rate (Sizonenko et al 2001). Arginine suppresses somatostatin tone (Rahim et al 1996, Sizonenko et al 2001) and may stimulate α-adrenoceptors, promoting GHRH release. Vomiting may feature occasionally (Sizonenko et al 2001) and the viscosity of the arginine infusion can cause venous occlusion (Galloway et al 2002). Exogenous GHRH is a potent stimulator of GH secretion and well-tolerated, apart from facial flushing (Sizonenko et al 2001). However, there is great variability in the GH response (Shalet et al 1998) and the sensitivity of GHRH testing is low (30% using a GH cut-off value of 7-10 μg/L [Sizonenko et al 2001]).

GHRH and arginine are increasingly used in combination as a GH provocation test in adults (Shalet et al 1998). Combining the two appears to reduce inter- and intra-individual variability of the GH response (Valetto et al 1996). Ghigo et al (1996b) established a GH cut-off value of 9 μg/L below which to define adult GHD. Corneli et al (2005) have since published BMI-dependent cut-offs. Schneider et al investigated 21 adult survivors of TBI with the ITT and GHRH-arginine test. Twelve failed the GHRH-arginine test (GH <9 μg/L) all of whom passed the ITT (GH >3 μg/L). All had BMIs greater than 28 and when BMI-dependent cut-offs were applied, only three of 12 still failed the test. One passed the GHRH-arginine but had a blunted response to ITT. There were no significant differences in IGF1 concentrations between obese and non-obese subjects.

In children, the combination of GHRH and arginine produces GH responses greater than 20 μg/L (Ghigo et al 1996a). However, the GHRH-arginine test tends not to be used frequently in paediatric endocrinology. In most children with isolated GHD, dysfunction of the GH axis appears to be hypothalamic rather than pituitary in origin (Sizonenko et al 2001). Whilst a low GH response to GHRH-arginine may indicate pituitary disease, a normal GH response does not exclude hypothalamic causes of GHD.

5.2.5.7 GH stimulation test cut-offs used by others

Cut-offs to GH stimulation tests are arbitrary and as previously mentioned there are limited data for children with normal height velocity (Ayling 2004). There is likely to be an overlap of values observed in normal children and those with partial GH insufficiency. Kaplan et el (1968) first defined GHD as a peak GH response less than 5 μg/L to insulin-induced hypoglycaemia. This value gave 88% specificity and 100% sensitivity for GHD. The

recommended cut-off was subsequently revised to 7 μg/L (20 mU/L, Fraser et al 1974) and some now use a cut-off as high as 10 μg/L. Whilst the latter cut-off is acknowledged in the GH Research Society consensus guidelines (2000), it is recommended that the value needs to be revised when using newer monoclonal-based assays and recombinant human GH reference preparations. NICE guidelines for childhood GHD (2002) suggest using the 7 μg/L cut-off, with similar qualification. Newer assays are discussed in more detail in section 5.2.5.8.

These cut-offs, generally based on GH response to the ITT, also need to be adjusted for the stimulation test used. Studies in children and adults have demonstrated that very different peak GH responses are obtained between individuals and in the same individual using different stimulation tests (Hoeck et al 1999, Hoeck et al 2000, Rahim et al 1996, Ghigo et al 1996a). Tillman et al (1997) reported that the best sensitivity for a single test was 85% at a peak cut-off level of 10 μg/L and the best specificity 92% at a cut-off level of 5 μg/L. Of note, only six of 170 children in that study underwent an ITT. The arginine test was used in 90, clonidine in 38, glucagon in 65 and the physiological GH response to exercise in two.

Evans and Gregory (2004) surveyed how children were investigated for short stature in 13 Welsh hospitals and found that practice and definitions varied widely. GH was measured in four laboratories using two assays yet nine different cut-offs were used to define GHD. The origin of cut-offs was unknown, unspecified or based on published work that was more than 15 years old. A European survey showed similar heterogeneity in terms of the diagnosis of GHD, particularly standardisation of assays and interpretation of cut-offs (Juul et al 2002). Eighty percent of paediatric endocrinologists used IGF1 concentrations in their initial evaluation of short stature whereas 22% used stimulation testing alone. Sixty-eight percent used two stimulation tests to confirm the diagnosis. The most common cut-offs used were 10 μg/L or 7 μg/L (20 mU/L) but it appeared that the use of these was arbitrary rather than specific to local assays and populations.

Ellis et al (2003), presented laboratories with aliquots of sera with GH concentrations likely to be found in an ITT and invited them to analyse these and present their findings. Approximately 90% of laboratories concluded (correctly) that the GH concentration was not indicative of GHD. Five labs gave an equivocal report. Several laboratories were using out-of-date cut-offs that bore little relation to their GH assay. Andersson et al (1995) reported a high level of correlation between normal and abnormal GH values across four different

laboratories using six different assays to measure the same samples. However, absolute GH estimates differed indicating the need for method-specific cut-off values. Individual laboratories should define these according to their local assay and reference population (Shalet et al 1998).

5.2.5.8 GH assays

As described above, the assays used in the analysis of GH test samples have a significant impact on the result obtained. Ninety percent of the GH formed in the pituitary is the 22 kDa form composed of 191 amino acid residues. The remainder is a 20 kDa form of 176 residues arising from an alternative splice site at exon 3. Other forms in the circulation are derived from post-translational modification, metabolic degradation and association with GH binding proteins (Ayling 2004). Assays using monoclonal antibodies to detect GH are less likely to show cross reactivity with these other forms (particularly 20 kDa human GH) than monoclonal/polyclonal pairs. Ranke et al (1999) have recommended using only GH assays that recognise only 22 kDa GH.

Early radioimmunoassays were replaced by two site non-competitive immunoradiometric assays. In some places, these were superceded by automated or semi-automated non-isotopic methods during the 1990s (Seth et al 1999). Ironically, use of more specific immunometric methods has led to a worsening of overall agreement between laboratories with the effect that there may be a two-fold difference in the way that the GH concentration in a serum sample is reported (Seth et al 1999). In spite of the fact that new assays can give GH concentrations two to three times lower than older radioimmunoassays (Celniker et al 1989), stimulation test cut-offs have not been revised in some centres.

Seth et al (1999) concluded an audit by calling for there to be agreements between manufacturers of GH assay kits to use antibodies of broadly the same specificity (at least with respect to known cross-reactants like 20 kDa GH). They also recommended that laboratories providing GH data to clinical centres should participate in a validated External Quality Assessment scheme. This is a condition of laboratory accreditation for all UK labs offering GH assays. Seth et al (1999) also advised that GH levels should be determined using correctly calibrated methods of known specificity and incorporated into national or internationally agreed guidelines.

5.2.5.9 GH reference standards

The recombinant 22 kDa human GH reference preparation used as a standard also has an impact on results observed. The new 98/574 standard reference preparation replaced the existing standard during the course of this study. The new standard is of known mass with a defined relationship of 1 μg/L equal to 3 mU/L. Previous standards were only assigned values in international (not mass) units, resulting in arbitrary conversion factors being used to convert GH concentrations from μg/L to mU/L using in-house methods. This has added to confusion in comparison of GH results between studies in the literature. It should be noted that in relation to my study, there was no significant shift in mU/L values following re-standardisation, allowing the same ITT cut-off to be used as previously established. All that was required was to divide the mU/L cut-off value by three to convert to ug/L. A summary of the evidence on which this judgment was based is presented in Appendix 2A.

5.2.5.10 Alternative measures of GH

Controversies over stimulation testing have led some to advocate greater use of GH profiling in the assessment of the GH axis. Pulsatile GH secretion can be measured by conducting serial sampling every 20 to 30 minutes, either overnight or for 24 hours. There are biological correlations between height, growth velocity and the frequency and amplitude of GH pulses (Sizonenko et al 2001). Yet, low overnight GH levels have been reported in normally growing pre-pubertal children (Lanes 1989). The reproducibility of GH profiling is poor although perhaps better than stimulation tests (Sizonenko et al 2001). Rose et al (1988) reported that GH profiling only identified 57% of children with GHD but this was using stimulation testing as the standard and some normal subjects might have failed to respond to the latter. The cost, intensive nature of the investigation and requirement for overnight stay mean that serial sampling is not often carried out in practice (Shalet et al 1998).

GH Research Society consensus guidelines (2000) suggest that GH profiling may have a role in situations where GH and IGF1 data conflict: for example, when IGF1 concentrations are low in spite of normal GH response to stimulation testing. Short children with reduced mean 24-hour serum GH concentrations but normal GH responses to stimulation have been proposed to have a form of neurosecretory dysfunction (Shalet et al 1998).

GH response to exercise has also been used to evaluate the GH axis. However there may be an absent response in up to a third of normal pre-pubertal children (Shalet et al 1998). Although Ghigo et al (1996a) suggested that GH results from exercise testing were not that unreliable compared to provocation tests, in practice, the former is used little. The positive predictive value is low (50%) even in a well-standardised test (Sizonenko et al 2001). The test requires subject cooperation and cannot be performed in a young child (Sizonenko et al 2001).

Lastly, GH is present in the urine in very low concentrations. Urinary GH levels can be measured but vary significantly from day to day and until recently, assay accuracy and reproducibility have been poor (Drake & Kelnar 2006). Renal function may account for up to 52% of the variability in urinary GH measurements (Shalet et al 1998). Whilst levels appear to be lower in children with hypopituitarism, urinary GH is also reduced in obese children (Georges et al 1997). Urinary GH measurements have been shown to be of little use in discriminating between short normal children and subjects with partial (Georges et al 1997) or definitive GHD (Tillman et al 1997).

5.2.6 GHD during the transition phase

One post-pubertal male subject had a suboptimal peak GH response to the ITT (3.2 μg/L). His height, weight and BMI SDS were -0.93, -1.28 and -0.76 respectively. Target and parental adjusted height SDS were -0.56 and -0.17 respectively. IGF1 concentrations were at the lower end of normal range for age and pubertal stage (207 [reference range 200-650] μg/L). No other hormone abnormalities were identified. This subject had sustained a severe TBI with evidence of a thalamic haemorrhage on CT scan. At assessment, he was found to be severely physically disabled (KOSCHI score 3b), 5.4 years post-injury. QoL-AGHDA score at assessment was 15 out of 25. A score of 11 or higher is required to qualify for adult GH therapy in the UK (NICE 2003).

Aimaretti et al (2005) have also demonstrated pituitary dysfunction in young post-pubertal subjects (aged 16-25 years) following TBI. They evaluated 23 subjects three and 12 months after mild, moderate or severe TBI. It is not clear how subjects were selected. Hypopituitarism was observed in 35% of subjects three months post-injury and in 30% at 12 months. New deficiencies emerged in three subjects between assessments and pituitary dysfunction resolved in two. Two subjects had total anterior hypopituitarism at 12 months, one who also had DI. Isolated HPA insufficiency was diagnosed in one subject on the basis

of a baseline early morning cortisol concentration less than 80 μg/L (220 nmol/L). Stimulation testing of the HPA axis was not undertaken. Two female subjects were diagnosed with central hypogonadism, one who also had severe GHD. Isolated severe GHD was seen in two subjects and defined as a peak GH response less than 9 μg/L to the GHRH-arginine test. Partial GHD was reported in two subjects (peak GH <16.5 μg/L).

In healthy individuals, GH secretion is maximal by mid-to-late puberty, levels then declining to reach 'adult concentrations' in the mid-twenties (Drake et al 2001, Clayton et al 2005). Somatic growth is completed during this transition phase with maturation of lean body mass and achievement of peak bone mass (Clayton et al 2005). There is increasing recognition that not replacing GH in deficient-individuals during this period has a negative impact on health outcomes. Adults with childhood-onset GHD have reduced BMD and LBM compared with individuals with adult-onset GHD (Attanasio et al 2002) and healthy controls (Lange et al 2005). This may be partly related to discontinuation of GH therapy once adult height has been achieved (Shalet et al 2003).

Consensus guidelines recommend re-evaluation of GH status (off GH) once linear growth is complete and continuation of GH therapy into the mid-twenties if severe GHD is confirmed (Clayton et al 2005, NICE 2003, Ho et al 2007). However, the definition of what constitutes severe GHD in the transition phase varies. NICE guidelines (2003) use the same definition as for adult GHD: a peak GH response of less than 3 μg/L during the ITT or a cross-validated GH threshold in an equivalent test. The ITT GH cut-off recommended by ESPE is 5 μg/L (Clayton et al 2005). The most recent GH Research Society consensus guidelines on adult GHD (Ho et al 2007) suggest using an ITT GH cut-off of 6 μg/L to define GHD during the transition phase. In patients with a high likelihood of GHD, an IGF1 value less than -2 SDS after four weeks off GH therapy may be considered sufficient evidence of severe GHD (Clayton et al 2005). In practice, there is wide variation in the management of GH-deficient subjects as they reach final height, with no consensus as to the efficacy and dose of GH that should be used during the transition phase.

5.2.6.1 Studies of GH therapy during transition

Several studies of GH therapy during transition have demonstrated improved body composition and increases in bone mineral content in severely GH-deficient adolescents. Vahl et al (2000) randomised 19 GH-deficient subjects aged between 16 and 26 (mean age 20) years to GH or placebo for 12 months, followed by a year of GH treatment in all. No

significant changes were observed in the GH-treated group but IGF1 levels suggested compliance problems. Total body fat increased in the placebo group with decreased IGF1 concentrations and increases in waist circumference measurements. Resumption of GH after a year resulted in significant increases in LBM. Drake et al (2003) randomised 24 subjects to GH therapy or placebo and reported a 6% increase in bone mineral content (BMC) in the treated group after 12 months. BMC remained unchanged in the untreated group. LBM also increased by 6% in treated subjects (Carroll et al 2004). The authors concluded that the changes observed with GH therapy mimicked the normal physiological accrual of bone mass and LBM in GH-sufficient individuals during the transition phase.

Shalet et al (2003) observed significant increases in BMC in subjects randomised to a high (paediatric) or low (adult) dose GH regimen for two years compared with subjects receiving no treatment. GHD was defined by a peak GH response less than 5 μg/L to stimulation testing at entry to the study. Significant gains in LBM and reduced FM were also reported in GH-treated subjects (Attanasio et al 2004). No dose-dependent effects were seen except that IGF1 concentrations were higher in the high dose GH group. By contrast, Underwood et al (2003) observed greater BMD increases in patients randomised to high rather than low dose GH therapy. However, study participants were older (adults <35 years, mean age 23.8 years) and had been off GH for longer (for at least a year, mean 5.6 years) than subjects in the previous study. Improvements in body composition were seen in both treatment groups compared with placebo but some of these benefits were later lost in the low dose GH group.

The benefits of GH therapy in transition have not been universally observed. Mauras et al (2005) found no advantage of GH therapy over placebo in terms of BMD, body composition, lipid and carbohydrate metabolism, cardiac function, muscle strength or quality of life over two years. A GH cut-off value of 5 μg/L was used to define GHD at entry to the study. Individuals who were GH-sufficient at re-testing were also followed-up, with no differences seen compared with subjects in any of the study variables. By comparison, Johannsson et al (1999) observed lower IGF1 levels, increased body fat and worse lipid profiles amongst 21 GH-deficient subjects aged between 16 and 21 years compared with 19 subjects who were GH-sufficient when re-tested and 16 healthy controls. Tauber et al (2003) reported that subjects with 'partial GHD' at re-testing had increased body fat and decreased LBM compared with GH-sufficient subjects. Partial GHD was defined as peak GH responses between 3 μg/L and 11.8 μg/L (equivalent to 20 mU/L in this study) to two provocation

tests. However, consensus guidelines (Ho et al 2007) state that partial GHD is not a well-defined clinical entity in adults.

It is unclear whether or not GH exposure during late puberty is critical to longer-term cardiovascular and related metabolic outcomes. GHD produces negative effects on cardiovascular function: directly on the heart and endothelium and indirectly via its impact on hypercoagulability, abdominal obesity, insulin resistance, serum lipid profile, atherosclerosis, decreased exercise performance, pulmonary capacity and endothelial function (Colao et al 2006). Serum IGF-1 concentrations are inversely related to the risk of congestive heart failure and ischaemic heart disease in healthy adults (Colao et al 2006). Pituitary insufficient adults have a reduced life expectancy, with a two-fold higher risk of death related to cardiovascular risk (Colao et al 2006). Yet in GH-treated individuals, a large retrospective Swedish study concluded that the overall mortality was similar to the general population, with apparent protection from myocardial infarction (Svensson et al 2004b). Colao et al (2002) reported deterioration in left ventricular mass index and cardiac function on withdrawal of GH treatment in deficient adolescents, results that were not substantiated by Mauras et al (2005). Findings in relation to the effects of GH therapy or its withdrawal on carotid artery intima media thickness (Colao et al 2005) and fibrinogen levels (Colao et al 2002) in this age group have been ambiguous.

5.2.6.2 QoL studies

As mentioned in section 1.11.2, the impact of GH on QoL status in deficient adolescents is also unclear. No QoL benefits have been observed in most studies of GH therapy in transition to date (Vahl et al 2000, Underwood et al 2003, Mauras et al 2005). However, Attanasio et al (2005) suggested that domains related to age-specific psychological problems are worse in untreated GH-deficient subjects than healthy young people, and appear to respond positively to GH therapy. Wiren et al (2001) assessed 40 subjects over a two-year period after discontinuation of GH therapy. Twenty-one were confirmed to be GH-deficient at retesting (definition not provided) and 19 were labelled GH-sufficient. Baseline QoL was more impaired in the GH-deficient group. However, there was no significant deterioration in QoL status over the two years. Anxiety scores were higher in GH-deficient subjects than GH-sufficient subjects at two years.

Stouthart et al (2003) evaluated QoL in 22 young people for 12 months after discontinuation of GH therapy. Only two thirds of the cohort (n=14) were severely GH-deficient on re-

testing, using a lower than usual GH cut-off (peak GH response to GHRH-arginine <5 μg/L). The peak GH responses of the eight subjects who were 'GH-sufficient' on re-testing were not specified. Depressive symptoms increased after six months off GH therapy and persisted at 12 months. Stouthart et al (2003) then investigated the effects of 12 months of GH therapy on nine of the original cohort (all with severe GHD) and 11 other subjects with childhood-onset GHD who had been off GH during the preceding year. Improved QoL was observed after a year of treatment. The authors concluded that discontinuation of GH therapy leads to a decrease in QoL that improves after treatment is restarted. However, the validity of this statement is questionable as less than 50% of subjects participated in both parts of the study.

Whilst the aforementioned subject in my study clearly perceived his QoL to be poor, it is difficult to know how much this was related to his borderline GHD. Yet GH therapy could still be of benefit. It has been established that peak bone mass is affected by environmental influences including physical activity (Rizzoli & Bonjour 1999). This subject was wheelchair-dependent, although he could transfer himself from this to a chair or a bed with assistance. BMD and LBM were likely to be reduced for his age and sex. Reduced peak bone mass is a major determinant of risk of osteoporosis in later life (National Osteoporosis Foundation 1998). GH therapy during the transition phase could have a positive impact on this subject's peak bone and muscle mass.

5.2.7 Summary of endocrine results

In summary, most of the abnormal endocrine findings in this study were of questionable significance. No subject had DI or central hypothyroidism. HPG function was unremarkable apart from low baseline LH concentrations in one male with probable constitutional delay of growth and puberty that was resolving. Several subjects had suboptimal cortisol responses to stimulation testing but only three of these were prescribed hydrocortisone cover during moderate or severe illness or injury. Individuals with partial ACTH deficiency are unlikely to require hydrocortisone replacement therapy on a day-to-day basis and even the rationale for corticosteroid replacement during physiological stress is based on limited evidence. Seven male subjects had sub-optimal GH responses to insulin-induced hypoglycaemia or glucagon. Six were in late pre-puberty or early puberty and the decision not to prime subjects with sex steroids prior to stimulation testing might have influenced GH response. One is now receiving GH therapy and linear growth in the other

five will remain under review. One post-pubertal male had a borderline GH response to ITT and arguments have been presented for and against GH replacement therapy in this case.

5.3 Evaluation of body composition methodology and results

5.3.1 Evaluation of body composition methodology

5.3.1.1 Use of BIA

I used BIA to measure body composition (section 1.12.2). Other methods that can be used were outlined in section 1.12.2. DXA, although accurate (Gately et al 2003) and used often in the measurement of body composition, involves a small dose of ionising radiation and cannot be used as a bedside tool. Furthermore, the cost involved in DXA scanning made it less suitable for use in this small-scale study, where comparison of body composition measurements between subjects with normal and abnormal pituitary function was very much an exploratory aim. BIA is a rapid, inexpensive, non-invasive test that can be performed at the bedside (McCarthy et al 2006b) and was felt to be more suitable in this context. It provides more accurate results than other bedside tests such as anthropometric measurements (e.g. skinfold thickness) (Tyrrell et al 2001, Gutin et al 1996).

BIA correlates well with DXA (Shaikh et al 2007, Pietrobelli et al 2003, Okasora et al 1999, Ellis et al 1996) but has a tendency to underestimate percentage fat mass (Lazzer et al 2008, Gutin et al 1996, Shaikh et al 2003). Furthermore, the difference in fat mass or percentage fat between DXA and BIA increases as weight increases (Shaikh et al 2003, Okasora et al 1999). Conversely, Okasora et al (1999) reported that in *underweight* children, bioelectrical impedance analysis appears to overestimate percentage fat. Fogelholm et al (1997) concluded that BIA could overestimate or underestimate percentage fat depending on the analyzer used and study methodology. Thus, the results of previous studies suggest that the DXA and BIA should not be used interchangeably (Gutin et al 1996, Ellis et al 1996, Lazzer et al 2008). When compared with gold standard techniques for measuring body composition such as the 4-component model (see section 5.3.1.2), the accuracy of DXA exceeds that of BIA (Wells et al 1999).

5.3.1.2 Rationale for the aggregate prediction approach

Resistance data were available for 28 subjects who underwent BIA. These values were used to determine the Resistance Index for each subject (section 3.4.3.1). I elected not to use the

RJL Systems Quantum II analyzer software to convert resistance data into values for FFM and FM. This software was developed using adult body composition data and intended for use in adult subjects. Instead, height, weight, BMI and Resistance Index values were used to determine body composition variables using age- and gender- specific equations from the literature (Wells et al 2009). These are summarised in section 3.4.3.2. All had been generated from samples of healthy children. The values obtained from these equations were combined and averaged to produce 'aggregate' estimates of TBW, FFM, FM and percentage body fat. The rationale behind this approach is explained below.

Research into the scientific basis of prediction has shown that accuracy is improved when four conditions are satisfied: (1) that many predictions based on diverse criteria are used, (2) that these predictions are independent of one another, (3) that the individual predictions are based on different underlying assumptions and (4) that these independent predictions are aggregated (Wells et al 2009). Error should be randomly distributed across the predictions and tend to cancel out. Using aggregate estimates minimises the likelihood that in the absence of any one equation being the most accurate for a given population, one with high bias is inadvertently selected.

Wells et al (2009) compared the individual results of 12 equations and the aggregate result against body composition measured by the gold standard 4-component method. Unlike 2-component approaches that rely on hydration and density constants to produce estimates of FM and FFM, the 4-component method distinguishes between the component parts of FFM, namely mineral, water and protein. It incorporates independent measurement of BMC, TBW and body density to enable estimation of FM (Gately 2002). Compared with 4-component-derived results for FM, FFM and percentage body fat, Wells et al (2009) found that aggregate predictions had lower mean biases and lower limits of agreement than individual predictions.

The 12 equations compiled by Wells et al (2009) use height and weight, BMI, age, gender, Resistance Index and skinfold thickness to determine body composition variables. As I did not measure skinfold thickness during this study, I used only nine of the 12. Fewer equations were applicable to the oldest and youngest subjects (section 3.4.3.2). These factors may have reduced the accuracy of the technique. The equations variously produced estimates of TBW, FFM, FM or percentage body fat, from which the other variables could be determined. A hydration constant was required to convert values for TBW into FFM.

5.3.1.3 Hydration constants

The hydration constant often used in adult studies is 73%. This derives from Pace and Rathbun's (1945) studies of the water content of mammalian fat-free tissue. However the hydration of FFM is not constant. Hewitt et al (1993) determined constants of 73.1% and 72.2% in pre-pubertal boys and girls respectively using a 3-component model, compared with 71% and 70.7% in young adult men and women aged 22-39. Paediatric hydration constants established in other studies are higher. Using a 4-component approach, Wells et al (1999) established hydration constants of 75.1% and 75.5% in boys and girls aged between eight and 12 years. Those reported by Boileau et al (1984) were similar: 75.1% and 76.0 in pre-pubertal boys and girls with a mean age of ten years.

In this study, I used age- and gender-specific hydration constants established by Foman et al (1982) for subjects aged between three and ten years. In fact, these values were extrapolated from data derived from three different populations of newborn babies, and children aged six-months and nine years at study. I used age- and gender-specific constants established by Lohman et al (1986) for subjects over the age of ten years. These were defined for children and young adults aged between seven and 25 years.

5.3.2 Evaluation of body composition results

GHD in children is associated with changes in BMI and body composition (section 1.12.1). I was investigating whether there were significant differences in BMI or body composition between childhood TBI survivors with abnormal GH responses to stimulation testing and those with normal responses. Adult TBI studies have reported BMI differences between subjects with normal versus abnormal pituitary function. In studies by Agha et al (2004b) and Tanriverdi et al (2006), BMI was significantly higher in GH-deficient TBI survivors than GH-sufficient subjects. Bavisetty et al (2008) observed that BMI was higher in subjects with pituitary hormone abnormalities than those without. Klose et al (2007b) also reported higher BMI values in subjects with pituitary dysfunction; all had GHD, some with additional hormone deficiencies.

In this study, I observed no significant differences in height, weight or BMI between groups with normal versus abnormal GH responses to stimulation (using a peak GH cut-off of 5 μg/L). Subjects' height, weight and BMI SDS were plotted against age at study (section 4.1.8); the results were proportional, regardless of GH status. Height SDS was noticeably

lower than weight and BMI SDS in one subject with constitutional delay of growth and puberty. Plotting Resistance Index values against age revealed no obvious difference between subjects with normal versus abnormal GH responses to stimulation. The Resistance index is related to FFM and has a linear relationship with increasing age. As discussed previously, most of the low GH responses in this study were elicited in unprimed children in late pre-puberty or early puberty and their significance is unclear. Thus, it is unsurprising that there were no obvious differences between subject groups.

Unfortunately, a lack of standardised UK reference ranges for FM and FFM limited the utility of the body composition data collected. Reference curves have been published for North American and European paediatric populations (Sala et al 2007, Faulkner et al 1996, Zanchetta et al 1995, Vab der Sluis et al 2002), however these may not be applicable to British children (McCarthy et al 2006b). FM and FFM should be adjusted for height to optimise comparisons between individuals or populations or within the same individuals or populations over time (Wells 2001). It has been suggested that using the indices of FM/HT^2 and FFM/HT^2 will in most cases be appropriate (Wells et al 2002). Suitable reference charts have yet to be developed.

UK age- and gender-specific reference curves have been established for percentage body fat using the Tanita Body Composition Analyser BC-418MA (McCarthy et al 2006b). McCarthy et al (2006b) demonstrated significant differences between UK reference data for percentage body fat and US data (Mueller et al 2004) derived from Caucasian American children of similar ages. The UK reference population comprised 1985 Caucasian children aged between five and 18 years from schools in Southern England. It has been reported that the Child Growth Foundation will publish these charts along with software to convert individual measurements to Z-scores but this has not yet occurred.

Three of my GH-sufficient subjects had percentage fat values greater than the 91st percentile, compared with four children in the group with suboptimal GH responses. Without a means of standardising FM and FFM results for age and gender, it was difficult to make further comparisons of body composition values between groups. This was compounded by the small size of the study sample. Had I collected longitudinal data, it might have been possible to compare mean change in FM, FFM or percentage body fat between groups over time.

5.4 Evaluation of quality of life data and self-reported symptoms

5.4.1 Self- or parent/guardian-reported symptoms

I compared physical and psychological symptoms between normal and abnormal pituitary hormone groups. This information was collected using a structured history-taking proforma. Irritability was more prevalent amongst subjects with normal pituitary function ($p=0.05$) and low mood tended to be a more common finding ($p=0.1$). Tiredness also featured more often amongst normal subjects, although the difference between groups was not statistically significant. Fifty-two percent of all subjects reported emotional difficulties and 53%, poor concentration. Short-term memory problems were reported in a third of subjects.

These findings may reflect a tendency for families of children with more difficulties to have volunteered to take part in the study. Aitken et al (2009) reported that caregivers are more likely to report family burden problems after TBI when child functioning is poor and health needs are unmet. However, my results are not dissimilar from other studies demonstrating a high prevalence of psychosocial and attentional difficulties after childhood TBI (Schwartz et al 2003, Anderson et al 2005b, Lehnung 2001, Verger et al 2000; Yeates et al 2005). It is not surprising, considering the doubtful significance of the pituitary hormone abnormalities encountered, that symptoms were not more prevalent in this group. The higher prevalence of several symptoms amongst normal subjects may reflect small sample bias.

5.4.2 The SDQ and alternative measures

I used the SDQ to evaluate behaviour and as a screening tool for mental health difficulties. The self-report version was completed by children aged 11 to 15 years, and one 16 year-old (Goodman et al 1998). There are two parent-report versions: one for parents of five to 16 year-olds (Goodman et al 1997) and an amended version for children aged three or four. The SDQ can be used to screen for hyperactivity disorders, depression, conduct disorders and some anxiety disorders (Goodman et al 2003). Sensitivity for specific phobias, panic disorder, eating disorders and separation anxiety is poor. Sensitivity is better using multi-informant rather than single-informant SDQs (Goodman et al 2003).

Twenty-six parents completed parent-report SDQs. As indicated in the previous section (5.4.1), emotional and behavioural difficulties were common across the whole sample. Fifty-four percent reported their children to be hyperactive and 46% described them as

having conduct problems. Thirty-one percent felt that their children had emotional difficulties. There was good correlation between parent- and self-reported SDQ scores for conduct and hyperactivity sub-scales and total overall difficulties. Fewer children identified themselves as having emotional symptoms. There were no significant differences in reported prevalence of difficulties between normal and abnormal pituitary hormone groups. Pro-social behaviour was preserved in the majority of subjects.

The most widely used alternative to the parent-report version of the SDQ is the Child Behaviour Checklist (Achenbach 1991). The latter is significantly longer. Several items in the SDQ are phrased positively to increase the questionnaire's acceptability to respondents (Goodman & Scott 1999). Goodman & Scott (1999) demonstrated that the two questionnaires were equally able to discriminate high risk from low risk cases. The SDQ was significantly better than the Child Behaviour Checklist at detecting inattention and hyperactivity. Further studies have confirmed the reliability and validity of the SDQ as a screening measure (Goodman 2001). However, Mellor (2004) reported that the internal reliability of the subscales was questionable for the self-report questionnaire. In spite of this, he suggested that the age range for the self-report SDQ could be extended 'cautiously' to include subjects between seven and 17 years. Kaptein et al (2008) suggested that the SDQ could be used as a screening tool for behaviour and mental health difficulties in children with learning disability.

5.4.3 Evaluation of QoL status in younger children

The self-report KIDSCREEN-52 questionnaire was used to evaluate QoL in children aged between eight and 16 years and in one 16 year-old (Ravens-Sieberer et al 2005). It has been validated in children aged between eight and 18 years. The KIDSCREEN-52 tool is a generic QoL measure; as yet, no questionnaire specific to children with hypopituitarism has been developed. I elected not to employ the parent-report version of the questionnaire, as I was primarily interested in subjects' perspectives regarding their QoL status. Parent-report SDQ data gave some indication of how parents felt their children were functioning (see section 5.4.2).

Twenty-four subjects completed the KIDSCREEN-52 questionnaire. Raw scores were converted into gender- and age-specific percentiles and T-scores for each of ten subscales. Low scores were more common in subjects with normal pituitary function. However, differences between normal and abnormal pituitary hormone groups were not statistically

significant. There were trends towards lower psychological wellbeing and lower satisfaction at school amongst subjects with normal pituitary function. Five normal subjects had T-scores more than -1 SD below the mean in three or more subscales compared with only one subject in the abnormal hormone group (p=0.2). Four normal subjects had scores below the 15th centile in three or more subscales compared with only one subject in the abnormal hormone group (p=0.3).

One of the disadvantages of the KIDSCREEN-52 questionnaire is that there are no reference ranges for the total score across all ten subscales. Probably the most suitable alternative to the KIDSCREEN-52 measure would have been the PedsQL questionnaire (Varni et al 2001). The generic component of the Peds QL contains only 23 items (grouped into physical, psychological, social and school functioning modules). However, extra modules can be added that evaluate worry (3 items), cognitive problems (7 items) and feelings about physical appearance (4 items).

I used KIDSCREEN-52 measure because it provided a more detailed assessment than the PedsQL. However, there is some experience of using the PedsQL in TBI follow-up studies (section 1.11.1). It has been shown to discriminate better than the Behaviour Rating Inventory of Executive Function (BRIEF) amongst survivors of childhood TBI (McCarthy et al 2005). The PedsQL can also be used in children aged five years and older. There is some evidence of KOSCHI score at discharge correlating with PedsQL score within the first year after TBI (Calvert et al 2008). It has been suggested that the PedsQL 4.0 generic core scale measure could be used as a routine outcome measure for paediatric trauma patients (Willis et al 2006).

5.4.4 Evaluation of QoL status in young people

All but one subject aged 16 years or over completed the QoL-AGHDA and QLS-H questionnaires. Both have been validated in adults with GHD but not in young people less than 18 years of age. It was unfortunate not to have been able to use the SF-36 questionnaire as planned. This generic QoL assessment tool has been validated for use in young people aged 14 years and over. However, the cost of the licence and analysis software precluded its use. Thus, only disease-specific QoL tools were employed amongst older participants.

The QLS-H questionnaire was developed using data from over 8,000 adults representative of the general population. It was validated using data from 957 GH-deficient adults taking part

in five clinical trials across seven different countries (Blum et al 2003). The average ratio of adult-onset to childhood-onset GHD across these trials was 3:1. The mean age at participation ranged from 39.6 years to 47.9 years. Thus, the QLS-H questionnaire may preferentially detect QoL issues in patients with adult-onset GHD and who are older than the subjects in my study. QLS-H raw scores were generally comparable between subjects with normal and abnormal pituitary hormone function. I elected to use raw score data alone because Z-scores can only be generated for subjects aged 18 years or over.

The QoL-AGHDA questionnaire was developed using material from in-depth interviews with 35 GH-deficient adults, 16 (46%) with childhood-onset GHD. The ages of the childhood-onset GHD subjects ranged from 20 to 35 years. There were obvious QoL differences between subjects with childhood-onset GHD and those with adult-onset disease. The instrument was validated in GH-deficient adults from five European countries (McKenna et al 1999). There was again a predominance of subjects with adult-onset GHD but the mean age range was slightly lower across participating sites than for the QLS-H (31.1-46.9 years). In my study, the QoL-AGHDA score was noticeably higher (15 out of 25) in one male with borderline (adult) GHD than in normal subjects or one subject with a suboptimal peak cortisol response to the ITT. A score greater than 11 is required to qualify for adult GH therapy in the UK (NICE 2003). Koltowska-Hallstrom et al (2005) reported mean-weighted QoL-AGHDA scores of 6.2 and 7.1 amongst healthy British men and women, compared with 13.6 and 15.7 for GH-deficient adults.

Several adult studies have investigated QoL outcomes in TBI survivors with hypopituitarism. These are described in more detail in the Introduction chapter (section 1.11.3). Wachter et al (2009) found no association between QoL and pituitary insufficiency. Similarly, Agha et al (2004b) observed no difference between GH-deficient and -sufficient subjects in terms of Qol-AGHDA score. However, Bavisetty et al (2008) reported a higher rate of depression and poorer QoL amongst GH-deficient TBI survivors than subjects with normal pituitary function. Klose et al (2007c) observed reduced QoL in subjects with hypopituitarism than in TBI subjects with normal pituitary function, or controls. Aimaretti et al (2005) evaluated neuroendocrine sequelae in 16 to 25 year-old survivors of TBI but did not examine the effects of pituitary dysfunction on QoL in this age group.

5.4.5 General criticisms of QoL methodology

Our study cohort was too small and the significance of pituitary abnormalities observed too ambiguous to comprehensively investigate the effects of pituitary dysfunction on QoL after TBI. The use of different assessment tools for different age groups further limited interpretation of QoL results. The study was too small to be able to evaluate gender differences in QoL outcomes. A better way of evaluating QoL might have been to investigate a larger sample of similarly aged children using the same measure(s) in all subjects. Collecting healthy control data would have helped to determine the significance of any QoL differences observed between TBI survivors with normal or abnormal pituitary function.

I evaluated self-reported QoL status. It is recommended that the perspective of the person under study be sought in QoL research (Ravens-Sieberer et al 2002). However, TBI survivors may lack insight into their difficulties, particularly if they have sustained cerebral lesions involving the frontal lobe regions (Klose et al 2007c). Stancin et al (2002) suggested that adolescents might minimise concerns about their own health and functioning, leading to under-recognition of poor QoL status. I observed good correlation between self- and parent-reported SDQ scores.

In this retrospective study, I had no means of measuring QoL status prior to TBI or development of pituitary dysfunction. McCarthy et al (2006a) reported pre-existing attentional problems in 12% of TBI subjects, learning disability in 11% and behavioural problems in 7%. Pre-morbid difficulties are associated with poor QoL scores after TBI (McCarthy et al 2006a, Stancin et al 2002). Longitudinal evaluation of QoL status may have enabled clearer associations to be made between emergence of pituitary dysfunction and its effects on QoL. The perception of a decline in QoL status may take months or years to emerge, particularly if baseline QoL is relatively high (Wiren et al 2001).

QoL was not assessed in either survivor of suspected NAHI due to their respective ages. Few, if any, QoL measures are applicable to children under five years. However, no significant emotional or behavioural problems were identified using the parent-report version of the SDQ (version for 3-4 year olds). This contrasts with follow-up studies demonstrating behavioural problems in as many as 52% of such children (Barlow et al 2005, see also

section 1.10.1). The post-injury environment may influence outcome (Barlow et al 2005). My sample was too small to draw any conclusions in this area.

5.5 Comparison of head injury characteristics between groups

I reviewed subjects' case records to determine whether there were any associations between the severity of injury, or the nature of the primary or secondary brain injury, and the emergence of pituitary hormone abnormalities. There were a variety of causes of TBI across both normal and abnormal pituitary hormone groups although no subjects with endocrine abnormalities had been injured as car passengers. I found no significant differences between groups in terms of mean age at injury or time since injury.

5.5.1 Severity of injury

Post-traumatic pituitary dysfunction in adults is associated with all grades of TBI severity. Although Bondanelli et al (2004) observed an association with severity of injury this has not been substantiated by other adult TBI studies (Agha et al 2004b, Tanriverdi et al 2006, Klose et al 2007a). However, DI may be more common after severe TBI (Agha et al 2004b). Niederland et al (2007) found no link between severity of childhood TBI and development of pituitary dysfunction. Einaudi et al (2006) evaluated 20 children at baseline, six and 12 months post-TBI, detecting abnormalities of the HPA or GH axes in two subjects, both survivors of minor TBI. Poomthavorn et al (2008) only investigated survivors of severe childhood TBI. I observed no significant difference between normal and abnormal pituitary hormone groups in terms of severity of TBI according to GCS classification. Nine of 12 survivors of severe TBI had normal endocrine function.

Full evaluation of pituitary function is intensive and should not be undertaken without just cause. The decision to include survivors of minor TBI in this study requires further justification. The vast majority of closed head injuries in children are of mild severity (Brookes et al 1990, Hawley et al 2003) yet only a minority may be at risk of endocrine complications. Studies have concluded that minor TBI has little impact on intellectual, neuropsychological, academic or health-related QoL outcomes in children (Fay et al 1993, Petersen et al 2008). However, Hawley et al (2004) observed that 43% of children admitted to hospital with minor head injuries had KOSCHI scores of 4a or 4b on follow-up (4b in most), consistent with a moderate level of disability. They found no evidence to suggest a threshold of injury severity below which the risk of late sequalae could be discounted.

I therefore felt that it was important not to exclude children from my study purely on the basis of high GCS scores. I evaluated six children with post-resuscitation/pre-intubation GCS scores between 13 and 15. All required intubation and admission to intensive care. Four had extradural haemorrhages, which were evacuated in three cases due to midline shift on CT scan. Two others had relatively minor brain injuries but other serious multi-system injuries with attendant hypoxia and/or hypotension.

Pituitary hormone abnormalities were seen in two of six children with minor TBI according to GCS classification, both males. Both had sustained extradural haemorrhages and were ventilated, although only one required neurosurgery. Pre-intubation GCS scores were 13 and 14. One subject was in late pre-puberty and had a suboptimal peak GH response to stimulation testing without prior priming with sex steroids (3.9 μg/L; cut-off 5 μg/L). The other had a suboptimal peak cortisol response to insulin-induced hypoglycaemia (458 nmol/L; cut-off 470 nmol/L in children ≥10 years) that was not considered low enough to require hydrocortisone cover during illness. Thus, in this small cohort, I found little evidence for the risk of signficant pituitary dysfunction following minor TBI in children.

5.5.1.1 Other measures of TBI severity

There was no significant difference in median ISS between normal and abnormal pituitary hormone groups. As described in section 3.1.1, the ISS is calculated by squaring the highest AIS scores for the three most severely injured body regions and adding these together (Baker et al, 1974). An alternative version of this scoring system is the New Injury Severity Score (NISS; Osler et al 1997). Squaring the three highest AIS scores in *any* body region and adding these together produces the NISS. Thus, in patients with multiple head injuries but few other body injuries, there is the potential for scoring a higher NISS than ISS. Studies comparing the two instruments in children have conflicted in their results. Grisoni et al (2001) reported that the NISS was no more accurate than the ISS in predicting mortality after injury. These findings were echoed in a study looking at the outcome after childhood falls (Bulut et al 2006). However, Sullivan et al (2003) found that the NISS performed as well as the ISS in pediatric patients with lower injury severity and outperformed the ISS in those with higher injury severity. Mortality, functional outcome, and discharge disposition were all endpoints evaluated in this large study.

I observed no correlation between pituitary hormone abnormalities and median PTS, RTS or TRISS scores. Scores could not be calculated for several subjects due to inadequate recording of data at the time of TBI admission. These scoring systems were developed to estimate the likelihood of mortality after trauma. The RTS and the TRISS were developed for use in adults; thus scores for variables like blood pressure and respiratory rate derive from reference ranges that are not applicable to young children. Their use in this study reflects the paucity of predictive physiological scoring systems appropriate for use in injured children of all ages. The Marshall scoring system is also based on observations in adult patients. However, it remains the main CT scoring system used for patients of all ages. There was no significant association between a Marshall score greater than one and post-traumatic pituitary dysfunction.

I did not investigate possible correlations between duration of post-traumatic amnesia and pituitary function. The former would have been impossible to measure retrospectively. It is defined as the period of time following emergence from coma during which the patient cannot recall information and/or store new memories. Duration of post-traumatic amnesia is considered a good predictor of outcome after TBI (McDonald et al 1994, Katz & Alexander 1994).

5.5.2 Primary brain injury

No subject in this study had evidence of hypothalamo-pituitary trauma on CT scan. Unlike Poomthavorn et al (2008) and contrary to expectation, I observed no association between basal skull fracture and development of pituitary dysfunction. Yet, in their recent metanalysis of adult TBI studies, Schneider et al (2007) concluded that there should be a low threshold for evaluation of the pituitary following basal skull fracture. I hypothesised that large contusions or extra-axial bleeds might be associated with pituitary dysfunction (as a consequence of contra-coup mechanism of injury, ensuing oedema or raised ICP). However, no significant differences were observed between groups with normal versus abnormal pituitary function. Borderline (adult) GHD was observed in one post-pubertal male as discussed in section 5.2.6. This subject had a brain stem lesion (thalamic haemorrhage) on CT scans taken during his head injury admission. No other subjects had brain stem injuries.

Springer and Chollet (2001) described a case of post-traumatic hypopituitarism in a man with frontal lobe injuries. I hypothesised that subjects with contra-coup frontal lobe injuries might be at risk of pituitary stalk injury via the contra-coup mechanism. Frontal lobe

injuries might themselves have an indirect effect on pituitary function: animal studies have suggested that the prefrontal cortex plays a role in the negative feedback regulation of the HPA axis (Diorio et al 1993). Tchiteya et al (2003) observed an association between frontal lobe injury and HPA axis dysregulation in a small group of adult subjects. However, no association was observed between frontal lobe injury and pituitary dysfunction in this study. Schneider et al (2007) suggested that patients with DAI might be at higher risk of post-traumatic hypopituitarism, however both subjects with DAI in my study had normal pituitary function.

5.5.3 Secondary brain injury

Kelly et al have demonstrated a relationship between diffuse brain swelling and emergence of pituitary dysfunction in two separate adult TBI studies (Kelly et al 2000, Bavisetty et al 2008). However, I found no association between oedema (± midline shift) and pituitary hormone abnormalities in this study. Generalised oedema is more common in children than adults after TBI but may follow a less severe course (Blumbergs 2005b). In my study, only two subjects had Marshall scores of 3 (n=2) or 4 (n=0), which indicate severe diffuse brain swelling. Midline shift was usually associated with extra-axial haemorrhages that were evacuated.

Klose et al (2007b) observed an association between raised ICP and the occurrence of pituitary dysfunction following TBI. Schneider et al (2007) concluded that raised ICP might be a risk factor for pituitary dysfunction from a metanalysis of adult TBI studies. I had planned to compare the duration and intensity of deranged ICP and CPP between normal and abnormal pituitary hormone groups, using the PTI to quantify the burden of secondary brain insult. However, only 16 subjects had ICP monitoring and physiological monitoring data were only retrievable in 12 of these, precluding any statistical comparisons. No trends were identifiable between groups.

5.5.4 Functional outcome after TBI

Where available, I documented KOSCHI score at six months post-TBI as a measure of outcome. A uniform time point was chosen to increase the accuracy of comparison between subjects. I was interested to see whether there were significant differences in outcome between normal and abnormal pituitary hormone groups. I also wanted to evaluate whether there had been any deterioration in KOSCHI score between the 6-month time point and study assessment. It was hypothesised that deterioration in outcome score might be related

to underlying pituitary dysfunction. Unfortunately, the 6-month KOSCHI score was only documented in or discernible from the follow-up records of 19 of 33 subjects. The rest either received no follow-up or were not followed-up locally, thus precluding any statistical evaluation.

KOSCHI scores were documented at study assessment and demonstrated a similar prevalence of significant disability across normal and abnormal pituitary hormone groups. A significant number of participants scored a KOSCHI score of 4a or 4b, both denoting moderate disability. This may reflect sample bias and a tendency for parents of children with residual problems to have put them forward for the study (McCullagh & Feinstein 2003, Aitken et al 2009). However, Hawley et al (2004) also reported moderate disability in significant numbers of childhood TBI survivors. This was a retrospective study in which there were responses from only 526 of 974 families invited to take part. At a mean of 2.2 years post-injury, moderate disability was reported in 43% after minor TBI (with admission to hospital), 64% after moderate TBI and 69% after severe TBI. The classification of moderate disability was usually applied to children with learning, behavioural or neurological sequelae affecting function, much like in my cohort. The authors of the KOSCHI emphasise that seemingly minor residual deficits are potentially much more damaging in children than adults, particularly in relation to social and learning behaviour (Crouchman et al 2001).

Crouchman et al (2001) acknowledge the limitations of using a short uni-dimensional scale to assess outcome. An alternative quick outcome measure, the modified Glasgow Outcome Score (GOS; Adelson et al 1997), is even less descriptive than the KOSCHI. Both scoring systems derive from the GOS, the predictive value of which has been questioned in children (Willis et al 2006). Further validation of the KOSCHI is required and evaluation of correlation with measures of TBI severity like the GCS. A recent study concluded that the KOSCHI scored at hospital discharge correlated with injury severity and some cognitive, health status, and QoL outcomes early after TBI but was not helpful at predicting later difficulties, or behavioural and emotional problems (Calvert et al 2008). I had no way of evaluating the impact of home environment on outcome. Taylor et al (1999) suggested that TBI outcomes are worse in families with higher levels of dysfunction.

5.5.5 NAHI group

Both survivors of suspected NAHI who participated in this study had moderate TBI using the GCS classification. The GCS can be difficult to apply accurately in infancy (Barlow et al 2005). Both subjects had evidence of bilateral subdural haemorrhages on imaging at the time of injury. One presented with early post-traumatic seizures (EPTS) and was moderately disabled at assessment (KOSCHI score 4a) with visual impairment and mild developmental delay. Barlow et al (2000) demonstrated that neurodevelopmental outcomes after NAHI correlate significantly with the presence and severity of EPTS. The other subject had a good outcome (KOSCHI score 5a).

Cumulative literature sources show that severe and moderate disability result in 34% and 25% of NAHI survivors respectively (Minns et al 2005). NAHI has been associated with worse outcomes than accidental TBI (Goldstein et al 1993, Keenan et al 2006a). Compared with infants with non-inflicted TBI, those with NAHI experience more frequent non-contact (rotational) injury mechanisms that result in deeper brain injuries and more frequent bilateral hypoxic-ischaemic brain injury (Hymel et al 2007). They present with lower GCS scores, experience more frequent and prolonged impairment of consciousness and more frequently manifest acute cardiorespiratory compromise (Hymel et al 2007). My sample included no children who presented with severe TBI due to suspected NAHI and, comprising only two subjects, was unlikely to be representative of NAHI survivors as a whole. Pituitary function was normal in both. I was unable to comment on factors affecting the likelihood of pituitary dysfunction following NAHI due to the size of the sample.

5.6 Summary and future studies

I evaluated 35 survivors of childhood TBI in this retrospective observational pilot study. Subjects underwent anthropometric and body composition measurements, evaluation of pituitary gland function and assessment of QoL status. Pituitary hormone abnormalities were detected in 13 of 33 survivors of accidental TBI (39%) and neither of two survivors of suspected NAHI. Endocrine abnormalities were of doubtful clinical significance. Abnormal findings were unrelated to age at injury, severity of TBI, type of primary or secondary brain injury or KOSCHI rating. There were no significant differences in anthropometric measurements or percentage body fat between normal and abnormal pituitary hormone groups. There were no associations between pituitary dysfunction and poorer QoL status in

subjects aged less than 16 years. QoL was poorer in one post-pubertal male with GHD than in other subjects aged ≥16 years.

Our results need to be validated in a larger sample. The natural history of pituitary dysfunction occurring after childhood TBI also needs further evaluation. Thus far, only one prospective study has investigated neuroendocrine sequelae following childhood TBI (Einaudi et al 2006). Thirty subjects underwent basal hormone evaluation within 72 hours of TBI, with follow-up in 26 and 20 subjects at six and 12 months post-injury respectively. Retention of subjects is clearly an issue. Conducting a multi-centre study would facilitate recruitment of more subjects but would require standardisation of investigations used and collection of comparative healthy control data in each centre. In particular, there would need to be agreement regarding dynamic testing of the HPA and GH axes and a consensus on sex steroid priming. Priming of pre- or early-pubertal children would be preferable prior to GH stimulation testing. Recruitment of healthy control subjects might be considered more acceptable if they were not subjected to stimulation tests. However, this would limit evaluation of the GH and HPA axes.

A prospective study would enable longitudinal evaluation of anthropometric, body composition and QoL data. It would be possible to detect intra-individual differences as well as comparing differences between groups of subjects with normal or abnormal pituitary function. In the absence of suitable age- and gender-specific reference ranges for body composition variables, subjects could be recruited within a narrower age band, with sex-matching. Alternatively, subjects and healthy control subjects spanning a wider range of ages could be stratified by age and sex, which would necessitate a much larger study. These strategies would facilitate more accurate comparisons between groups. It might also be preferable to use the Tanita Body Composition Analyzer over other models, as preliminary UK reference ranges for percentage body fat derive from studies using this analyzer.

Recruiting subjects within a narrower age band would reduce the need to use different QoL measures for different age groups. It might be preferable to use the PedsQL measure over the KIDSCREEN-52 questionnaire as there is more experience of its use in TBI outcome studies. In this study, I evaluated QoL solely from the perspective of the subjects but it might have been useful to have parent-reported QoL data for comparison. My findings agree with those of other studies that suggest that there is a high prevalence of emotional and behavioural difficulties amongst childhood TBI survivors. There have been too few studies

evaluating QoL in GH-deficient children to conclude that QoL is not impaired in childhood GHD. The lack of a validated QoL measure specific to children with GHD is an issue in this respect.

A larger study would be required to determine and confirm any associations between post-traumatic pituitary dysfunction and head injury characteristics. In my study, there were no significant differences in severity of TBI or type of primary brain injury between normal and abnormal pituitary hormone groups. There was no association between post-traumatic seizures or the presence of cerebral oedema (± midline shift) on CT scan and pituitary dysfunction. I was unable to make more detailed comparisons of the severity of secondary brain insult between groups: many subjects did not undergo invasive ICP monitoring for any significant length of time, if at all. This issue could again be addressed by narrowing eligibility criteria. However, too few children might then be eligible to take part. The need to optimise recruitment must be balanced against the likelihood of obtaining meaningful results. Prospective longitudinal follow-up would facilitate evaluation of KOSCHI rating at comparable time points and identify intra-individual changes in outcome over time.

I was unable to properly evaluate the risk of pituitary dysfunction occurring after suspected NAHI due to inadequate recruitment. Many children are followed-up by neurologists or community paediatricians after TBI. Survivors of NAHI may be more likely to participate in a study if perceived to be part of routine follow-up. This could be facilitated through collaboration with colleagues from other disciplines.

Chapter 6: Conclusions

I conducted a retrospective observational pilot study to determine the prevalence and clinical significance of pituitary dysfunction following moderate or severe childhood TBI, or minor TBI with an ISS ≥16. Thirty-five subjects took part in the study, from a cohort of 151 eligible TBI survivors. I evaluated 33 of 102 (32%) accidental TBI survivors invited to take part. Whilst the recruitment rate was relatively low, the sample recruited was representative of the wider accidental TBI cohort. I recruited only two of 12 invited subjects with a history of suspected NAHI (17%), highlighting the challenges of conducting research among this group.

Subjects were assessed at between 1.4 and 7.8 years following TBI. They underwent anthropometric and body composition measurements, comprehensive evaluation of pituitary gland function and assessment of QoL status. Head injury characteristics were compared between subjects with normal endocrine function and those with pituitary hormone abnormalities. In all, pituitary hormone abnormalities were detected in 13 of 33 survivors of accidental TBI (39%). Suboptimal GH responses to stimulation were seen in seven subjects, abnormalities of the HPA axis in nine and prolactin deficiency in one. Multiple abnormalities were seen in four of 33 subjects (12%). In each case, two hormone axes were affected: GH and HPA axes in three, and prolactin and HPA axes in the fourth. Both survivors of suspected NAHI had normal endocrine function.

Preliminary studies have reported long-term pituitary dysfunction in between 10% and 61% of childhood TBI survivors. The study reporting the highest prevalence of pituitary insufficiency (Neiderland et al 2007) provided the least information about methods of patient selection, cut-offs used to define HPA insufficiency and it was unclear whether subjects were primed with sex steroids. Critical evaluation and interpretation of results, where possible in relation to locally validated assays and age- and pubertal stage-related reference ranges, is important in determining the likely clinical significance of abnormalities detected. Whilst the prevalence of pituitary 'dysfunction' in my study was high, many of the abnormalities seen were of doubtful significance.

Stimulation tests are difficult to standardise, may give false positive or false negative results and the individual response is variable. I used local evidence-based cut-offs for peak GH and cortisol responses to the ITT. However, I did not prime peri-pubertal subjects and 'normal' physiological blunting of GH secretion may explain the low GH responses to

stimulation testing observed in six pre- or early-pubertal subjects during my study. Only one of the six had inappropriately slow growth on follow-up; this subject is now receiving GH therapy. It will be important to keep all six under review. Peak GH response to the ITT was also borderline low in one post-pubertal male (according to the NICE cut-off used to define adult or transitional GHD). Although his QoL-AGHDA score was high, it is not clear whether treatment with GH would be beneficial in this case. Further evaluation of this subject's wellbeing, BMD and cardiovascular status are required. Greater clarification is required in this age group in terms of criteria for GH replacement, GH dosing and aims of treatment.

There were suboptimal peak cortisol responses to glucagon or ITT in one and eight subjects respectively. These results are of uncertain clinical significance and are difficult to interpret in the absence of data from healthy controls; physiological and pathological cortisol responses may overlap within a biological continuum. No clear guidelines exist for treatment or retesting. Three subjects were prescribed hydrocortisone cover during moderate or severe illness or injury; there is no evidence that regular hydrocortisone therapy is required in partial ACTH insufficiency. Glucagon gives a variable stimulus for cortisol and I would have elected to evaluate the HPA axis further in one subject who had a flat cortisol response rather than commence treatment. However, this subject cancelled his follow-up appointment. Lastly, prolactin deficiency observed in one male subject was of uncertain clinical significance.

Adult consensus guidelines recommend performing basal endocrine investigations at three and 12 months post-TBI (Ghigo et al 2005). More recent guidelines suggest performing dynamic testing of the HPA axis at between three and six months (Behan et al 2008) and of the GH axis at 12 months post-injury (Ho et al 2007, Behan et al 2008). Whilst basal screening may be justified, I do not feel that the findings in my study justify a recommendation to perform dynamic testing in childhood TBI survivors routinely. These results need to be validated in a larger sample. The natural history of pituitary dysfunction occurring after childhood TBI also needs further evaluation. Clarification is required of risk factors for and time to development of pituitary dysfunction, and of the likelihood of abnormalities detected being permanent.

I observed no significant differences in anthropometric measurements or QoL status between normal and abnormal pituitary hormone groups. There were trends towards poorer psychological wellbeing and lower satisfaction at school amongst subjects with normal

pituitary function. Again, these results are difficult to interpret without comparative data from healthy controls. It was not possible to perform statistical analyses comparing body composition variables between groups. A prospective study including heathy control subjects would enable more accurate, longitudinal evaluation of anthropometric, body composition and QoL data.

A larger study would be required to determine and confirm any associations between post-traumatic pituitary dysfunction and head injury characteristics. In this study, there were no significant differences in TBI severity or type of primary or secondary brain injury between normal and abnormal pituitary hormone groups. I was unable to make detailed comparisons of the severity of secondary brain insult between groups: many subjects did not undergo invasive ICP monitoring for any significant length of time, if at all. I was unable to properly evaluate the risk of pituitary dysfunction occurring after suspected NAHI due to inadequate recruitment.

In conclusion, mild pituitary 'dysfunction' was common (39%) amongst survivors of accidental childhood TBI evaluated at between 1.4 and 7.8 years post-injury. However, no unequivocal clinically significant endocrinopathies were found, although the GH and HPA axes may be vulnerable. Prospective longitudinal studies are required.

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Appendix 1: Local laboratory information regarding hormone assays used

Local laboratory information about assay quality control, lower limit of detection and cross-reactivity is provided below. Data relating to detectable cross-reactivity has been proritised over non-detectable. Information about the GH reference standard used is also provided.

1. GH (Immulite 2000 analyzer (Siemens Healthcare Diagnostics UK))

Lower limit of detection: 0.1 μg/L

Quality control:

Biorad Lyphocheck Lot 40221/2/3 (reported in terms of WHO NIBSC IS 98/574)

Pool	Target	No	Mean	Sd	Bias %	CV%	PCV%
lypho 1	3.16	23	3.01	0.16	-4.7	5.1	4.0
lypho 2	6.76	22	6.57	0.27	-2.8	4.2	6.6
lypho 3	13.9	22	13.4	0.60	-3.6	4.5	3.7

Cross reactivity:

- 0.001% cross-reactivity with FSH at a concentration of 10,000 ng/mL
- 0.03% cross-reactivity with human placental lactogen at a concentration of 100 ng/mL
- 0.5% cross-reactivity with LH at a concentration of 3,663 ng/mL
- 0.01% cross-reactivity with TSH at a concentration of 11,125 ng/mL

GH standard: Until 2008, a GH standard traceable to the previous GH international reference preparation 80/505 was used (values assigned in mU/L only). In 2008, the Royal Infirmary of Edinburgh GH assay was re-standardised using a standard traceable to the new recombinant international reference preparation 98/574 with values assigned in both mU/L and ug/L (mU/L = 3 x ug/L). This was part of the UK-wide switch to the new standard, reporting in ug/L. There was no significant shift in mU/L values following re-standardisation. A summary of the evidence on which this judgment was based is presented in Appendix 2A.

2. Free T4 (Architect analyzer (Abbott Diagnostics, Maidenhead, UK)).

Lower limit of detection: 5 pmol/L

Quality control:

Biorad lyphochek. lot 40221/2

CTRL	TARGET	NO	MEAN	SD	BIAS%	CV	PCV
LY 1	13.2	108	12.9	0.5	-2.3	3.8	3.8
LY 2	28.0	98	27.6	1.0	-3.6	3.7	3.7

Cross reactivity: Mean analytical specificity of $\leq 0.0035\%$ cross reactivity with triiodothyronine (T3) at a concentration of 12,000 ng/dL in a sample containing 0.5 ng/dL of Free T4.

3. TSH (Architect analyzer (Abbott Diagnostics, Maidenhead, UK)).

Lower limit of detection: 0.01 mU/L

Quality control:

Biorad lyphochek. lot 40221/2

CTRL	TARGET	NO	MEAN	SD	BIAS%	CV	PCV
LY 1	0.557	111	0.546	0.018	-2.0	3.3	3.3
LY 2	5.36	102	5.32	0.14	-0.7	2.7	3.2

Cross reactivity: Analytical specificity of $< 10\%$ cross reactivity with the following substances, at the concentration levels listed, in human serum samples containing TSH in the normal range:

- FSH < 500 mIU/mL
- LH < 500 mIU/mL
- hCG $< 200{,}000$ mIU/mL

4. Oestradiol (Architect analyzer (Abbott Diagnostics, Maidenhead, UK)).

Lower limit of detection: 50 pmol/L

Quality control:

Abbott Multiconstituent Control Lot 95331/2/3

CTRL	TARGET	NO	MEAN	SD	BIAS%	CV	PCV
LOW	134	70	118	16.9	-11.9	14.0	11.3
MED	981	67	954	25.0	-2.8	2.6	2.6
HIGH	1912	72	1832	60.6	-4.2	3.3	3.7

Cross reactivity: Synthetic specimens containing essentially no residual estradiol were supplemented with potential cross reactants at the concentrations listed and tested for estradiol. There was 0.1% cross reactivity with 17β-Estradiol 3-sulfate at a concentration of 50 ng/mL and 0.7% crossreactivity with Estrone at a concentration of 1500 pg/mL.

5. Testosterone (Centaur analyzer (Siemens Healthcare Diagnostics UK)).

Lower limit of detection: 0.4 nmol/L

Quality control:

Biorad lyphochek. lot 40221/2/3

CTRL	TARGET	NO	MEAN	SD	BIAS%	CV	PCV
LY 1	5.2	71	5.3	0.3	+1.9	5.3	5.3
LY 2	17.5	84	16.8	1.7	-4.0	10.3	7.8
LY 3	31.2	94	32.6	2.9	+4.5	8.9	7.8

Cross reactivity: The following compounds were spiked into Multi-Diluent 3 and the following results were obtained:

- 5.4% cross-reactivity with 5α-dihydrotestosterone at a concentration of 100 ng/mL
- 0.94% cross-reactivity with androstenedione at a concentration of 100 ng/mL
- 0.68% cross-reactivity with methytestosterone at a concentration of 100 ng/mL
- 0.02% cross-reactivity with estradiol-17β at a concentration of 100 ng/mL

6. Prolactin (Centaur analyzer (Siemens Healthcare Diagnostics UK)).

Lower limit of detection: 50 mU/L

Quality control:

Biorad Lyphochek lot 40221/2/3

CTRL	TARGET	NO	MEAN	SD	BIAS%	CV	PCV
LY1	105	48	104	3.4	-1.0	3.3	3.6
LY2	312	53	302	17.7	-3.2	5.9	4.3
LY3	631	56	610	29.4	-3.3	4.8	4.2

Cross reactivity: The cross-reactivity of the ADVIA Centaur Prolactin assay with TSH, LH, hCG, FSH, hGH, and hPL was determined by adding these hormones to serum samples containing prolactin. The level of prolactin in the samples was then determined (see table below).

	Prolactin value without cross-reactant		*Prolactin value with cross-reactant*	
Cross-reactant	*(ng/mL)*	*(µIU/mL)*	*(ng/mL)*	*(µIU/mL)*
TSH; 1000 µIU/mL	8.16	173	8.55	181
	42.82	908	43.40	920
	109.68	2325	107.30	2275
LH; 250 mIU/mL	8.16	173	8.84	187
	42.82	908	43.13	914
	109.68	2325	104.88	2223
hCG; 200,000 mIU/mL	8.16	173	8.30	176
	42.82	908	40.28	854
	109.68	2325	109.60	2324
FSH; 250 mIU/mL	8.16	173	8.40	178
	42.82	908	42.66	904
	110.08	2325	105.05	2227
hGH; 500 ng/mL	4.51	96	4.56	97
	42.82	908	42.22	895
	109.68	2325	108.22	2294
hPL; 12.5 µg/mL	2.50	53	2.51	53
	35.13	745	36.40	772
	88.67	1880	88.18	1869

7. FSH (Centaur analyzer (Siemens Healthcare Diagnostics UK)).

Lower limit of detection: 0.5 U/L

Quality control:

Biorad Lyphochek lot 40221/2/3

CTRL	TARGET	NO	MEAN	SD	BIAS%	CV	PCV
LY1	13.3	56	13.0	0.5	-2.3	4.0	3.6
LY2	34.1	66	33.8	1.3	-0.9	3.9	4.2
LY3	63.3	62	62.7	2.3	-0.9	3.7	3.7

Cross reactivity: The cross-reactivity of the ADVIA Centaur FSH assay with TSH, LH, hCG, prolactin, and hGH was determined by adding these hormones to serum samples containing FSH. The level of FSH in the samples was then determined (see table overleaf).

Cross-reactant	*FSH value without cross-reactant (mIU/mL) (IU/L)*	*FSH value with cross-reactant (mIU/mL) (IU/L)*
TSH; 1000 µIU/mL	2.7	3.4
	29.7	30.3
	62.0	63.0
LH; 300 mIU/mL	2.7	2.8
	30.3	29.9
	62.5	64.3
hCG; 200,000 mIU/mL	2.7	4.9
	29.5	31.7
	62.2	65.5
Prolactin; 400 ng/mL	2.8	2.7
	30.2	30.0
	61.9	62.3
hGH; 100 ng/mL	2.7	2.7
	30.5	30.3
	64.5	63.1

8. LH (Centaur analyzer (Siemens Healthcare Diagnostics UK)).

Lower limit of detection: 0.5 U/L

Quality control:

Biorad Lyphochek lot 40221/2/3

CTRL	TARGET	NO	MEAN	SD	BIAS%	CV	PCV
LY1	2.7	52	2.7	0.1	0	4.6	4.1
LY2	15.7	50	15.6	0.7	-0.6	4.6	4.9
LY3	56.2	56	54.9	2.5	-1.8	4.5	4.6

Cross reactivity: The cross-reactivity of the ADVIA Centaur LH assay with TSH, β-LH, hCG, β-hCG, FSH, prolactin, and hGH was determined by adding these hormones to samples containing LH. The level of LH in the samples was then determined (see table below).

Cross-reactant	*LH value without cross-reactant (mIU/mL) (IU/L)*	*LH value with cross-reactant (mIU/mL) (IU/L)*
TSH; 1000 µIU/mL	13.3	13.1
	26.8	27.4
	46.3	47.2
β-LH; 5 ng/mL	8.4	9.6
	20.4	21.6
	39.5	41.4
	71.8	71.1
hCG; 200,000 mIU/mL	13.3	12.6
	26.8	24.5
	46.3	43.4
β-hCG; 10,000 mIU/mL	3.0	2.6
	8.6	7.4
	75.8	68.4
FSH; 200 mIU/mL	13.3	13.5
	26.8	27.4
	46.3	48.2
Prolactin; 400 ng/mL	13.3	12.8
	26.8	26.5
	46.3	46.4
hGH; 100 ng/mL	13.3	13.6
	26.8	27.1
	46.3	48.3

9. Cortisol (Centaur analyzer (Siemens Healthcare Diagnostics UK)).

Lower limit of detection: 50 nmol/L

Quality control:

Biorad lyphochek. lot 40221/2/3

CTRL	TARGET	NO	MEAN	SD	BIAS%	CV	PCV
LY 1	83	57	88	5.6	+6.0	6.4	7.0
LY 2	558	64	556	22.2	-0.4	4.0	4.7
LY 3	899	67	911	45.8	+1.3	5.0	5.8

Cross reactivity: Cross-reactivity by structurally related compounds and pharmaceuticals was determined by spiking each compound into separate human serum samples to a final level of 1000 µg/dL (27,590 nmol/L), unless otherwise noted. See table below.

Compound	% Cross-reactivity	Compound	% Cross-reactivity
Endogenous steroids		***Endogenous steroids***	
Aldosterone	0.3	11ß-hydroxyprogesterone	1.0
Allotetrahydrocortisol (100 µg/dL)	6.5	17α-hydroxyprogesterone	1.2
Androstenedione	ND*	17ß-hydroxypregnanolone	ND
Corticosterone	5.3	11-keto-androsterone	ND
Cortisone (100 µg/dL)	31.1	11-keto-etiocholanolone	1.8
α-cortol	ND	Pregnanetriol	ND
α-cortolone	ND	Pregnenolone	0.5
ß-cortol	ND	Progesterone	ND
ß-cortolone	ND	Spironolactone	ND
Dehydrocorticosterone	1.9	Testosterone	0.2
11-deoxycorticosterone	1.8	Tetrahydrocortisol	ND
11-deoxycortisol (100 µg/dL)	23.3	Tetrahydrocortisone	0.3
21-deoxycortisol (100 µg/dL)	8.1	Tetrahydro-11-deoxycortisol	ND
20α-dihydrocortisol	0.50		
20ß-dihydrocortisol	1.3	***Synthetic steroids***	
20α-dihydrocortisone	0.2	Prednisolone (50 µg/dL)	109
20ß-dihydrocortisone	ND	6-methyl-prednisolone (100 µg/dL)	26.2
11ß-hydroxyandrosterone	ND	Dexamethasone	0.20
6ß-hydroxycortisol	6.8	Prednisone (100 µg/dL)	34.0
11ß-hydroxyetiocholanolone	ND	Canrenone	0.3

* ND = ≤ 0.14

10. IGF1 (OCTEIA IGF1 (Immunodiagnostic Systems Ltd, Tyne and Wear, UK))

Lower limit of detection: 3 µg/L

Quality control: Intra assay precision is <5% and inter-assay precision <11% over the assay range (3–1000µg/L).

Cross reactivity: Cross-reactivity and interference by IGF-II, insulin and proinsulin were tested by adding 4444 µg/L IGF-II, 25 IU/L insulin and 1000µg/L proinsulin to the '0' and '150' ug/L calibrators. No interference was observed. In addition haemoglobin (500mg/dl), bilirubuin (30mg/L and triglyceride 94000mg/dL) did not interfere.

Appendix 2A: Derivation of cut-offs for peak GH response to the ITT

The locally determined cut-off for GH response to the ITT was established using data from Scottish children and the Immulite 2000 GH assay. These data are presented below. Of note, they are based on a GH standard traceable to the previous GH international reference preparation 80/505 (values assigned in mU/L only). In 2008, the Royal Infirmary of Edinburgh GH assay was re-standardised using a standard traceable to the new recombinant international reference preparation 98/574 with values assigned in both mU/L and ug/L (mU/L = 3 x ug/L). This was part of the UK-wide switch to the new standard, reporting in ug/L.

In 47 adult patient samples (GH range 0.3 - 47.8 mU/L) the relationship between results (in mU/L) obtained using the previous and new standard was New = 1.012 + 0.09, with a mean difference of 0.2 mU/L. In an additional 10 paediatric samples (GH range 0.5 - 44.7 mU/L) the relationship between results (in mU/L) obtained using the previous and new standard was New = 1.007 + 0.07, with a mean difference of 0.25 mU/L. There was therefore no significant shift in mU/L values following re-standardisation, allowing the same ITT cut-off to be used as previously established. All that was required was to divide the mU/L cut-off value by 3 to convert to ug/L.

Audit of GH responses to Clonidine and Insulin Hypoglycaemia Tests

Background

A previous audit has established that the maximum cortisol and GH responses to an insulin hypoglycaemia test (ITT) usually occur by 60min after insulin injection and always by 90min, allowing the test to be shortened to 90min post-injection compared with the current 150min. Peak cortisol response to an ITT shows a marked inverse correlation with age in our paediatric population.

Although the ITT is currently the most commonly used test for assessment of GH secretion in RHSC, allowing simultaneous assessment of the hypothalamic-pituitary-adrenal axis, there are some patients in whom it is unsuitable or unsafe. In such patients, the clonidine test may be used as an alternative. However, the timing of the GH response to clonidine has not been systematically investigated and there remain uncertainties regarding whether the two

tests are equivalent in terms of interpreting the GH response. It is also unknown whether there is a relationship between peak GH response and age in our paediatric population.

Aims of audit

1. Can the clonidine test be shortened from its current 150min?
2. Is the peak GH response to clonidine and ITT age-dependent?
3. What cut-offs should we use in determining an adequate GH response to clonidine & ITT?

Methods

Computerised search of laboratory 4D database for all ITT and clonidine tests carried out over the 6-year period 1/11/96 - 31/10/02 inclusive. One 20-year-old woman was excluded from the ITT data set, together with 9 tests in whom adequate hypoglycaemia (<2.2 mmol/L or decrease >50% compared with baseline) was not achieved. One clonidine test was excluded because samples were only collected up to 60min instead of 150min. All the remaining ITT and clonidine tests were included in this audit, regardless of diagnosis or GH response.

33 clonidine tests and 68 ITT tests were included. Median (range) ages were 11.3y (1.8 - 16.1y) for clonidine and 10.7y (0.9 - 16.0y) for ITT. The male to female ratios were 22:11 for clonidine and 45:23 for ITT. GH was measured by a two-site chemiluminescence enzyme immunoassay on the Immulite analyser (DPC) throughout the audit period.

Data were expressed as median (range). Non-parametric statistics were used throughout. Comparison of peak GH response between clonidine & ITT tests was by the Mann-Whitney U test. The relationship between peak GH response and age was explored using Spearman correlation. Statistical significance was accepted at $P < 0.05$.

Results

There was little relationship between maximum GH response and age for either clonidine ($r_s = 0.32$, $P = 0.07$, Fig 1) or ITT ($r_s = 0.08$, $P = 0.5$, Fig 2). The time course of GH response to clonidine was quite variable but generally showed a sustained elevation over 60 - 150min post-dose (Fig 3), unlike the response to ITT, which frequently showed elevations at a single time point only (data not shown). The time of peak GH response to clonidine showed a gaussian distribution, with a median of 90min (range 30 - 150min): - see Fig 4. This

contrasts with the earlier non-gaussian timing of the peak GH response to ITT (excuding 4 ITT tests in which no GH peak occurred) - Fig 4.

18% of patients who underwent a clonidine test had clear GH insufficiency with peak GH responses <10 mU/L, compared with 35% of patients who had an ITT (Fig 5). Excluding those GH-insufficient patients, the peak GH response to both clonidine and ITT was skewed, with a tendency for higher peak GH responses to clonidine (Fig 5). Median (range) peak GH response for those with responses >10 mU/L was 45.5 (14.9 - >104.0) for clonidine versus 22.3 (11.5 - 83.3) for ITT (P <0.0001).

Among subjects with peak GH responses >10 mU/L, 93% subjects had peak GH responses to clonidine stimulation >20 mU/L compared with only 66% for the ITT. However, 96% subjects had peak GH responses to ITT >15 mU/L.

Only two subjects had both a clonidine test and an ITT done, 2 months and 4 months apart respectively. The first subject had peak GH responses of 8.6 to clonidine and 4.1 mU/L to ITT and was subsequently treated with GH. The second (with a posterior fossa ependymoma treated with CNS irradiation) had an ITT done first with a peak GH response of 3.9 mU/, followed by a clonidine test with a peak response of 27.6 mU/L. Discordant results were therefore obtained by the two tests in this case, as has been described in the literature.

Conclusions

1. Maximal GH response to clonidine occurs from 30 - 150 min post-dose.
2. There is no age-dependency in GH responses to either clonidine or ITT in our paediatric population.
3. GH responses to clonidine tend to be higher than those to ITT.
4. 93% non-GH-deficient subjects had a peak GH response to clonidine >20mU/L. 96% non-GH-deficient subjects had a peak GH response to ITT >15mU/L.

Recommendations

1. The clonidine test cannot be shortened from its current length of 150min.
2. Age-related cut-offs for GH responses to clonidine & ITT are not required.
3. The peak GH response to define an adequate response should be >15 mU/L for an ITT and >20 mU/L for a clonidine test.

Pat Crofton
19/11/2002

Figure 1: Clonidine: Peak GH versus Age

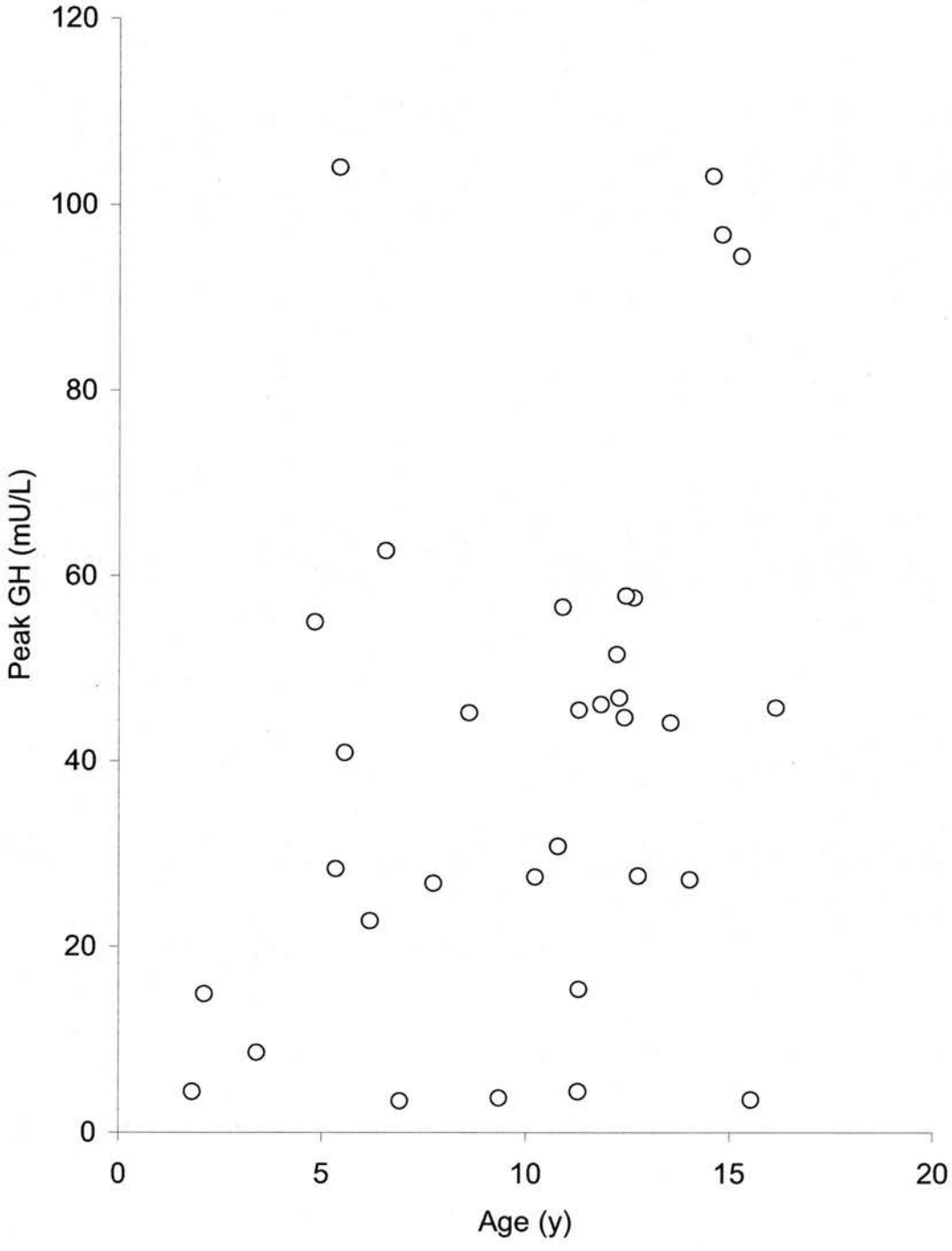


Figure 2: ITT: Peak GH versus Age

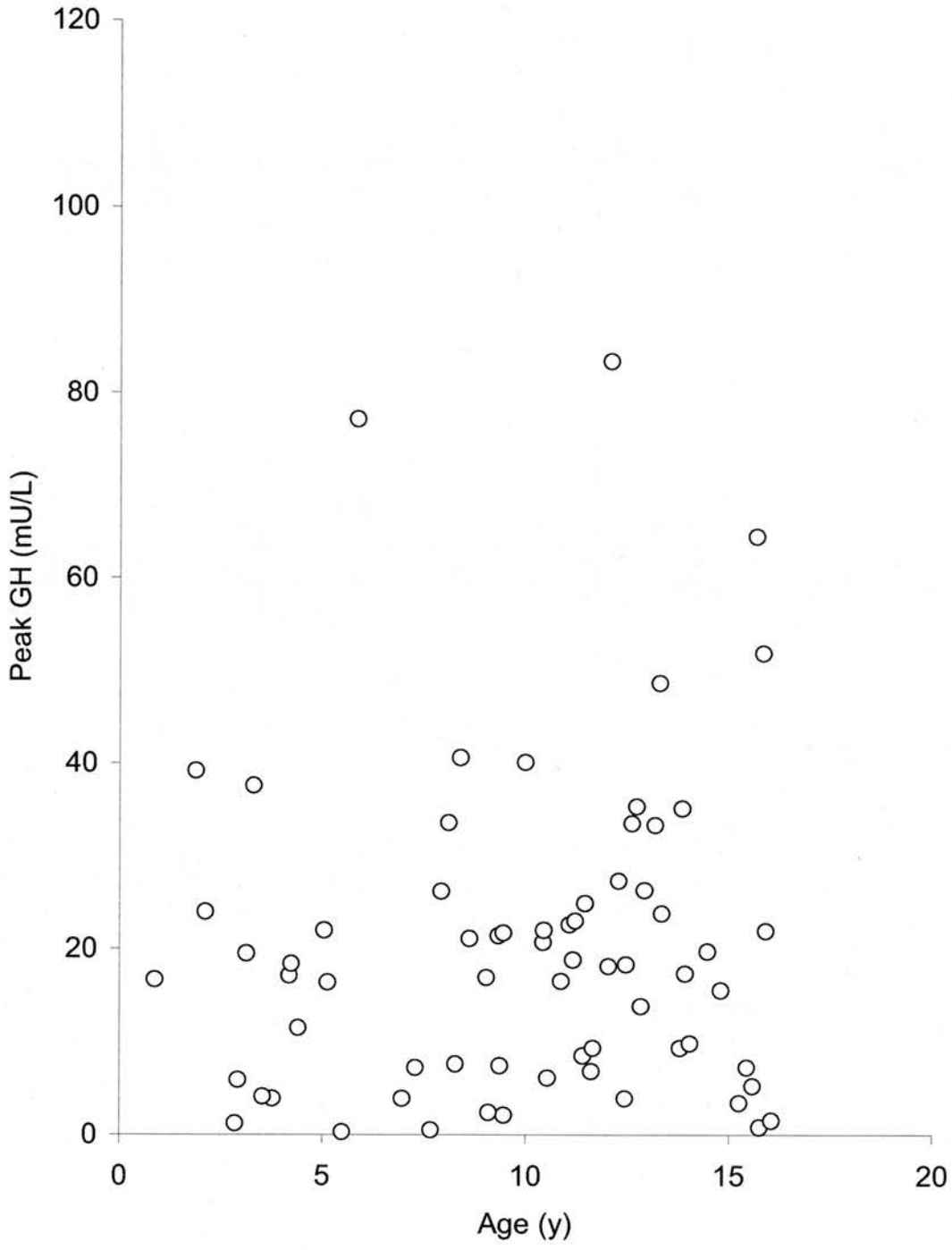


Figure 3: Clonidine Test: GH individual responses (n=33, thick line = median)

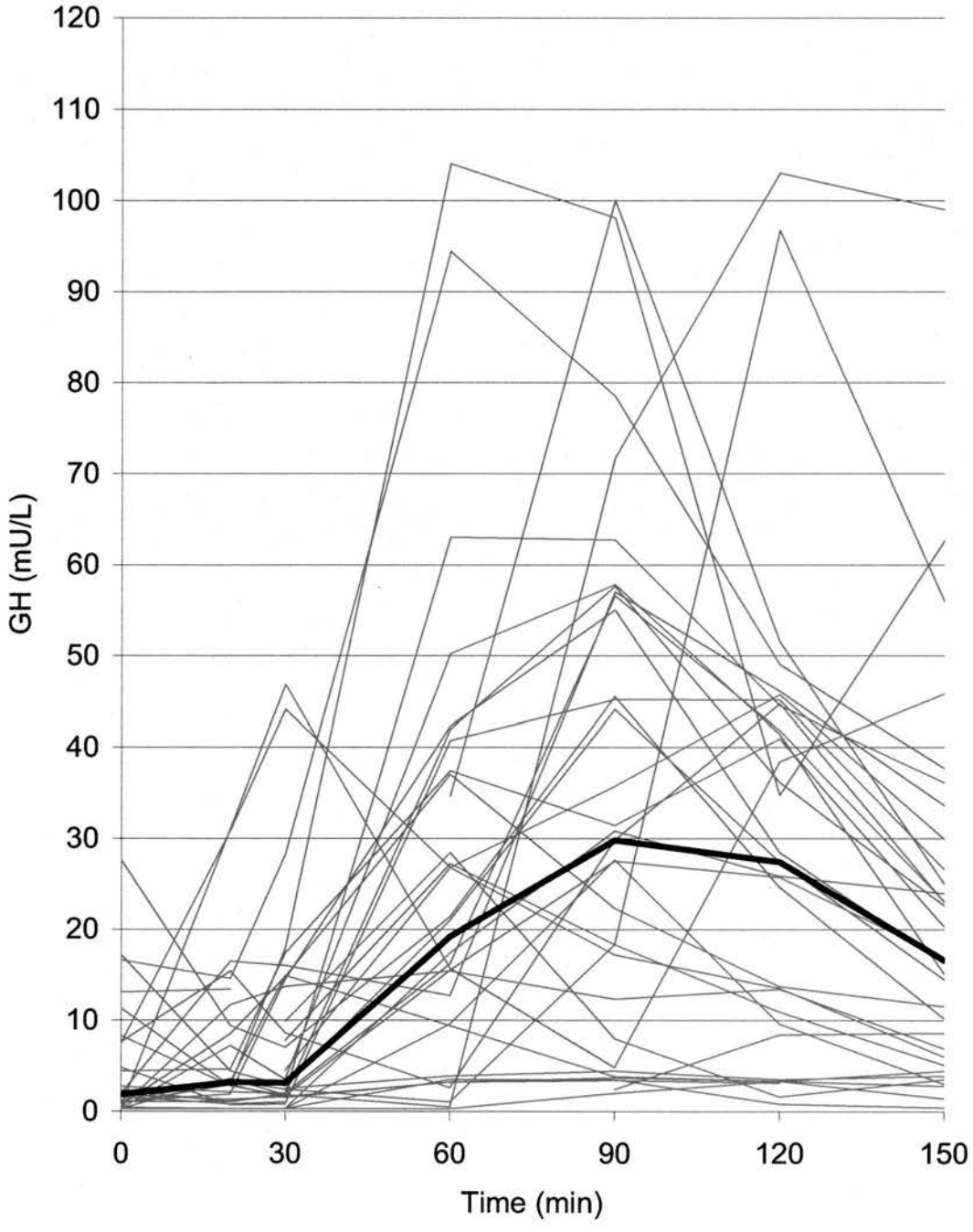


Figure 4: Time of peak GH response to Clonidine & ITT

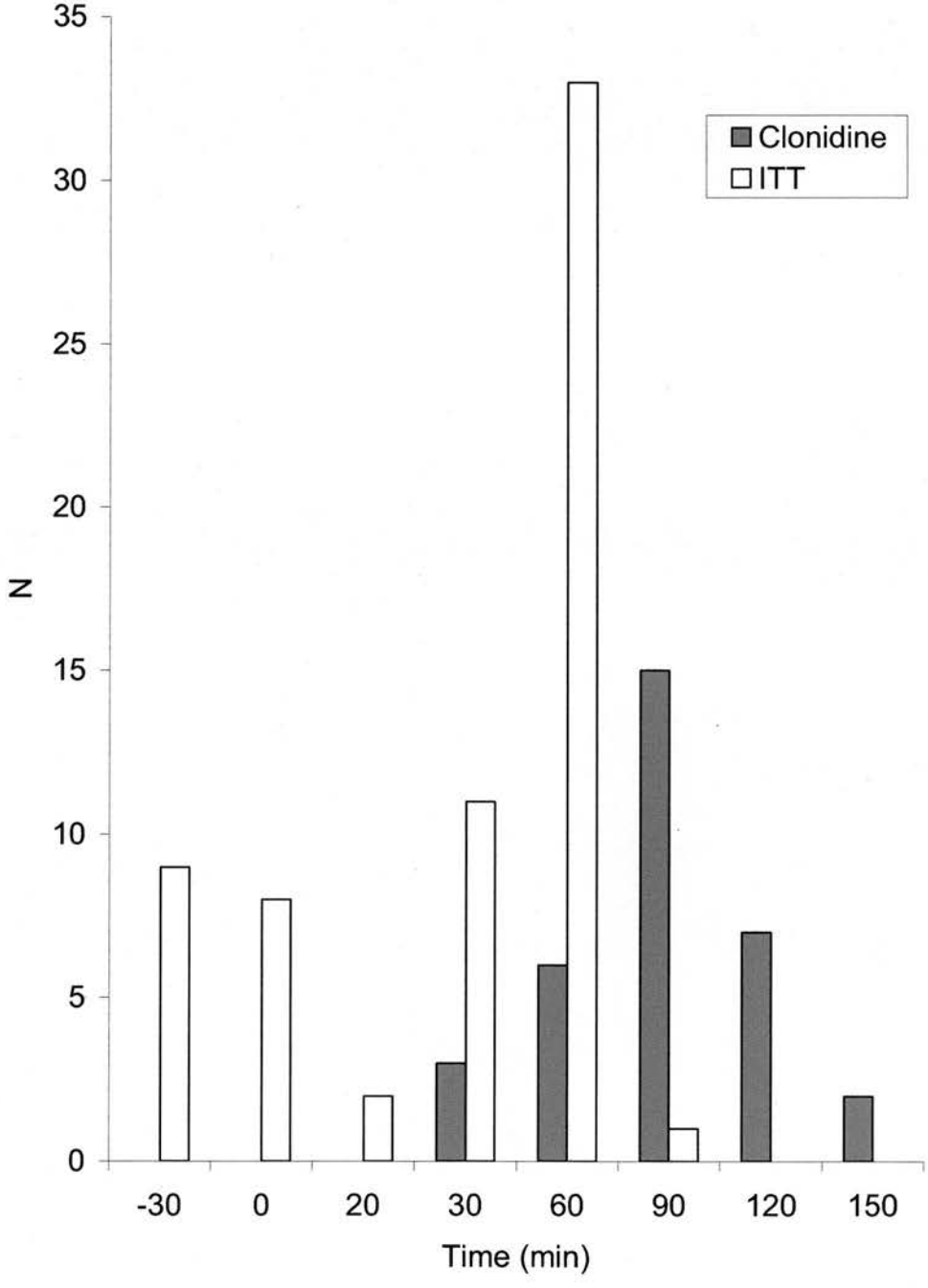
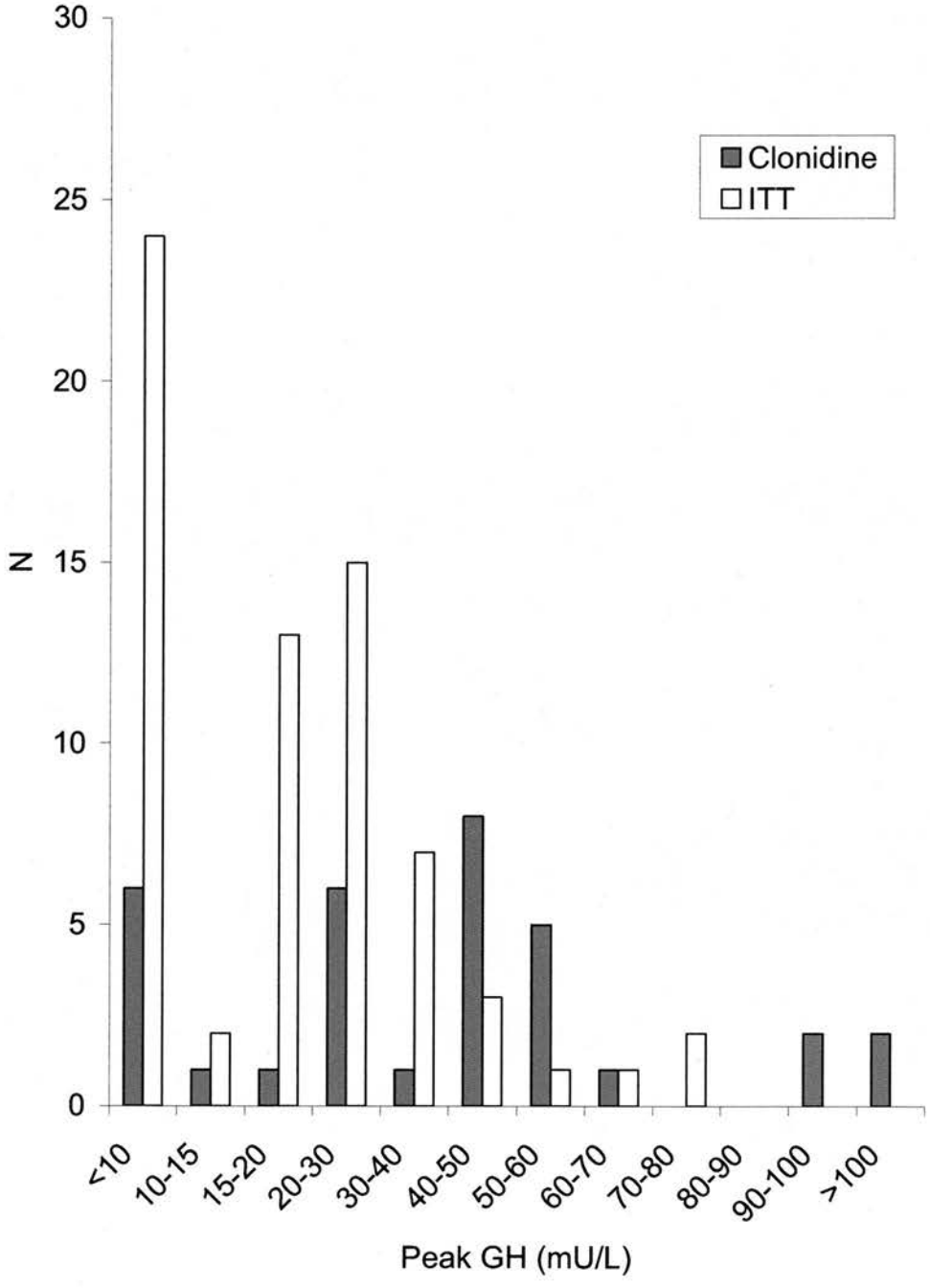


Figure 5: Peak GH response to Clonidine & ITT



Appendix 2B: Derivation of cut-offs for peak cortisol response to the ITT

Locally determined age-dependent cut-offs were used to evaluate peak cortisol response to the ITT: >470 nmol/L in subjects ≥10 years; >550 nmol/L in subjects <10 years. These data were published in the paper cited below:

Crofton PM, Don-Wauchope AC, Bath LB, Kelnar CJ. Cortisol responses to the insulin hypoglycaemia test in children. Horm Res 2004;61:92-7.

The abstract from this paper is below.

Abstract

Aim: To determine the timing of the peak cortisol response to the insulin hypoglycaemia (IH) test in children and to establish paediatric reference data. Methods: We retrospectively reviewed all IH tests in a tertiary paediatric endocrine referral centre over a 6-year period. Inclusion criteria were age <16 years and adequate hypoglycaemia (glucose ≤ 2.0 mmol/l). Patients with an impaired hypothalamic-pituitary-adrenal axis or receiving glucocorticoid medication were excluded. Fifty-four subjects (35 males) met the criteria. Blood samples were collected at –30, 0, 20, 30, 60, 90, 120, and 150 min in relation to insulin bolus injection (0.15 U/kg) at 0 min. Glucose, cortisol, and growth hormone (GH) were measured in all samples. Results: Peak cortisol and GH responses occurred by 90 min in all subjects. Peak cortisol was inversely correlated with age (rs –0.65, $p < 0.0001$). The median (5th centile) peak cortisol value was 689 nmol/l (547 nmol/l) in children younger than 10 years as compared with 555 nmol/l (468 nmol/l) in those older than 10 years ($p < 0.0001$). Peak cortisol was not related to peak GH (rs –0.20, $p = 0.15$). Conclusions: Blood sampling in the IH test may be curtailed 90 min after injection. The peak cortisol response to IH is age related.

The peak cortisol response data are presented in the figure overleaf, extracted from the paper.

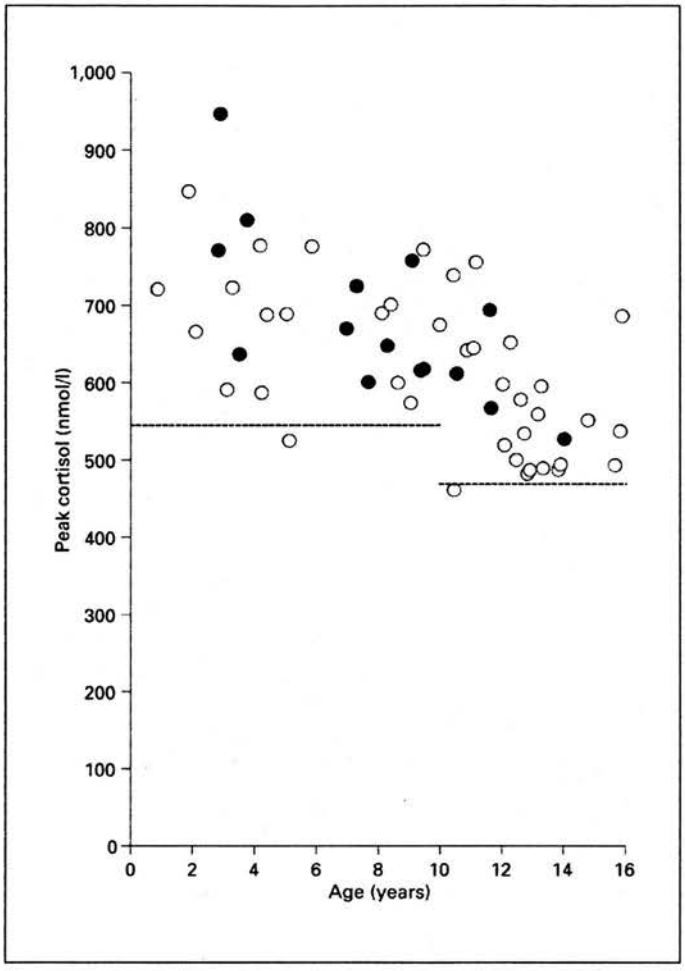


Fig. 1. Peak cortisol response to IH plotted against age. Solid circles: patients with peak GH concentrations <10 mU/l; open circles: patients with peak GH concentrations >10 mU/l. The cortisol concentrations corresponding to the 5th centiles in the younger (<10 years) and older (>10 years) age groups are shown as dashed lines. To convert cortisol in nanomoles per liter to micrograms per deciliter, divide by 27.6.

Appendix 3A: Stimulation test results in full by test

Endocrine results follow for each stimulation test in full. Peak hormone responses are highlighted in bold.

Table A: Complete ITT results for GH by subject

Subject no.	Age (y) at study	GH concentration (µg/L) at specified time point (mins)					
		-30	0	20	30	60	90
Accidental TBI group							
2	18.0	<0.1	<0.1	0.2	5.6	**15.1**	9.4
3	16.4	0.2	0.2	0.4	8.8	**11.0**	3.8
4	12.2	<0.1	1.2	1.3	5.5	**7.9**	4.3
5	13.1	0.2	0.9	0.3	1.8	**5.2**	3.2
6	17.2	0.1	2.5	7.7	**14.8**	13.6	5.4
7	13.2	0.1	0.3	0.2	0.1	**7.0**	1.5
8	17.0	<0.1	<0.1	<0.1	0.9	**3.2** [a]	0.7
12	9.9	0.4	**5.6** [b]	1.3	1.6	0.9	0.2
13	9.0	3.9	0.7	0.3	0.8	**5.1**	1.1
14	12.0	0.2	2.4	0.5	0.8	**2.9** [a]	0.7
15	8.4	1.1	0.2	1.1	**6.1**	2.8	0.5
16	12.6	0.2	1.4	1.1	**7.1**	4.2	1.0
17	11.1	0.1	0.2	0.1	2.1	**3.5** [a]	0.8
19	14.8	0.1	0.2	<0.1	0.3	**2.8** [a]	1.3
20	12.9	<0.1	0.2	<0.1	0.2	**4.4** [a]	1.2
21	18.0	13.5	**20.6** [b]	9.0	6.6	9.5	3.7
22	12.9	0.1	0.6	10.3	**15.8**	9.2	2.5
23	5.4	5.1	1.2	3.4	**7.5**	5.2	1.3
24	8.5	0.8	0.2	0.2	0.4	3.8	**3.9** [a]
28	11.7	0.3	0.4	1.8	**13.0**	8.8	3.2
29	15.0	0.2	0.3	5.7	13.9	**25.4**	14.1
30	15.3	0.8	0.3	0.3	0.7	**10.6**	6.5
31	13.0	0.2	3.8	2.3	**5.7**	<0.1	<0.1
32	14.6	0.1	0.3	5.4	**13.2**	10.5	4.3
33	21.7	8.2	3.0	1.2	2.2	**13.8**	8.6
Suspected NAHI group							
Subject B	3.7	0.3	0.3	4.4	**8.0**	4.0	0.9

y, years; [a] GH peak <5 µg/L; [b] value refers to spontaneous GH peak at baseline

Table B: Complete ITT results for cortisol by subject

Subject	Age (y) at study	Cortisol concentration (nmol/L) at specified time point (mins)					
		-30	0	20	30	60	90
Accidental TBI group							
2	18.0	471	434	355	553	**717**	574
3	16.4	341	209	209	267	**462** [c]	336
4	12.2	218	146	95	375	**538**	537
5	13.1	248	164	151	309	**564**	498
6	17.2	281	173	147	185	**443** [c]	319
7	13.2	202	152	150	173	**488**	356
8	17.0	494	295	219	395	**598**	449
12	9.9	320	293	284	379	**593**	381
13	9.0	356	301	282	378	**546** [c]	380
14	12.0	402	223	163	298	**367** [d]	246
15	8.4	624 [b]	431	446	**459** [b]	434	254
16	12.6	214	203	261	301	**522**	392
17	11.1	261	225	175	359	**545**	391
19	14.8	110 [a]	129	98	128	**458** [c]	333
20	12.9	262	282	226	194	**385** [d]	223
21	18.0	342	314	253	277	**507**	364
22	12.9	290	192	174	362	**458** [c]	329
23	5.4	294	337	551	606	**716**	554
24	8.5	373	243	211	483	**645**	638
28	11.7	236	225	211	334	**528**	406
29	15.0	622	473	369	369	**526**	418
30	15.3	146 [a]	99	88	96	**414** [d]	382
31	13.0	298	225	220	294	**542**	372
32	14.6	562	458	297	611	**660**	485
33	21.7	589	439	340	337	643	**707**
Suspected NAHI group							
Subject B	3.7	309	283	252	540	**688**	492

y, years; [a] low basal early morning cortisol; [b] basal early morning cortisol high so inadequate peak response discounted; [c] sub-optimal response to ITT, peak cortisol within 50 mmol/L below cut-off; [d] sub-optimal response to ITT, peak cortisol 50-100 mmol/L below cut-off; See text for reference ranges.

Table C: Complete glucagon test results for GH by subject

Subject	Age (y) at study	GH concentration (µg/L) at specified time point (mins)							
		-30	0	20	60	90	120	150	180
Accidental TBI group									
1	10.3	<0.1	<0.1	<0.1	<0.1	0.1	**5.9**	5.2	2.0
9	15.1	0.1	1.7	2.2	0.3	0.9	**14.2**	13.4	6.1
10	9.8	1.5	0.3	0.3	<0.1	0.6	**2.5** [a]	1.7	0.8
11	16.3	9.4	**9.5** [b]	6.8	1.6	0.7	0.4	3.7	1.6
18	15.1	2.4	0.7	0.4	0.2	0.4	6.7	**15.3**	13.3
25	6.5	1.9	4.1	1.7	1.5	1.9	**14.0**	5.7	2.3
26	15.6	2.0	-	0.3	0.3	0.2	4.8	**9.4**	3.4
27	18.9	1.0	0.2	0.2	-	-	-	**10.9**	-
Suspected NAHI group									
Subject A	5.0	1.9	1.6	1.1	1.7	**8.7**	<0.1	1.9	1.2

y, years; [a] GH peak <5 µg/L; [b] value refers to spontaneous GH peak at baseline

Table D: Complete glucagon test results for cortisol by subject

Subject	Age (y) at study	Cortisol concentration (nmol/L) at specified time point (mins)							
		-30	0	20	60	90	120	150	180
Accidental TBI group									
1	10.3	166	167	239	267	349	330	**517**	503
9	15.1	487	341	289	338	352	349	**466**	421
10	9.8	150	265	311	273	316	**496**	343	376
11	16.3	**722** [a]	622	494	391	302	245	177	141
18	15.1	210	198	168	214	**289** [b]	252	215	277
25	6.5	415	485	408	297	322	559	**729**	673
26	15.6	152	-	106	262	279	378	561	**606**
27	18.9	316	252	IS	-		-	**624**	-
Suspected NAHI group									
Subject A	5.0	266	266	280	375	520	719	790	**830**

y, years; IS, insufficient; [a] basal early morning cortisol high so inadequate peak response discounted; [b] sub-optimal cortisol response to glucagon. See text for reference ranges.

Table E: Complete GnRH test results for FSH by subject

Subject	Sex	Age (y) at study	Pubertal stage	FSH concentration (U/L) at specified time point (mins)		
				0	20	60
Accidental TBI group						
1	M	10.3	Pre-	<0.5	**3.1**	2.8
2	M	18.0	Late/post-	1.1	2.6	**2.8**
3	M	16.4	Late/post-	3.1	**4.7**	4.6
4	M	12.2	Pre-	3.1	**6.1**	5.9
5	M	13.1	Early/Mid-	1.4	**1.7**	1.5
6	M	17.2	Late/post-	1.7	**2.3**	2.1
7	M	13.2	Late/post-	2.7	**4.0**	3.6
8	M	17.0	Late/post-	4.3	10.9	**11.2**
9	M	15.1	Late/post-	1.6	**2.4**	2.4
10	M	9.8	Pre-	0.5	**3.6**	3.5
11	M	16.3	Late/post-	7.2	9.1	**9.8**
12	M	9.9	Pre-	<0.5	**1.9**	1.5
13	M	9.0	Pre-	<0.5	2.1	**2.4**
14	M	12.0	Early/Mid-	4.5	9.3	**9.6**
15	M	8.4	Pre-	<0.5	**3.0**	2.8
16	M	12.6	Early/Mid-	<0.5	1.4	**1.5**
17	M	11.1	Pre-	1.3	2.7	**2.8**
18	M	15.1	Late/post-	1.8	2.2	**2.5**
19	M	14.8	Early/Mid-	1.5	**2.1**	2.1
20	M	12.9	Early/Mid-	1.5	2.1	**2.5**
21	M	18.0	Late/post-	2.2	3.1	**3.3**
22	M	12.9	Early/Mid-	2.4	3.9	**4.3**
23	M	5.4	Pre-	0.5	2.9	**3.2**
24	M	8.5	Pre-	0.5	2.6	**3.0**
25	M	6.5	Pre-	1.1	3.5	**4.4**
26	F	15.6	Late/post-	4.2	11.6	**13.8**
27	F	18.9	Late/post-	6.1	IS	-
28	F	11.7	Early/Mid-	6.9	**14.6**	14.1
29	F	15.0	Late/post-	3.4	**7.5**	7.4
30	F	15.3	Late/post-	2.3	2.4	**2.5**
31	F	13.0	Late/post-	5.8	**7.9**	6.8
32	F	14.6	Late/post-	3.5	**5.9**	5.7
33	F	21.7	Late/post-	3.3	6.5	**8.2**
Suspected NAHI group						
A	M	5.0	Pre-	<0.5	3.5	**3.8**
B	M	3.7	Pre-	0.5	5.3	**7.4**

M, male; F, female; y, years; IS, insufficient; [a] probable constitutional delay of puberty, resolving spontaneously

Table F: Complete GnRH test results for LH by subject

Subject	Sex	Age (y) at study	Pubertal stage	LH concentration (U/L) at specified time point (mins)		
				0	20	60
Accidental TBI group						
1	M	10.3	Pre-	1.9	4.9	**5.9**
2	M	18.0	Late/post-	1.6	**25.1**	18.1
3	M	16.4	Late/post-	1.4	**10.7**	8.4
4	M	12.2	Pre-	<0.5	**4.0**	2.7
5	M	13.1	Early/Mid-	1.5	**6.8**	6.2
6	M	17.2	Late/post-	2.1	**10.6**	7.0
7	M	13.2	Late/post-	2.7	**14.6**	9.0
8	M	17.0	Late/post-	2.8	**35.3**	26.9
9	M	15.1	Late/post-	2.1	**11.8**	9.5
10	M	9.8	Pre-	<0.5	**1.9**	1.0
11	M	16.3	Late/post-	3.2	**14.0**	10.1
12	M	9.9	Pre-	<0.5	**1.0**	0.6
13	M	9.0	Pre-	<0.5	**1.0**	0.7
14	M	12.0	Early/Mid-	0.9	**11.5**	9.6
15	M	8.4	Pre-	<0.5	**1.3**	0.6
16	M	12.6	Early/Mid-	0.7	**3.7**	2.4
17	M	11.1	Pre-	<0.5	**4.7**	3.3
18	M	15.1	Late/post-	1.9	**6.8**	5.3
19	M	14.8	Early/Mid-	<0.5 [a]	**4.2**	3.4
20	M	12.9	Early/Mid-	1.6	**10.1**	7.0
21	M	18.0	Late/post-	1.3	**9.2**	7.2
22	M	12.9	Early/Mid-	0.5	**10.7**	7.3
23	M	5.4	Pre-	<0.5	**2.0**	1.6
24	M	8.5	Pre-	<0.5	<0.5	**1.1**
25	M	6.5	Pre-	<0.5	**0.7**	0.5
26	F	15.6	Late/post-	12.2	**106.0**	89.6
27	F	18.9	Late/post-	10.2	IS	-
28	F	11.7	Early/Mid-	4.2	**25.8**	17.2
29	F	15.0	Late/post-	2.4	**20.9**	17.4
30	F	15.3	Late/post-	1.0	**15.6**	10.6
31	F	13.0	Late/post-	7.7	**20.4**	12.3
32	F	14.6	Late/post-	9.5	**38.9**	25.1
33	F	21.7	Late/post-	6.3	**65.8**	56.1
Suspected NAHI group						
A	M	5.0	Pre-	<0.5	**1.4**	0.9
B	M	3.7	Pre-	<0.5	**1.6**	1.4

M, male; F, female; y, years; IS, insufficient; [a] probable constitutional delay of puberty, resolving spontaneously

Appendix 3B: Stimulation test results in full by subject

Complete stimulation test results follow for each subject. Peak hormone responses and lowest glucose concentrations are highlighted in bold.

Accidental TBI group

Subject 1 (male, 10.3 years)

Glucagon test and GnRH test

Time (mins)	-30	0	20	60	90	120	150	180
Time (actual)	09:00	09:30	09:55	10:35	11:05	11:35	12:05	12:35
Glucose, mmol/L	4.8	4.9	8.1	5.3	4.4	**4.3**	4.6	4.7
GH, µg/L	<0.1	<0.1	<0.1	<0.1	0.1	**5.9**	5.2	2.0
Cortisol, nmol/L	166	167	239	267	349	330	**517**	503
FSH, U/L		<0.5	**3.1**	2.8				
LH, U/L		1.9	4.9	**5.9**				

Glucagon and GnRH administered at 09:35

Subject 2 (male, 18.0 years)

ITT and GnRH test

Time (mins)	-40	0	20	30	60	90
Time (actual)	08:35	09:15	09:35	09:45	10:15	10:45
Glucose, mmol/L	4.7	4.4	**1.4**	2.2	6.3	4.9
GH, µg/L	<0.1	<0.1	0.2	5.6	**15.1**	9.4
Cortisol, nmol/L	471	434	355	553	**717**	574
FSH, U/L		1.1	2.6		**2.8**	
LH, U/L		1.6	**25.1**		18.1	

Insulin and GnRH administered at 09:15

Subject 3 (male, 16.4 years)

ITT and GnRH test

Time (mins)	-50	0	20	30	60	90
Time (actual)	09:20	10:10	10:30	10:40	11:10	11:40
Glucose, mmol/L	4.9	4.7	**1.0**	2.0	4.4	4.3
GH, µg/L	0.2	0.2	0.4	8.8	**11.0**	3.8
Cortisol, nmol/L	341	209	209	267	**462**	336
FSH, U/L		3.1	**4.7**		4.6	
LH, U/L		1.4	**10.7**		8.4	

Insulin and GnRH administered at 10:10

Subject: 4 (male, 12.2 years)

ITT and GnRH test

Time (mins)	-40	0	20	30	60	90
Time (actual)	09:05	09:45	10:05	10:15	10:45	11:15
Glucose, mmol/L	4.3	4.4	**1.2**	2.2	5.0	7.1
GH, µg/L	<0.1	1.2	1.3	5.5	**7.9**	4.3
Cortisol, nmol/L	218	146	95	375	**538**	537
FSH, U/L		3.1	**6.1**		5.9	
LH, U/L		<0.5	**4.0**		2.7	

Insulin and GnRH administered at 09:45

Subject 5 (male, 13.1 years)

ITT and GnRH test

Time (mins)	-40	0	20	30	60	90
Time (actual)	09:25	10:05	10:25	10:35	11:05	11:35
Glucose, mmol/L	4.4	4.5	**1.5**	2.6	4.1	5.3
GH, µg/L	0.2	0.9	0.3	1.8	**5.2**	3.2
Cortisol, nmol/L	248	164	151	309	**564**	498
FSH, U/L		1.4	**1.7**		1.5	
LH, U/L		1.5	**6.8**		6.2	

Insulin and GnRH administered at 10:05

Subject 6 (male, 17.2 years)

ITT and GnRH test

Time (mins)	-30	0	20	30	60	90
Time (actual)	08:45	09:15	09:40	09:50	10:20	10:50
Glucose, mmol/L	4.5	4.6	**0.8**	2.7	5.0	3.7
GH, µg/L	0.1	2.5	7.7	**14.8**	13.6	5.4
Cortisol, nmol/L	281	173	147	185	**443**	319
FSH, U/L		1.7	**2.3**		2.1	
LH, U/L		2.1	**10.6**		7.0	

Insulin and GnRH administered at 09:20

Subject 7 (male, 13.2 years)

ITT and GnRH test

Time (mins)	-30	0	20	30	60	90
Time (actual)	09:30	10:00	10:25	10:35	11:05	11:35
Glucose, mmol/L	5.1	5.1	1.4	**1.0***	5.9	3.7
GH, µg/L	0.1	0.3	0.2	0.1	**7.0**	1.5
Cortisol, nmol/L	202	152	150	173	**488**	356
FSH, U/L		2.7	**4.0**		3.6	
LH, U/L		2.7	**14.6**		9.0	

Insulin and GnRH administered at 10:05. *Glucose meter reading at 35 min.: 2.4 mmol/L

Subject 8 (male, 17.0 years)

ITT and GnRH test

Time (mins)	-30	0	20	30	60	90
Time (actual)	09:15	09:45	10:05	10:15	10:45	11:15
Glucose, mmol/L	4.5	4.5	**1.5**	1.8	6.3	4.5
GH, µg/L	<0.1	<0.1	<0.1	0.9	**3.2**	0.7
Cortisol, nmol/L	494	295	219	395	**598**	449
FSH, U/L		4.3	10.9		**11.2**	
LH, U/L		2.8	**35.3**		26.9	

Insulin and GnRH administered at 09:45

Subject 9 (male, 15.1 years)

Glucagon test and GnRH test

Time (mins)	**-30**	**0**	**20**	**60**	**90**	**120**	**150**	**180**
Time (actual)	09:25	09:55	10:20	11:00	11:30	12:00	12:30	13:00
Glucose, mmol/L	4.5	4.4	7.2	**3.0**	3.3	4.0	3.9	4.3
GH, µg/L	0.1	1.7	2.2	0.3	0.9	**14.2**	13.4	6.1
Cortisol, nmol/L	487	341	289	338	352	349	**466**	421
FSH, U/L		1.6	**2.4**	2.4				
LH, U/L		2.1	**11.8**	9.5				

Glucagon and GnRH administered at 10:00

Subject 10 (male, 9.8 years)

Glucagon test and GnRH test

Time (mins)	**-30**	**0**	**20**	**60**	**90**	**120**	**150**	**180**
Time (actual)	09:10	09:40	10:05	10:45	11:15	11:45	12:15	12:45
Glucose, mmol/L	4.7	4.7	8.1	5.6	**3.4**	4.3	4.4	4.7
GH, µg/L	1.5	0.3	0.3	<0.1	0.6	**2.5**	1.7	0.8
Cortisol, nmol/L	150	265	311	273	316	**496**	343	376
FSH, U/L		0.5	**3.6**	3.5				
LH, U/L		<0.5	**1.9**	1.0				

Glucagon and GnRH administered at 09:45

Subject 11 (male, 16.3 years)

Glucagon test and GnRH test

Time (mins)	-30	0	20	60	90	120	150	180
Time (actual)	08:20	08:50	09:15	09:55	10:25	10:55	11:25	11:55
Glucose, mmol/L	5.0	4.9	7.8	6.7	5.2	4.7	4.1	**3.7**
GH, µg/L	9.4	**9.5**	6.8	1.6	0.7	0.4	3.7	1.6
Cortisol, nmol/L	**722**	622	494	391	302	245	177	141
FSH, U/L		7.2	9.1	**9.8**				
LH, U/L		3.2	**14.0**	10.1				

Glucagon and GnRH administered at 08:55

Subject 12 (male, 9.9 years)

ITT and GnRH test

Time (mins)	-35	0	20	30	60	90
Time (actual)	10:00	10:35	10:55	11:05	11:35	12:05
Glucose, mmol/L	4.6	4.4	**1.4**	4.2	6.1	5.6
GH, µg/L	0.4	**5.6**	1.3	1.6	0.9	0.2
Cortisol, nmol/L	320	293	284	379	**593**	381
FSH, U/L		<0.5	**1.9**		1.5	
LH, U/L		<0.5	**1.0**		0.6	

Insulin and GnRH administered at 10:35

Subject 13 (male, 9.0 years)

ITT and GnRH test

Time (mins)	-35	0	20	30	60	90
Time (actual)	09:40	10:15	10:35	10:45	11:15	11:45
Glucose, mmol/L	4.3	4.4	**0.9**	3.9	6.4	4.1
GH, µg/L	3.9	0.7	0.3	0.8	**5.1**	1.1
Cortisol, nmol/L	356	301	282	378	**546**	380
FSH, U/L		<0.5	2.1		**2.4**	
LH, U/L		<0.5	**1.0**		0.7	

Insulin and GnRH administered at 10:15

Subject 14 (male, 12.0 years)

ITT and GnRH test

Time (mins)	-45	0	20	30	60	90
Time (actual)	09:35	10:20	10:45	10:55	11:25	11:55
Glucose, mmol/L	4.5	4.5	**1.5**	3.5	6.4	6.7
GH, µg/L	0.2	2.4	0.5	0.8	**2.9**	0.7
Cortisol, nmol/L	402	223	163	298	**367**	246
FSH, U/L		4.5	9.3		**9.6**	
LH, U/L		0.9	**11.5**		9.6	

Insulin and GnRH administered at 10:25

Subject 15 (male, 8.4 years)

ITT and GnRH test

Time (mins)	-40	0	20	30	60	90
Time (actual)	09:35	10:15	10:35	10:45	11:15	11:45
Glucose, mmol/L	4.8	4.7	**1.5**	4.6	7.3	7.1
GH, µg/L	1.1	0.2	1.1	**6.1**	2.8	0.5
Cortisol, nmol/L	624	431	446	**459**	434	254
FSH, U/L		<0.5	**3.0**		2.8	
LH, U/L		<0.5	**1.3**		0.6	

Insulin and GnRH administered at 10:15

Subject 16 (male, 12.6 years)

ITT and GnRH test

Time (mins)	-30	0	20	30	60	90
Time (actual)	09:40	10:10	10:30	10:40	11:10	11:40
Glucose, mmol/L	4.0	4.2	**1.5**	4.2	4.7	4.8
GH, µg/L	0.2	1.4	1.1	**7.1**	4.2	1.0
Cortisol, nmol/L	214	203	261	301	**522**	392
FSH, U/L		<0.5	1.4		**1.5**	
LH, U/L		0.7	**3.7**		2.4	

Insulin and GnRH administered at 10:10

Subject 17 (male, 11.1 years)

ITT and GnRH test

Time (mins)	-40	0	20	30	60	90
Time (actual)	09:45	10:25	10:45	10:55	11:25	11:55
Glucose, mmol/L	4.7	4.6	**1.1**	4.1	8.9	7.8
GH, µg/L	0.1	0.2	0.1	2.1	**3.5**	0.8
Cortisol, nmol/L	261	225	175	359	**545**	391
FSH, U/L		1.3	2.7		**2.8**	
LH, U/L		<0.5	**4.7**		3.3	

Insulin and GnRH administered at 10:25

Subject 18 (male, 15.1 years)

Glucagon test and GnRH test

Time (mins)	-30	0	20	60	90	120	150	180
Time (actual)	09:10	09:40	10:00	10:40	11:10	11:40	12:10	12:40
Glucose, mmol/L	3.7	3.9	6.0	4.4	3.5	**3.3**	4.0	3.9
GH, µg/L	2.4	0.7	0.4	0.2	0.4	6.7	**15.3**	13.3
Cortisol, nmol/L	210	198	168	214	**289**	252	215	277
FSH, U/L		1.8	2.2	**2.5**				
LH, U/L		1.9	**6.8**	5.3				

Glucagon and GnRH administered at 09:40

Subject 19 (male, 14.8 years)

ITT and GnRH test

Time (mins)	**-55**	**0**	**20**	**30**	**60**	**90**
Time (actual)	09:20	10:15	10:35	10:45	11:5	11:45
Glucose, mmol/L	4.5	4.4	**1.2**	1.7*	7.4	3.4
GH, µg/L	0.1	0.2	<0.1	0.3	**2.8**	1.3
Cortisol, nmol/L	110	129	98	128	**458**	333
FSH, U/L		1.5	**2.1**		2.1	
LH, U/L		<0.5	**4.2**		3.4	

Insulin and GnRH administered at 10:15. *Glucose meter reading at 45 min.: 5.4 mmol/L.

Subject 20 (male, 12.9 years)

ITT and GnRH test

Time (mins)	**-40**	**0**	**20**	**30**	**60**	**90**
Time (actual)	09:05	09:45	10:05	10:15	10:45	11:15
Glucose, mmol/L	5.0	5.3	**1.8**	2.9	8.0	5.8
GH, µg/L	<0.1	0.2	<0.1	0.2	**4.4**	1.2
Cortisol, nmol/L	262	282	226	194	**385**	223
FSH, U/L		1.5	2.1		**2.5**	
LH, U/L		1.6	**10.1**		7.0	

Insulin and GnRH administered at 09:45

Subject 21 (male, 18.0 years)

ITT and GnRH test

Time (mins)	-35	0	20	30	60	90
Time (actual)	09:20	09:55	10:15	10:25	10:55	11:25
Glucose, mmol/L	4.5	4.4	**1.2**	3.2	4.6	3.8
GH, µg/L	13.5	**20.6**	9.0	6.6	9.5	3.7
Cortisol, nmol/L	342	314	253	277	**507**	364
FSH, U/L		2.2	3.1		**3.3**	
LH, U/L		1.3	**9.2**		7.2	

Insulin and GnRH administered at 09:55

Subject 22 (male, 12.9 years)

ITT and GnRH test

Time (mins)	-55	0	20	30	60	90
Time (actual)	09:45	10:40	11:00	11:10	11:40	12:10
Glucose, mmol/L	3.8	4.2	**1.4**	3.6	6.0	6.1
GH, µg/L	0.1	0.6	10.3	**15.8**	9.2	2.5
Cortisol, nmol/L	290	192	174	362	**458**	329
FSH, U/L		2.4	3.9		**4.3**	
LH, U/L		0.5	**10.7**		7.3	

Insulin and GnRH administered at 10:40

Subject 23 (male, 5.4 years)

ITT and GnRH test

Time (mins)	-55	0	20	30	60	90
Time (actual)	09:45	10:50	11:10	11:20	11:50	12:20
Glucose, mmol/L	4.2	4.2	**2.0**	3.4	9.4	11.2
GH, µg/L	5.1	1.2	3.4	**7.5**	5.2	1.3
Cortisol, nmol/L	294	337	551	606	**716**	554
FSH, U/L		0.5	2.9		**3.2**	
LH, U/L		<0.5	**2.0**		1.6	

Insulin and GnRH administered at 10:50

Subject 24 (male, 8.5 years)

ITT and GnRH test

Time (mins)	-35	0	20	30	60	90
Time (actual)	09:45	10:20	10:40	10:50	11:20	11:50
Glucose, mmol/L	4.3	4.7	**1.0**	3.6	5.1	4.4
GH, µg/L	0.8	0.2	0.2	0.4	3.8	**3.9**
Cortisol, nmol/L	373	243	211	483	**645**	638
FSH, U/L		0.5	2.6		**3.0**	
LH, U/L		<0.5	<0.5		**1.1**	

Insulin and GnRH administered at 10:20

Subject 25 (male, 6.5 years)

Glucagon test and GnRH test

Time (mins)	-50	0	20	60	90	120	150	180
Time (actual)	09:30	10:20	10:40	11:20	11:50	12:20	12:50	13:20
Glucose, mmol/L	4.3	4.2	7.0	5.5	**3.1**	3.6	4.4	4.1
GH, µg/L	1.9	4.1	1.7	1.5	1.9	**14.0**	5.7	2.3
Cortisol, nmol/L	415	485	408	297	322	559	**729**	673
FSH, U/L		1.1	3.5	**4.4**				
LH, U/L		<0.5	**0.7**	0.5				

Glucagon and GnRH administered at 10:20

Subject 26 (female, 15.6 years)

Glucagon test and GnRH test

Time (mins)	-45	0	20	60	90	120	150	180
Time (actual)	10:10	10:55	11:15	11:55	12:25	12:55	13:25	13:55
Glucose, mmol/L	3.9	4.2	8.1	6.4	5.0	3.8	**3.2**	6.0
GH, µg/L	2.0	-	0.3	0.3	0.2	4.8	**9.4**	3.4
Cortisol, nmol/L	152	-	106	262	279	378	561	**606**
FSH, U/L	4.2*		11.6	**13.8**				
LH, U/L	12.2*		**106.0**	89.6				

Glucagon and GnRH administered at 10:55. Day 17 of 35-day menstrual cycle (ovulating).
*0 min. sample broke in the centrifuge therefore -30 min. sample used for baseline LH and FSH

Subject 27 (female, 18.9 years)

Glucagon test and GnRH test

Time (mins)	-45	0	20	60	90	120	150	180
Time (actual)	09:55	10:40	11:15	-	-	-	13:30*	-
Glucose, mmol/L	4.5	4.4	7.6	-	-	-	-	-
GH, µg/L	1.0	0.2	0.2	-	-	-	**10.9***	-
Cortisol, nmol/L	316	252	IS	-		-	**624***	-
FSH, U/L		6.1	IS	-				
LH, U/L		10.2	IS	-				

IS, insufficient. Glucagon and GnRH administered at 10:50. *N.B. Cannula came out at 40 min. Re-cannulation attempts failed but a venous blood sample was obtained at 140 min.

Subject 28 (female, 11.7 years)

ITT and GnRH test

Time (mins)	-35	0	20	30	60	90
Time (actual)	09:40	10:15	10:35	10:45	11:15	11:45
Glucose, mmol/L	5.1	4.9	**1.1**	2.1	3.7	5.8
GH, µg/L	0.3	0.4	1.8	**13.0**	8.8	3.2
Cortisol, nmol/L	236	225	211	334	**528**	406
FSH, U/L		6.9	**14.6**		14.1	
LH, U/L		4.2	**25.8**		17.2	

Insulin and GnRH administered at 10:15

Subject 29 (female, 15.0 years)

ITT and GnRH test

Time (mins)	-35	0	20	30	60	90
Time (actual)	09:15	09:50	10:15	10:25	10:55	11:25
Glucose, mmol/L	4.6	4.7	**1.8**	2.1	4.9	6.7
GH, µg/L	0.2	0.3	5.7	13.9	**25.4**	14.1
Cortisol, nmol/L	622	473	369	369	**526**	418
FSH, U/L		3.4	**7.5**		7.4	
LH, U/L		2.4	**20.9**		17.4	

Insulin and GnRH administered at 09:55

Subject 30 (female, 15.3 years)

ITT and GnRH test

Time (mins)	-30	0	20	30	60	90
Time (actual)	10:00	10:30	10:55	11:05	11:35	12:05
Glucose, mmol/L	3.9	4.0	1.6	**1.2***	3.1	3.7
GH, µg/L	0.8	0.3	0.3	0.7	**10.6**	6.5
Cortisol, nmol/L	146	99	88	96	**414**	382
FSH, U/L		2.3	2.4		**2.5**	
LH, U/L		1.0	**15.6**		10.6	

Insulin and GnRH administered at 10:35. Day 21 of 35-day menstrual cycle.
*Glucose meter readings at 35 and 45 min.: 2.0 and 3.2 mmol/L.

Subject 31 (female, 13.0 years)

ITT and GnRH test

Time (mins)	-85	0	20	30	60	90
Time (actual)	09:20	10:45	11:05	11:15	11:45	12:15
Glucose, mmol/L	4.2	4.2	**1.0**	2.2	4.7	4.1
GH, µg/L	0.2	3.8	2.3	**5.7**	<0.1	<0.1
Cortisol, nmol/L	298	225	220	294	**542**	372
FSH, U/L		5.8	**7.9**		6.8	
LH, U/L		7.7	**20.4**		12.3	

Insulin and GnRH administered at 10:45. Day 20 of 28-day menstrual cycle.

Subject 32 (female, 14.6 years)

ITT and GnRH test

Time (mins)	-50	0	20	30	60	90
Time (actual)	09:20	10:10	10:30	10:40	11:10	11:40
Glucose, mmol/L	4.2	4.1	**1.3**	4.7	6.4	3.5
GH, µg/L	0.1	0.3	5.4	**13.2**	10.5	4.3
Cortisol, nmol/L	562	458	297	611	**660**	485
FSH, U/L		3.5	**5.9**		5.7	
LH, U/L		9.5	**38.9**		25.1	

Insulin and GnRH administered at 10:10. Menstrual irregularities; unclear where in cycle.

Subject 33 (female, 21.7 years)

ITT and GnRH test

Time (mins)	**-35**	**0**	**20**	**30**	**60**	**90**
Time (actual)	08:55	09:30	09:50	10:00	10:30	11:00
Glucose, mmol/L	4.9	4.6	**1.4**	1.6*	3.4	4.0
GH, µg/L	8.2	3.0	1.2	2.2	**13.8**	8.6
Cortisol, nmol/L	589	439	340	337	643	**707**
FSH, U/L		3.3	6.5		**8.2**	
LH, U/L		6.3	**65.8**		56.1	

Insulin and GnRH administered at 09:30. Day 16 of 28-day menstrual cycle.
*Glucose meter readings at 35 and 50 mins: 3.5 and 4.1 mmol/L

Suspected NAHI group

Subject A (male, 5.0 years)

Glucagon test and GnRH test

Time (mins)	-35	0	20	60	90	120	150	180
Time (actual)	09:45	10:20	10:50	11:25	11:55	12:25	12:55	13:25
Glucose, mmol/L	3.9	4.0	8.3	6.0	**2.5**	4.0	3.8	4.8
GH, µg/L	1.9	1.6	1.1	1.7	**8.7**	<0.1	1.9	1.2
Cortisol, nmol/L	266	266	280	375	520	719	790	**830**
FSH, U/L		<0.5	3.5	**3.8**				
LH, U/L		<0.5	**1.4**	0.9				

Glucagon and GnRH administered at 10:25

Subject B (male, 3.7 years)

ITT and GnRH test

Time (mins)	-30	0	20	30	60	90
Time (actual)	09:30	10:00	10:25	10:35	11:05	11:35
Glucose, mmol/L	4.4	4.3	**2.0**	4.3	6.2	7.2
GH, µg/L	0.3	0.3	4.4	**8.0**	4.0	0.9
Cortisol, nmol/L	309	283	252	540	**688**	492
FSH, U/L		0.5	5.3		**7.4**	
LH, U/L		<0.5	**1.6**		1.4	

Insulin and GnRH administered at 10:05