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The prevalence of endocrine dysfunction in head injured children and adolescents and its impact on health, cognition, emotional/behavioural status and health related quality of life (Kid's Head Injury Neuro-Endocrine Study, "KHINES")

The prevalence of endocrine dysfunction in head injured children and adolescents and its impact on health, cognition, emotional/behavioural status and health related quality of life (Kid's Head Injury Neuro-Endocrine Study, "KHINES")

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Research Governance Statement

This study was conducted in accordance with the Research Governance Framework for Health and Social Care and Good Clinical Practice.

ABSTRACT

Background: Traumatic Brain Injury (TBI) is a major cause of death and acquired disability. Adult studies demonstrate a high prevalence of endocrine dysfunction post-TBI with associated clinical morbidity but paediatric data are sparse. Pituitary hormone deficiencies adversely affect growth, sexual maturation, energy levels but also cognitive function, particularly memory and concentration.

Hypotheses: 1) Long-term endocrine dysfunction occurs in children following severe/moderate TBI, and possibly mild TBI. 2) There is a relationship between neuro-endocrine dysfunction in TBI children and impaired physical, cognitive and psychological functioning and reduced health related quality of life (HRQL).

Aims: To investigate in an established well-characterised prospective cohort of severe/moderate/mild TBI adolescents and non-injured controls endocrine status and relationship of neuro-endocrine function and neuroimaging changes post-TBI to measures of outcome including cognition, psychological status, fatigue and Health-Related Quality of Life (HRQL).

Methods: TBI participants who had previously participated to KHIS (Kid's Head Injury Study, Bristol 2002-2004). Seventy-two participants (age 10-26y, time from TBI 6-11y) completed the study. (Group 1 [control group n=17, 14M], Group 2 [mild TBI n=24, 14M] and Group 3 [moderate/severe TBI n=31, 20M]). The following assessments were completed (a-c group 3 only, d-e all groups): a) Baseline endocrine status b) GH status (Insulin Tolerance Test [ITT] and overnight 12 hour GH profile), c) HPA axis status (ITT, overnight 12 hour cortisol profile and salivary cortisol profile), d) cognitive, psychological and HRQL assessment, e) pituitary and hippocampus imaging (MRI).

Results: No auxological differences between groups (height, weight, BMI, body fat percentage). No cases of precocious puberty or diabetes insipidus. One female from group 3 had primary amenorrhea and GH deficiency. In group 3, GH response to ITT was abnormal in 7/25. Mean 12-hour GH secretion was low (<1mcg/L) in 4/22 profiles. Peak spontaneous GH secretion was low in 1/22 profiles. There was no correlation between stimulated and spontaneous GH levels. Spontaneous (but not stimulated) GH secretion correlated with IGF1 levels. Cortisol response was suboptimal in 2/25 ITT. Peak spontaneous cortisol was under 500 nmol/l in 9/22 profiles, stimulated levels were normal in 7 of these. Spontaneous and stimulated cortisol levels correlated strongly. Salivary cortisol analysis did not demonstrate differences between groups in regards to diurnal rhythm, awakening response or suppression with dexamethasone. Verbal IQ was lower in the mod/sev TBI group who also showed difficulties with both externalising (conflict, aggression, rule-breaking), internalising behaviour (withdrawal, anxiety, depression) and working memory. TBI survivors reported high levels of depression (14/46) and fatigue (21/46). HRQL was lower in TBI participants mainly because of lower psychosocial scores. Neuroimaging did not demonstrate any structural pituitary abnormality. Voxel based morphometry showed reduced grey matter and right hippocampus volume in the mod/sev TBI group.

Conclusions: Childhood TBI is associated with long term endocrine dysfunction. Although all had structurally normal pituitary glands, GH status in mod/sev TBI survivors based on ITT was abnormal in 28% and in 18% based on overnight GH profile. This did not affect their growth or body composition. Fatigue correlated with measures of spontaneous but not stimulated GH. High rates of behaviour problems including aggression, rule breaking behaviour but also depression, reduced HRQL and poor working memory were observed.

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1 LIST OF ABBREVIATIONS

ABCL	Adult Behaviour Checklist
ACTH	Adrenocorticotropic hormone
ApEn	Approximate Entropy
AUC	Area Under the Curve
BAI	Beck Anxiety Inventory
BDI	Beck Depression Inventory
BIA	Bioelectrical Impedance Analysis
BRIEF	Behaviour Rating Inventory of Executive Function
BSA	Body Surface Area
BMI	Body Mass Index
CAR	Cortisol Awakening Response
CBCL	Child Behaviour Checklist
CI	Confidence Interval
COR	Cortisol
CRH	Corticotropin Releasing Hormone
CFS	Chronic fatigue syndrome
CMS	Children's Memory Scales
CSF	Cerebrospinal fluid
CT	Computer Tomography
DAI	Diffuse Axonal Injury
DEXA	Dual-energy x-ray absorptiometry
DTI	Diffusion Tensor Imaging
DWI	Diffusion weighted imaging
F	Female
FA	Fractional anisotropy
FLAIR	Fluid-attenuated inversion recovery
fMRI	Functional MRI
fT3/fT4	Free T3 / free T4
FSH	Follicle stimulating hormone
FOV	Field of View
GCS	Glasgow Coma Scale
GH	Growth Hormone
GHRH	Growth hormone releasing hormone
GM	Grey matter
hGH	Human Growth Hormone
HRQL	Health Related Quality of Life
HPA	Hypothalamus-Pituitary-Adrenal
HUI	Health Utilities Index
IGF1	Insulin Growth Factor 1
IGF-BP3	Insulin Growth Factor Binding Globulin 3
IQ	Intelligence Quotient
ITT	Insulin tolerance test
i.v.	intravenous
KHINES	Kid's Head Injury NeuroEndocrine Study
KHIS	Kid's Head Injury Study
LH	Luteinising hormone
LOC	Loss of consciousness
M	Male
mg	milligram
mmol	millimole

mU	milli unit
MRI	Magnetic resonance imaging
pmol	picomole
PRL	Prolactin
PTHP	Post-Traumatic Hypopituitarism
PTSD	Post-Traumatic Stress Disorder
PVN	Paraventricular Nucleus
REM sleep	Rapid Eye Movement sleep
SCN	Suprachiasmatic Nucleus
SD/SDS	Standard deviation / standard deviation score
SS	Somatostatin
TBI	Traumatic Brain Injury
TEACh	Test of Everyday Attention in Children
TEA	Test of Everyday Attention
TIV	Total intracranial volume
TSE	Turbo Spin Echo
TR/TE	Repetition time / Echo time
TSH	Thyroid stimulating hormone
VBM	Voxel Based Morphometry
WASI-II	Wechsler Abbreviated Scale of Intelligence - Second Edition
WM	White matter
WMS	Wechsler Memory Scales
WRAT-4	Wide Range Achievement Test - Fourth Edition
y	year
>	Greater than
<	Less than
>	Equal or greater than
<	Equal or less than
=	Equal
%	Percent

KHINES STUDY

1 CHAPTER 1 (INTRODUCTION)

1.1 TBI EPIDEMIOLOGY

Traumatic brain injury (TBI) - an acute brain injury resulting from mechanical energy forces to the head from external sources - is a major cause of hospital admission in children and the leading cause of acquired neurological morbidity. Previous studies reported that 1 in 100-200 children are admitted to hospital each year following TBI of all severities [1] with 80% of them being mild and 20% severe or moderate according to the Glasgow Coma Scale [2]. The [GCS] – a neurological scale aiming to offer an objective way to record the level of consciousness - has been conventionally used to classify brain injury in adults. A modified version [Table 1] has been developed for children (Paediatric GCS) [3] to account for the poor verbal performance of even health very young children. An admission GCS score of 13-15 signifies mild, 9-12 moderate and 8 or below severe TBI. Although mortality rates are higher among patients with lower admission GCS TBI (up to 100% with admission GCS of 3), the GCS on its own cannot reliably predict the neuropsychological outcome in children with mild [4] or even severe TBI in the absence of prolonged hypoxemia [5]. When compared to adults, severe TBI in children is associated with lower mortality rates and better functional outcome [6].

	>5 years	<5 years
Eye opening		
E4	Spontaneous	
E3	To voice	
E2	To pain	
E1	None	
C	Eyes closed (by swelling or bandage)	
Verbal		
V5	Orientated(in person or place)	Alert, babbles, usual ability
V4	Confused	Less than usual ability, irritable cry
V3	Inappropriate words	Cries to pain
V2	Incomprehensible sounds	Moans to pain
V1	No response to pain	No response to pain
T	Intubated	Intubated
Motor		
M6	Obeys commands	Normal spontaneous movements
M5	Localises to supraorbital pain (>9 months of age) or withdraws to touch	
M4	Withdraws from nailbed pain	
M3	Flexion to supraorbital pain (decorticate)	
M2	Extension to supraorbital pain (decerebrate)	
M1	No response to supraorbital pain (flaccid)	

Table 1 Paediatric Glasgow Coma Scale [3]

TBI in the UK is more prevalent amongst children living in deprived areas with falls accounting for more than half of TBI in the under 5 years age group while in the 10-15 years age group road traffic accidents are the most common cause [7]. TBI as a result

of child abuse is more likely to occur in infants [8]. These data collected from a regional UK register of all children admitted with TBI still represents a very conservative estimate of the true incidence of TBI in childhood and an overestimate of the proportion of severe TBI, as most cases of mild TBI are not seen in hospital. Population studies estimate that 3.8% of the population experienced at least one hospital admission due to TBI by 35 years of age [9]. Almost a third of the general population will have sustained a TBI by 25 years of age as reported in recent prospective population studies such as the Christchurch Birth Cohort study [10], BIONIC (Brain Injury Outcomes New Zealand In the Community) study group in New Zealand [11] and Olmsted County (MN, USA) [12]. These large, population-based studies showed the incidence of TBI to be 550-800 per 100,000 people per year. In addition, even cases of mild TBI that present to hospital emergency departments are likely to be misdiagnosed by medical personnel who sensibly focus on ruling out a more severe brain injury that may require additional monitoring or intervention. It appears however that once moderate/severe brain injury is ruled out (normal CT scan, only brief loss of consciousness [LOC] with apparently good recovery) patients with no obvious clinical signs of mild TBI by the time they arrive at the ED are more likely *not* to be diagnosed with mild TBI (greatest discrepancy when assessing degree of confusion) [13].

For more serious types of injury, requiring admission to paediatric intensive care units, the incidence of severe TBI is 5.6 per 100,000 population per year. There was a summer peak in admission in children under 10 years with timing of the TBI being mostly in the evening [14]. Extrapolation from these data suggest the UK incidence of severe/moderate TBI in the paediatric population to be 11.2 per 100,000 per year.

Causes of childhood TBI in developed countries vary with age. Falls (interestingly the major cause of TBI in adults older than 65 years of age), pedestrian road accidents and inflicted injuries are the most common causes in infants and children younger than 14 years of age while motor vehicle accidents, assaults and contact sports injuries predominate at older ages. Male children and adolescents have a greater risk of TBI due to transport accidents, exposure to mechanical forces or assaults compared to girls and women of the same age groups [11].

1.2 TBI PATHOPHYSIOLOGY

In TBI, acceleration–deceleration motions over different planes (sometimes without actual impact) apply forces to the brain, which can deform white matter. Deformation of white matter results in diffuse axonal injury (DAI) through shearing forces [15]. With impact injuries, basal skull fractures may damage the pituitary and/or the

hypothalamus directly. Even without skull fracture, tethering of the pituitary to the hypothalamus with the pituitary stalk makes it delicate and vulnerable to trauma. Blood supply to the anterior pituitary is via the short and long portal vessels. In particular, areas containing the somatotropic and gonadotropic cells (usually in the periphery of the pituitary) are supplied mainly by the long portal vessels making them more vulnerable to ischaemic injury compared to the corticotropic cells which receive their blood supply by both long and short portal vessels [16]. This would suggest that the somatotropic and gonadotropic cells are more likely to be affected by disruptions in pituitary blood supply. Following DAI, oedema (which may cause herniation through the diaphragmatic opening and secondary compression [17]), ischaemia and excitogenic neurotransmitters may also contribute to the resulting diffuse damage [18]. In the chronic TBI phase, loss of pituitary gland volume, perfusion deficits, absence of posterior pituitary signal have been described in 80% of patients with hypopituitarism and 29% of those without hypopituitarism indicating that pituitary imaging abnormalities are more common in TBI patients with hypopituitarism than those without [19]. Although several studies suggest that severe TBI is more likely to be associated with post-traumatic hypopituitarism (PTHP) [20-22] other studies have not found any correlation between PTHP and injury severity as assessed with the GCS [23-25]. Interestingly repeated mild TBI may also be implicated in the development of PTHP as reported in studies of amateur boxers [26, 27].

1.3 TBI AND HYPOPITUITARISM

The relationship between head trauma and endocrine dysfunction was first described in case reports early in the beginning of the last century [Cryan E. 1918] but further work on this subject was not published for half a century [28]. Although the wide range of pathological changes to the post-TBI brain had been described in detail [17], it was only after advances in intensive care and subsequent improved survival of TBI patients that more cases of hypopituitarism started being reported [29]. Following these initial case reports of PTHP, there has been an increasing recognition that TBI in adults can cause endocrine dysfunction as well as neurological, cognitive and psychological sequelae [30]. Prospective follow-up studies published over the last decade have now conclusively demonstrated hypopituitarism as a cause of endocrine dysfunction following TBI in adults and an association with major negative impacts on health. Although not as common as anterior pituitary damage, posterior pituitary dysfunction has a more dramatic presentation and will prompt further neuroendocrine assessment [31]. Benvenga et al (2000) reviewed 367 literature case reports and 15 new cases and

concluded that almost half of TBI patients showed evidence of adrenocorticotropic hormone (ACTH) or growth hormone (GH) deficiency during the first year after injury and delay in diagnosis was a common feature. Kelly et al (2000) described similar findings in almost half of 22 head-injured patients with GH and gonadotrophin deficiencies being most common [21]. A subsequent study by Lieberman (2001) of 70 TBI patients from a transitional learning community found that 45% of them had subnormal morning cortisol levels, and an abnormal cortisol response to a high-dose Synacthen test in 7% [23]. Subsequent follow up studies have reported 20-30% prevalence of PTHP in adults following severe/moderate TBI [32-34] with some however reporting much higher (up to 80% in the acute post TBI period [35] and 50% after a year [36]) and some as low as 5% [37] - a reflection of differences in inclusion criteria, time since TBI, use of different diagnostic tests and cut-off values [38]. Although multiple hormone deficiencies have been described a year post TBI (range 3-50%) most participants were deficient in a single axis. Isolated GH deficiency is more prevalent (8-38% [39]) which is probably an indication of the susceptibility of somatotroph cells to ischaemia or hypoxia.

Despite however the increasing number of studies in adult PTHP, data regarding PTHP in childhood are limited. Aimaretti et al (2005) studied 23 adolescents and young adults after TBI and reported PTHP in a third of the patients 3 months post TBI that persisted after one year [40]. Ulutabanca et al (2014) in a prospective study of 41 children (age 7±4 years) with TBI found PTHP in 44% (18/41) of them in the acute post TBI phase. 10/41 had low baseline cortisol levels (<15 µg/dL) with inappropriately low ACTH, 7/41 were diagnosed with central hypothyroidism (low TSH and fT4) and 1/41 with hypogonadism. IGF1 was measured in all patients but no GH provocation tests were undertaken in the acute phase. When patients were reviewed 12 months post TBI, thyroid status was normal in all patients. GH stimulation testing (GHRH-arginine) was abnormal in two patients (2/22, 9.1 %) but only one demonstrated slow growth post-TBI. One of these patients had also low morning cortisol (<5 µg/dL – a different, lower cut-off was used for diagnosing adrenal insufficiency in the chronic vs acute phase) with inappropriately low ACTH. This study however was limited by the absence of dynamic testing for GH (acute phase) and cortisol deficiency (both in the acute and chronic phase). In addition, only half of patients initially recruited were reviewed after 12 months (22/41) [41].

In a cross-sectional study of 36 children with mod/sev TBI who were discharged from a Paediatric Intensive Care Unit (all patients has either skull fracture or intracranial haemorrhage), no clinical evidence of PTHP was observed 3 years post injury. The

mean age at injury was 4 years so it is possible that the low incidence of PTHP in this study is a reflection of the different injury mechanism seen in this age group (falls rather than high velocity collisions) but also elective use of stimulation tests. In this study, stimulation tests were performed only if baseline, blood hormone levels were abnormal or when clinical findings suggestive of endocrine dysfunction were present [42]. In a prospective study of an older group of 87 children and adolescents (median age 6.7, range 0.8-15.2 years) who were hospitalised with severe TBI, PTHP was found in 6% a year after TBI applying strict criteria (two stimulation tests with GH response <5 mcg/L and IGF-1≤2SDS) [43]. In another prospective study, 23 TBI children (age >6 years) underwent two stimulation tests (glucagon and clonidine). Almost half (47.8%) showed a suboptimal GH response (using however a 10 mcg/L cut-off [44]) three months post TBI which persisted in most of them (8 out of 11) after 1 year. No endocrine abnormalities were found in a subgroup of younger patients (<6 years) who were not however assessed with stimulation tests [45].

Although older age and male gender appear to increase the risk of PTHP, the risk of endocrine dysfunction appears to be much higher if the mechanism of injury is non-accidental. Auble et al (2014) found endocrine dysfunction in 80% in children (57%, if hyperprolactinaemia was excluded) with moderate/severe inflicted TBI. Half of them had multiple hormone deficiencies [46].

In contrast to the above study, Heather et al (2012) concluded that permanent PTHP is rare after both non-accidental (33%) and accidental (67%) TBI in early childhood, in one of the largest cross-sectional studies with longitudinal follow-up. Almost two hundred survivors of structural TBI were thoroughly assessed 3-9 years post injury. The endocrine assessment for GH included two dynamic tests of pituitary function (clonidine and arginine) using a cut-off of 5 mcg/L. Eight percent of children demonstrated an abnormal GH response but had normal height and IGF-1 (Insulin Growth Factor 1) and IGF-BP3 (Insulin Growth Factor Binding Globulin 3). This group was followed-up for a period of 6–36 months, and all children showed normal height velocity and had normal GH response on repeat testing [47].

Khadr et al (2010) in their retrospective exploratory study of 33 TBI survivors (mean age 13.4 years) did not find any clinically significant endocrinopathy 1–8 years post injury, but minor abnormalities of the pituitary axes were observed [48]. Finally, in two of the first paediatric TBI studies Poomthavorn et al. (2008) detected endocrine abnormalities in 17% of severe TBI survivors but the study was limited by selection bias as only half of the participants had baseline blood tests and only 8/54 stimulation

tests [49]. An earlier study by Einaudi et al (2006) found that 10.4% of the 48 patients developed thalamo-hypophysial dysfunction 6 months or more after TBI [50].

The nature of the relationship between hormone deficiencies and clinical outcome in paediatric TBI survivors remains poorly understood. Reviews of the subject confirm the pressing need to obtain such data [51] as hormone deficiencies may produce additional morbidity in TBI patients who already have a high burden of physical, cognitive and psychiatric disability.

The mismatch between expected and diagnosed PTHP cases has also been a matter of debate. The relatively small number of reported cases with PTHP (mainly GH deficiency) that are registered in international databases, suggest that PTHP is underestimated or under diagnosed [52]. Certainly growth monitoring - the most sensitive indicator of pituitary function in children – is not consistently reported in every clinical setting, highlighting the need for a co-ordinated approach in any setting (hospital or primary care), that is involved in the follow-up of children with TBI [53]. Although there is no agreement that the GCS is a reliable predictor of PTHP, it has been suggested that all adult patients with moderate to severe TBI should have systematic screening of pituitary function to benefit from treatment of PTHP [54]. There are not sufficient data however to support similar practice for childhood TBI. The studies by Heather et al showing permanent hypopituitarism as being a rare consequence after both inflicted (abusive head trauma) and accidental structural TBI in early childhood [47] and Salomon-Estebanez showing absence of endocrine sequelae in children with TBI related skull fracture or intracranial haemorrhage [42] support the above practice. The former study however targeted a group of very young children (mean age of 1.7 ± 1.5 yr) and these findings are probably not applicable to adolescents, whose physical characteristics and TBI mechanism are similar to those seen in adults. In the latter study (which also included prepubertal children), only participants who had low IGF1 were reassessed after one year. In all of them IGF1 levels normalised after a year so no further tests were undertaken. Once again, the authors highlight the importance of growth monitoring in children after TBI and suggest that “invasive assessments should be reserved for selected cases where there is slow growth or other clinical suspicion of hypopituitarism”.

Assessments of endocrine function can be complex and difficult to interpret. Although thyroid, gonadotropin and posterior pituitary function can be reliably assessed with a baseline blood test, diagnosis of GH or ACTH deficiency is more problematic. For practical reasons dynamic, non-physiological and potentially dangerous tests are still being used. Unfortunately, interpretation of these tests and their numerous

combinations remains complex. By using the same and sometimes arbitrary diagnostic cut-offs in different populations (age, weight, pubertal stage) with different assays, it would be reasonable to assume that part of the variability of the reported prevalence of PTHP is related to different study methodologies and interpretation of tests. Although alternative approaches for diagnosing GHD based on combinations of auxological, radiological, biochemical and genetic measures have been proposed [55], stimulation tests remain the standard for assessing GH status as GHD is a diagnosis based on biochemical evidence of reduced/absent GH secretion.

1.3.1 Growth hormone

1.3.1.1 Growth hormone structure

Growth hormone (GH) is a 191 amino acid polypeptide with a molecular weight of 22-kDa. In addition to the 22-kDa isoform that constitutes 75-90% of pituitary GH, several other molecular isoforms of GH exist (20-, 17-, 5-kDa), the physiological significance of which remains mostly unknown [56]. The 20-kDa isoform, which represents 10% of circulating GH, is co-secreted with the 22-kDa GH lacking amino acids 32-46. The biological activity of both forms is thought to be comparable [57]. Some of these isoforms are detected to a variable degree by available GH assays. As a result measured levels of GH differ depending on the assay used [58]. The main GH isoform and these variants circulate partially bound (up to 50% [59]) to binding proteins (growth hormone-binding protein, GHBP), which have a structure identical to the extra-cellular domain of the GH receptor [60].

1.3.1.2 Growth hormone physiology

GH is secreted from somatotroph pituitary cells in a pulsatile pattern following constantly changing equilibrium between stimulatory (GH-releasing hormone [GHRH] alone or in combination with ghrelin [61]) and inhibitory (somatostatin [SS]) signals [62] [Figure 1].

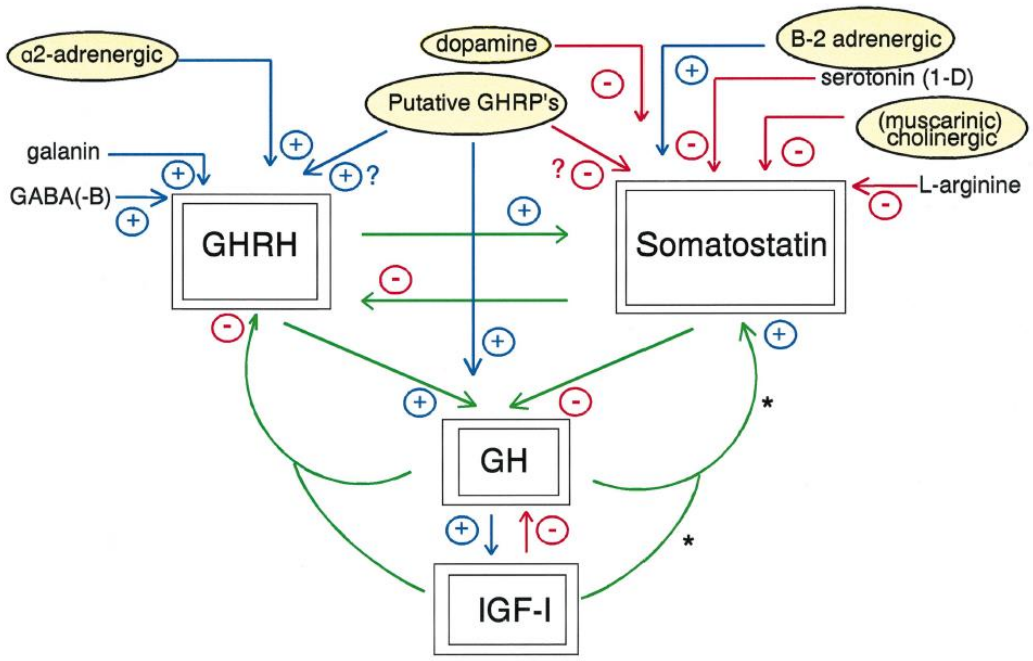


Figure 1 Human GH axis – neuromodulators

GHRH is predominantly produced in the arcuate nucleus while SS in the paraventricular nucleus [Figure 2]. SS determines the trough levels of GH by inhibiting GHRH release. Pulses of GH occur when SS tone is withdrawn – this usually every 3-4 hours. A variety of neurotransmitters, metabolites (glucose, amino acids), hormones (oestrogen, testosterone, insulin) but also GH itself and IGF-1 may exert a positive or negative effect when binding to specific hypothalamic receptors and therefore mediate GH secretion [63]. Although fasting normally increases GH secretion via inhibition of SS release, this effect is not seen in obese patients. This suggests that metabolic changes (insulin resistance) rather than hypothalamic mechanisms are responsible for the low GH levels seen in obese patients [64].

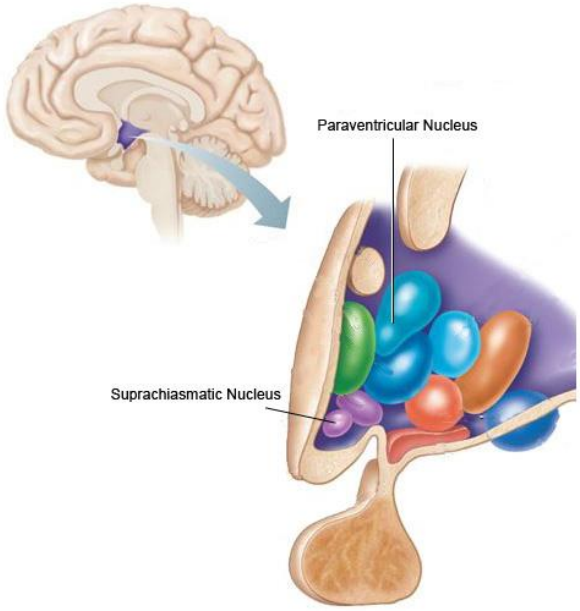


Figure 2 Growth hormone related hypothalamic nuclei

The pulsatile pattern of GH secretion has been confirmed in other species from foetal life [65]. In humans, GH is secreted in markedly amplified GH-secretory bursts from the early neonatal period [66]. In the first day after birth, there is GH hypersecretion with increased frequency (every 70 min) and amplitude of GH pulses. Gradually and with the development of inhibitory mechanisms (mainly SS release), GH release becomes pulsatile. The effect of SS is not only on the pituitary level but also on hypothalamic GHRH and insulin release [67]. The net result of high circulating SS is a decrease in the mass and frequency of GH secretory events. As with other hormones (gonadotrophins), pulsatile GH secretion may be necessary for tissue specific responses.

Although the pulsatile pattern of GH secretion continues throughout childhood and adult life, the frequency and amplitude of GH pulses evolves. During puberty there is a marked increase in GH secretion. This is due to increased amplitude of GH pulses with some notable differences between men and women. In men, GH pulsatility is more evident during the night with large pulses and low trough levels while in women there is less diurnal variation with higher trough levels and nocturnal pulses of lower amplitude [68].

Single measurements therefore do not reflect endogenous GH secretion and will produce an uninterpretable result in virtually all cases except during the early neonatal

period when SS tone is not yet established. Various protocols have therefore been developed to access GH secretion in a more predictable and controlled way.

1.3.1.3 Assessing GH status

Testing methods used for diagnosing impaired GH secretion include pharmacological (stimulated GH secretion) and physiological (measurement of spontaneous GH secretion over a period of time – usually 12 or 24 hours). There is considerable variability in GH status even when repeating the same test in the same individual, which suggests that the variability is test- rather than patient-related [69].

1.3.1.3.1 Pharmacologic tests for GH secretion

A number of different stimulation tests using various stimuli have been used for assessing GH status. In decreasing frequency based on a pharmaco-epidemiological survey these include pharmacological agents like insulin, arginine, clonidine, L-dopa, glucagon, GHRH, ornithine [70].

Historically insulin was used as an indicator of growth hormone status based on the ability to recover from the induced hypoglycaemia. Roth et al [71] was the first to demonstrate the GH response to insulin-induced hypoglycaemia (Insulin Tolerance Test [ITT]) in 6 healthy adults. By sampling every 30 min for several hours, he was able to demonstrate a rise in GH levels 30 min after hypoglycaemia in all except one participant, to levels usually found in “random plasma samples from acromegalic subjects”. This study became the basis of using insulin-induced hypoglycaemia as a direct test of pituitary somatotropic function. Further studies [72] confirmed this observation in both healthy volunteers and patients with hypothalamic or pituitary dysfunction. The author also described – in his healthy, adult volunteer group - markedly variable, individual GH levels but also poor reproducibility of the GH response in comparison to cortisol, free fatty acid and plasma glucose.

The test is regarded as safe when done by appropriately trained staff, in a safe environment and in patients without a history of seizures or heart disease. An audit of over 500 tests over a 10 year period (1989–99) from a tertiary paediatric centre in the UK, reported no serious adverse events [73]. An earlier serious incident associated with the ITT involved the administration of inappropriately large quantities of intravenous dextrose to correct the insulin induced hypoglycaemia [74].

When interpreting the result of an ITT it is important to consider the following factors to avoid misclassification.

- The test is sensitive to weight and therefore not as reliable in diagnosing GH deficiency in obese children [75].
- As sex steroids enhance GH secretion during puberty, the practise of “priming” children of almost normal pubertal age with sex steroids for the purpose of the test, may have a similar effect.
- Finally, age appropriate cut-offs should be used. In adults having an ITT, a GH cut-off of 3mcg/L has been accepted [76] to diagnose GHD, while in children 7-10 mcg/L is being used in most centres. During the transition phase, a cut-off of 5 mcg/L achieved the best sensitivity and specificity [77].

1.3.1.3.2 Physiologic tests for GH secretion

The first detailed studies in physiological GH secretion patterns in healthy adult participants, showed increased GH secretion with the onset of sleep. Sleep consists of 5 stages (1, 2, 3, 4 and REM [Rapid Eye Movement]) and sleep cycle is defined as the period of time it takes for an individual to progress through all stages before returning to stage 1. A typical full cycle last about 100 minutes [78]. GH is released mainly during sleep stages 3 or 4 and is suppressed during periods of awakeness [79, 80]. The described GH secretion pattern is relatively uniform, starting 1-2 hours after sleep onset. The observed peak GH levels are reported to be comparable to those seen during stimulation tests. Interestingly this pattern of GH secretion is related only to sleep onset without any obvious circadian rhythm [81] [Figure 3].

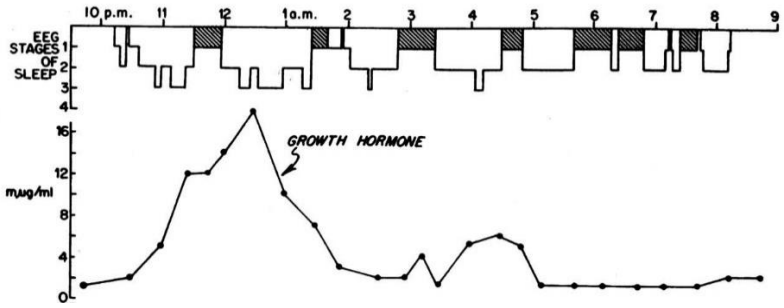


Figure 3 Growth hormone secretion in relation to sleep stages (Takahashi et al)

A similar pattern has been described in children where GH secretion during the day was lower than during the night period with the difference being more marked in short children [82] and some authors concluding that by using a single sample 1 hour after the onset of deep sleep, GHD could be precluded in as many as 70% of children [83]. In another study with similar design of short, prepubertal children without GHD (i.e. normal GH response to one or more pharmacological tests), **at least one** GH secretory

peak was observed in all children during sleep which was above the cut-off being used in that centre for pharmacological tests [84].

Early studies in children comparing peak GH response to ITT with peak GH concentration during the first cycle of stage 4 sleep, showed that results were discordant in less than 15% of children [85]. The authors suggest that the sensitivity of sleep sampling under EEG monitoring could be improved with information obtained from a complete 12 hour overnight profile as the maximum GH peak is not necessarily associated with the first cycle of stage 4 sleep. Growth velocity was also found to correlate with the mean GH level over 12 hours (or area under the curve), number of GH concentration peaks ≥ 5mcg/L and peak GH concentration 1 hour from sleep onset. Comparisons between normally growing and poorly growing children have shown that the difference in GH secretion is due to lower amplitude of same number of GH peaks rather than reduced number of GH secretory episodes [86].

When compared to GH stimulation tests, mean overnight GH secretion was found to have 100% specificity but variable sensitivity (54-95%) [87, 88]. Studies of spontaneous GH hormone secretion in normal children have shown that GH secretion depends on pubertal stage, sex, BMI and bone age and interpretation of data should therefore be done in relation to appropriate normative data which adds to the complexity of interpretation. These differences however become less significant at later states of puberty and mean overnight GH levels above 1mcg/L (using polyclonal RIA, Hazleton Biotechnologies, Vienna, VA) were seen in all healthy participants irrespective of bone age, sex or pubertal stage [89] [Figure 4].

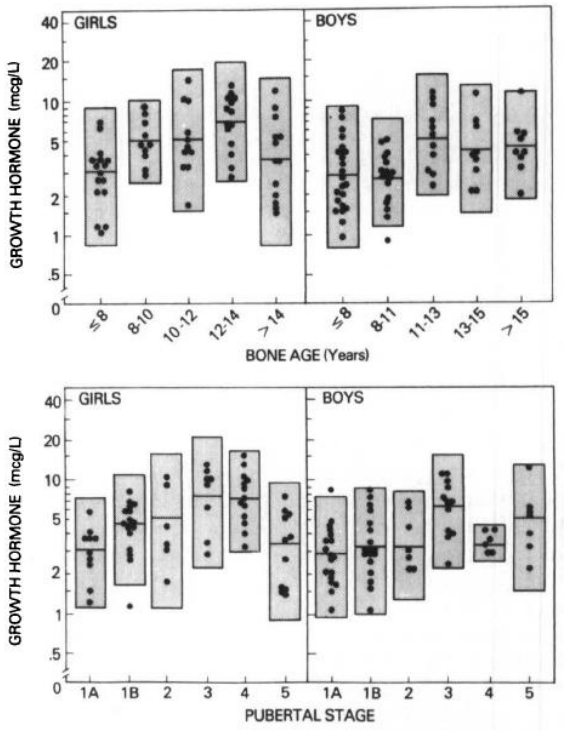


*Figure 4 Spontaneous growth hormone during puberty in normal girls and boys (*Reproduced from Rose, S.R., et al. J Clin Endocrinol Metab, 1991. 73(2): p. 428-35)

Other researchers have found a strong correlation between mean 12-hour nocturnal GH concentration and growth velocity in all slowly growing children (height velocity less than 3rd percentile for bone age) having a mean nocturnal GH concentration less than 4 mcg/L [90]. This led to the measurement of endogenous GH secretion in short children who were not GH deficient by conventional criteria (i.e. abnormal GH response to stimulation test). Results from these studies identified a subgroup of children where sleep-associated GH release was reduced and who benefited from GH therapy [91]. In another study spontaneous maximum GH concentration during night-time sampling in children was a better predictor for the response to GH treatment than peak GH concentration following provocation testing [92].

In a more recent study [93] comparing nocturnal GH secretion in pre-pubertal and post-pubertal lean and obese study participants using a modern, automated chemiluminescence assay (Nichols Luma Tag hGH), mean overnight GH concentration was 5.7±0.3 and 3.6±0.5 mcg/L for the lean and overweight pubertal participants respectively. The reduction in GH secretion in overweight participants was secondary to reduced GH burst mass (metric from deconvolution analysis of GH profiles) and half-life but not because of changes in number of secretory events. The former is controlled

by GHRH secretion while the latter by SS and the same pattern has been demonstrated in studies of obese adults [94].

Although the pattern of growth hormone secretion in pre-pubertal and post-pubertal boys and girls is similar, during puberty sex-specific increases in GH secretion rate occur (sexual dimorphism). These appear at an earlier pubertal stage and are more pronounced in girls than in boys [95]. Not only the secretion rate but also the orderliness (as measured by Approximate Entropy [96]) of GH release is controlled by sex steroid hormones with the pattern of GH release becoming more irregular in pubertal boys as sex steroid concentrations rise [97]. The increased GH secretion rates during puberty are a result of a 2-3 fold increase in the mean serum GH concentration peak amplitude. The frequency of detected GH pulses using discrete peak detection methodologies remains unchanged [98]. The same pattern has also been described in boys with constitutional delay of puberty treated with testosterone. In these boys, treatment with testosterone increased the amplitude of GH-secretory peaks without changing pulse frequency, or pulse duration [99, 100]. In normal girls, pubertal GH elevations are proportionate to the rise in serum oestradiol levels and reach a peak 2- to 3- fold increase compared to pre-puberty at menarche [101]. Interestingly, the enhanced GH secretion of puberty gradually declines to below prepubertal levels, despite continuing adult sex-steroid hormone concentrations [100] Figure 5.

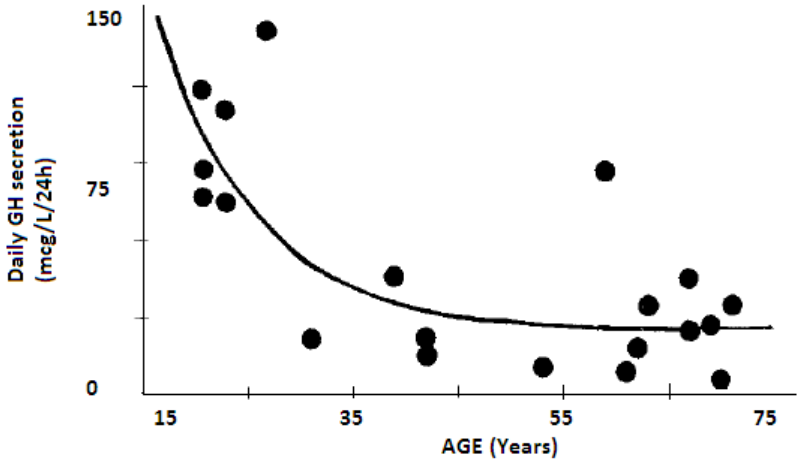


Figure 5 Daily GH secretion rates by age (Adapted from A. Iranmanesh et al. [102])

Spontaneous GH secretion rate estimates seem to be stable and reproducible [103, 104] and according to some researchers even more consistently reproducible compared to pharmacological tests [88]. Others however question the advantage of

spontaneous GH secretion compared to stimulation tests in prepubertal children, as measurements of spontaneous GH secretion were considerably less sensitive (sensitivity 57%; specificity 100%; predictive value of a low mean nocturnal GH level 100%; predictive value of a normal mean nocturnal GH level 76% when using a mean GH cut-off level of 1mcg/L) [87, 105]. These results did not change significantly even when the cut-off level for diagnosing GHD with the use of a stimulation test was lowered from 7 mcg/L to 5 mcg/L. According to the same authors, testing of spontaneous GH secretion did not identify any GH deficient children that had not been already identified by a stimulation test. They did however suggest that overnight GH studies could be useful in selected clinical settings such as cranial irradiation in oncology patients or other central nervous system disorders which may include inflammatory conditions and possibly TBI [106].

As stimulation tests are less labour intensive, require a smaller number of samples and considered to be equally reproducible by some authors they were adopted early [71] and became the standard method of estimating GH secretion despite not being able to provide an explanation of the slow growth in children with “normal” GH response to stimulation test. This condition, referred to as GH “neurosecretory dysfunction” highlighted the importance of assessment of spontaneous GH secretion especially as it was shown to correlate closely with growth rate [107-109] and subsequent clinical course in comparison to stimulation tests [110].

In line with pharmacologic tests for GH secretion, it is reasonable to accept that using a universal cut-off value to define normality when assessing spontaneous GH secretion is misleading, as GH secretion is a continuous variable (notwithstanding the inherent problems associated with the performance of GH assays and how the test is performed) [111, 112].

To complicate interpretation of GH secretion normality, measured plasma GH levels do not reflect actual GH secretion as the plasma concentration at any time point is the result of GH secretion from somatotroph cells in the pituitary, distribution of GH in various compartments, binding, metabolism and excretion/elimination. The exact timing of GHRH release will therefore be inevitably blurred by the secretory and post-secretory events.

Advances in computer technology made it possible to undertake complex analyses – initially separating pulsatile signal from noise and then deconvolution analyses - and obtain reliable estimates of the pulsatile pattern of endocrine systems including GH secretion [113]. This still requires sampling at optimal intervals, which are mainly

determined by the half-life of GH, for which the elimination mechanisms are still largely unidentified. Fortunately, endogenous GH hormone disappearance rates as determined by deconvolution, agree well with those reported after exogenous GH hormone injections but also from GH kinetic studies where GH and somatostatin were given in combination (somatostatin given in order to supress endogenous GH secretion). The half-life of GH is estimated to be between 9-15 min with a mono-exponential disappearance curve. This would suggest that 10-15 min GH sampling intervals are optimal in order to determine the pattern of secretion when using discrete sample methodology [114, 115]. With integrated sampling, in which blood is withdrawn continuously over time periods and assayed in defined time segments, 20 min subsampling frequency would seem to capture the essential major details of profiles [116]. More intensive 5-min or even 1-min sampling has been found to detect a higher number of peaks (all additional peaks however were detected within the GH major secretory peaks) when using an objective, statistically based pulse detection algorithm (Cluster). This suggests that major secretory episodes of GH release (detected fairly consistently with 10-30 min sampling) incorporate high-frequency GH secretory activity [117]. If however the focus is the secretion rate alone, longer sample intervals (up to 30-min) are sufficient [Figure 6].

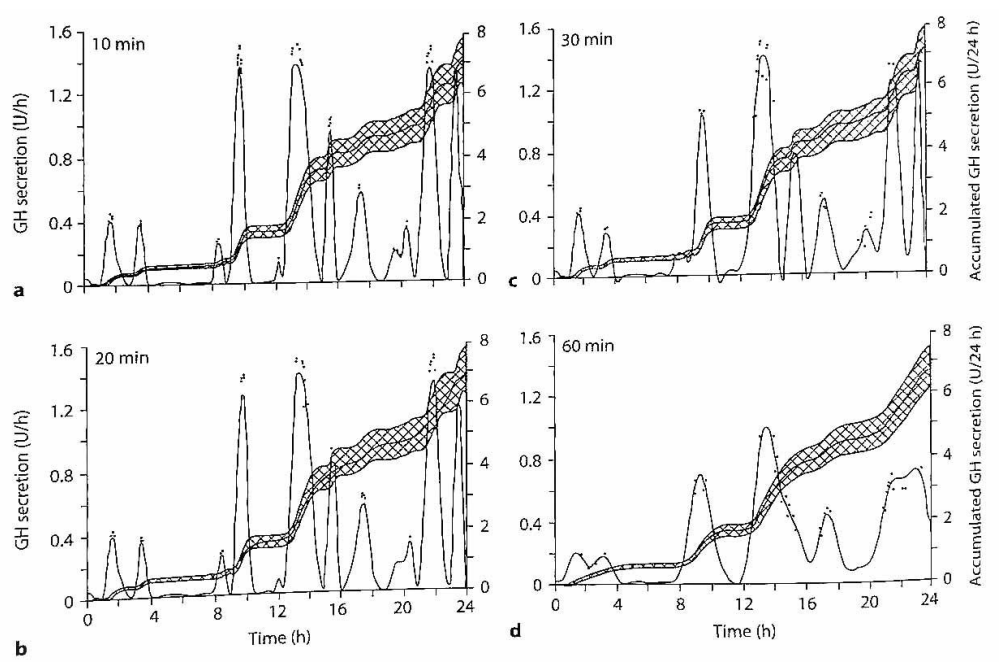


Figure 6 Simulated GH profile with different sampling intervals (adapted from Diagnostics of Endocrine function in children and adolescents 4th Edition)

In early attempts to characterise GH secretion patterns, samples would be obtained serially over a period of hours or days, assayed and a “scorer” would identify peaks

(arbitrarily defined as a deviation from baseline of at least 20% [118]) by inspection and count them for analysis. This manual and highly subjective method of identifying GH peaks complicated communication among different researchers and was subject to bias at many different levels.

Time series analysis was one of the first methods used; the principle behind time series being that any complex waveform can be expressed as a combination of sine and cosine waves with various frequencies. Following time series analysis, Fourier transformation can be applied to reveal dominant features [119]. Such methods, however, cannot deal with irregular cycles, which is the typical pattern seen with most biological systems.

Pulse detection algorithms (Pulsar [120] and Cluster [121] being the most widely used) were subsequently developed to characterise GH secretion. Pulsar would identify secretory episodes by comparing the height from a baseline while taking into account the assay standard deviation. The Cluster program would define a pulse as a "statistically significant increase in a cluster of hormone values followed by a significant decrease in a second cluster of values" by using a sliding, grouped t-test. This method is limited in that it cannot adjust for varying trough hormone concentration, pulse amplitude and duration. Both Cluster and Pulsar algorithms operate at a similarly peak detection efficacy with 20-min GH sampling [120].

Development of sensitive GH assays that are able to detect low concentrations of GH and newer computer algorithms that are able to define parameters of GH secretion by an automated, statistically based approach, has enabled representation of hormone secretory episodes by a Gaussian distribution of brief molecular secretory episodes around a particular time point.

Deconvolution methods take pulse detection algorithms a step further, as they are able to expose underlying GH secretion rates by taking away the influence of GH elimination on the measured plasma concentration [122, 123] [Figure 7]. They can therefore present information about regulation of secretory activity and enable detailed analysis of GH pulsatile secretion (AutoDecon) [124].

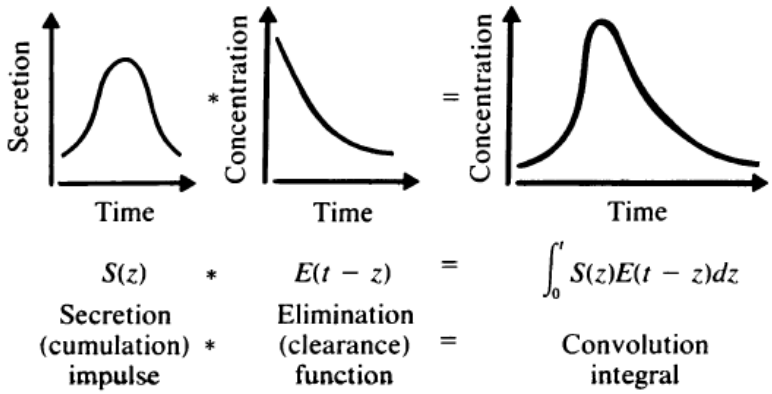


Figure 7 Multiple-parameter deconvolution model

1.3.2 Cortisol

1.3.2.1 Cortisol physiology

The hypothalamus–pituitary–adrenal (HPA) axis is a major endocrine system, which plays a crucial role in maintaining homeostasis and adaptability to physical and environmental challenges [125]. Cortisol, the final product of the HPA axis, binds to glucocorticoid receptors that are abundant in almost every bodily tissue and facilitates metabolic processes related to glucose utilization and delivery to the brain and muscles in addition to maintaining blood pressure and hence peripheral blood perfusion.

Corticotrophin-releasing-hormone (CRH) is synthesized at the level of the paraventricular nucleus (PVN). The PVN receives input from the suprachiasmatic nucleus (SCN) in the hypothalamus which synchronises the body's circadian rhythms [126]. Via the portal circulation, CRH reaches the anterior pituitary and stimulates the secretion of adrenocorticotropic hormone (ACTH) which in turn reaches the adrenal cortex and stimulates the synthesis and secretion of glucocorticoids [127].

The HPA axis has a distinct diurnal rhythm with short secretory episodes of high amplitude starting in the second half of the night with peak cortisol levels in the early morning [128]. An additional brisk increase in cortisol levels, embedded in this circadian pattern but relatively distinct from components of circadian cortisol secretion, is seen 20-30 minutes after awakening (cortisol awakening response [CAR]) [129]. Cortisol levels during this brief period rise by almost two fold and remain elevated for at least 60 minutes [130]. CAR shows high intra-individual stability (r= .45 - .70) even when measured at monthly intervals and in different age groups [131]. Thereafter,

cortisol levels gradually decline throughout the day with lowest levels seen around midnight [132].

The CAR is therefore considered a useful indicator of the integrity of the HPA axis. The cortisol increase can be supressed after intake of a low-dose dexamethasone the night before suggesting that CAR is mainly driven by hormonal release from the pituitary [133] influenced by orientation in time [134]. This is supported by the increased post-awakening cortisol production seen by using a dawn simulator producing increasing light levels before awakening [135].

Although stress and depression can result in an enhanced CAR the results from various studies are mixed [136]. It is possible that differences in sleep and gender could account for these differences as although depressive symptoms are associated with an elevated CAR in females, in males the result is a blunted CAR [137].

Another important factor that needs to be taken into account whenever interpreting CAR data is the temporal accuracy of saliva sampling. Although the first sample should be taken immediately after awakening, studies that were able to verify awakening and sampling times found delays in sample collection by >30 min on 14% of sampling days [138]. This is clearly demonstrated in Figure 8.

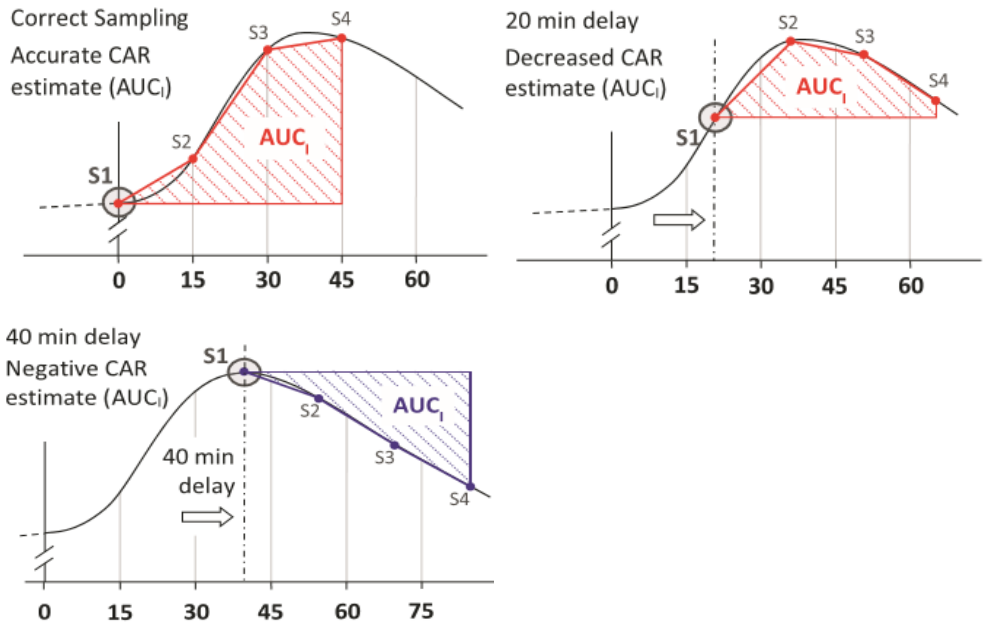


Figure 8 Illustration of the impact of sampling in CAR estimation.S1 denotes the first sample on awakening. Differences in the estimated Area under the Curve (AUC) can be seen with delays of 20 and 40 min between awakening and collection of first sample.

Although the HPA axis is mainly regulated at the level of the PVN with input from the SCN, other brain regions including the hippocampus have been found to play a role in modifying HPA axis activity. Reduced hippocampal volume is associated with impairment of hippocampus-dependent cognitive processes and persistently elevated cortisol levels while larger hippocampal volume is associated with a greater CAR [139]. Taken together, these results suggest a central role of the hippocampus in regulation of the CAR.

1.3.3 Impact of post traumatic hypopituitarism (PTHP)

In addition to its growth promoting effects, GH plays an important role in maintaining a healthy metabolic profile, body composition with increases in lean body mass and bone density but also memory, cognition, mood and HRQL [140, 141]. Treatment with GH in GH-deficient children has been shown to improve cognition, especially performance IQ and processing speed [142].

GHD is associated with increased risk of developing adverse cardiovascular and cerebrovascular events - a combined result of various factors including reduced left ventricular mass, endothelial dysfunction and increased visceral fat [143]. Treatment with GH has a beneficial effect possibly by improving endothelial function and reducing arterial stiffness [144]. The beneficial effect of hormone replacement in patients with hypopituitarism is well established [145].

Hypogonadism is associated with infertility, adverse changes in body composition/bone mineralisation [146] and increased risk for cardiovascular and cerebrovascular events. Depending upon the age and gender of the TBI individual, the symptoms of hypogonadism can vary. Women can exhibit amenorrhea, decreased libido, fertility and reduction in bone density. Men can present with fatigue, reduced lean/muscle mass, loss of secondary sexually characteristics and decreased libido and fertility. Gonadotrophin deficiency has been found to be a significant independent factor for development of cerebrovascular disease, essentially increasing mortality two fold compared to the general population [147]. It is also associated with impairment of some aspects of cognitive function, particularly verbal fluency [148].

Glucocorticoid deficiency can cause lethargy, weakness, fatigue and cognitive dysfunction [149]. With secondary hormone deficiencies as seen with TBI, most mineralocorticoid secretion remains intact. Corticotropin deficiency is therefore less evident than primary adrenal deficiency.

Hypothyroidism causes fatigue, poor concentration and memory problems which can be ameliorated by treatment [150, 151], but again the clinical phenotype is less severe than that seen in primary hypothyroidism.

1.3.3.1 Fatigue, HRQL, cognitive function and PTHP

Persisting mental and physical fatigue has been reported in up to 80% of traumatic brain injury patients even at 5 years post TBI [152, 153] . This is much higher when compared to the lifetime prevalence of fatigue in the general population (24%) [154]. In the first year post-TBI, fatigue levels are more likely to improve, remain stable or increase following mild, moderate and severe TBI respectively and correlate strongly with depression, insomnia, and cognitive difficulties [155]. Various mechanisms have been proposed to explain fatigue in patients with TBI including excess mental effort to overcome memory, problem solving and attention impairment, disordered sleep and hypopituitarism [156]. Although fatigue levels between GHD and non-GHD TBI survivors (irrespective of TBI severity) have been reported to be comparable [157, 158], studies of GHD patients from non-TBI causes indicate an association between GH status and fatigue. The discrepancy could be related to the small number of patients recruited in the formal studies as it is well described that GHD is associated by a described reduction in cardiorespiratory endurance which can present as physical fatigue [159, 160]. It would be reasonable to assume that treatment with GH would reduce fatigue in these patients. This has been confirmed with double blind, placebo controlled studies that have demonstrated an overall improvement in energy levels with GH replacement therapy in GHD [161-163].

The potential benefit to patients of correcting post-TBI endocrine deficits is further highlighted by evidence that adults with GHD following TBI, show a marked improvement in HRQL (including social and psychological wellbeing) but also in cognitive function, organisational skills, co-ordination and fine motor dexterity after treatment with GH [164]. Analysis of the German International Metabolic database (KIMS) in 2006 identified 84 TBI patients on GH treatment (54 adult-onset and 24 childhood-onset). When compared to a matched group of GHD patients due to non-functioning pituitary adenoma, they showed a similar benefit from GH replacement in terms of improved HRQL [172].

Although impaired cognitive function is a well-recognised complication of TBI, the relationship between TBI-related hypopituitarism and cognitive dysfunction is not clear. Children with congenital hypopituitarism have an intellectual ability that is in the low-average range compared to the population norm but not significantly different when

compared to their siblings except for performance IQ (reflection of reduced ability to perform tasks requiring perceptual organisational skills) [165]. These children are susceptible to hypoglycaemia and other hormone deficiencies early in life that can affect the developing brain and are recognised causes of neurocognitive dysfunction [166] without forgetting that children with congenital hypopituitarism are also likely to have an abnormally developed brain to start with. Furthermore, intelligence in children with GH insensitivity due to abnormal GH receptor is not different compared to controls indicating that GH-induced IGF-1 production is not required for normal brain growth or for postnatal intellectual development [167].

Considering that GH and IGF-1 receptors are found in the thalamus, hypothalamus, hippocampus and parahippocampal areas it is still possible that GH exerts a neurotrophic effect in childhood but also during adulthood [168]. Although some of GH actions are mediated through IGF-1, the expression of GH receptors in the brain suggests a direct effect on neural cells especially as GH receptor is upregulated following brain injury and in response to GH administration [169]. Experimental research raises the possibility that at least some of the adverse effects of hormone deficiencies following TBI might be related to an impact upon endogenous neuro reparative processes [170, 171].

1.4 TBI AND QUALITY OF LIFE

The impact of endocrine dysfunction following TBI is likely to be even more significant in children than in adults because of the crucial roles of pituitary hormones in growth, puberty and the successful physical and psychological transition from childhood to adulthood. TBI children have significantly lower HRQL than non-injured controls several years post injury [172] but in contrast to parent report they do not always rate their HRQL much differently compared to their peers when assessed a mean of 4 years post TBI [173]. The reduction in HRQL correlates not only with injury severity but also with levels of cognitive and psychological dysfunction.

Kokshoorn et al (2011) [37] reported differences in HRQL between patients diagnosed with and without hypopituitarism at least one year after TBI. From the 112 patients that were included in the study (age 19-69 years, time since TBI 4±3 years) six were diagnosed with one or more pituitary hormone deficiencies following a single dynamic test (3 GHD, 1 GHD and cortisol, 1 cortisol, 1 hypogonadism). As a group, patients with PTHP had problems with depression, social isolation, reduced activity and reduced general health perception. In another retrospective study of 97 symptomatic (presence of psychiatric or neuropsychological deficits like chronic fatigue, impairment

of alertness, listlessness) TBI patients, Nourollahi et al [174] found that all patients had significantly lower HRQL compared to the standard population. In addition patients with PTHP had also worse HRQL compared to patients without PTHP.

1.5 TBI AND BEHAVIOURAL OUTCOMES

Behavioural and psychosocial problems following TBI in childhood have not received the same attention as the physical and cognitive consequences. As damage to neural substrate after TBI can affect neurocognitive skills that mediate behaviour, children following TBI are at increased risk of adverse behavioural outcomes. The effects of injury severity are not clear [175, 176] for most behavioural outcomes except possibly for personality changes that may be noticeable shortly after injury [177]. With modern imaging modalities (DTI and fMRI) it is now possible to assess subtle changes in white matter integrity. Executive functioning (task planning, inhibition, attention control, sustained effort, and mental flexibility) for example relies on frontal–striatal networks that are particularly susceptible to diffuse axonal injury [178]. Approximately 20 to 40% of young children (5-15 years of age) with TBI show significant executive dysfunction within the first year of injury [179]. Frontal and temporal area networks are also important for processing emotional information. Acquired brain injuries to these areas (e.g. TBI, stroke, meningitis) can predict deficits in processing and recognizing emotions, executive function and general cognitive functioning [180, 181].

Early studies of children following severe TBI suggested that the risk of experiencing TBI is influenced by the child's pre-accident behaviour, intellectual level, and psychosocial circumstances [182, 183]. Other studies have reported a threefold increase of new psychiatric disorders compared to children with mild TBI (62% vs 20%) or orthopaedic injury controls [182, 184]. These studies however have relied on post-injury collection of pre-injury data and it is possible that parental perception of the child's past behaviour was influenced by the child's current behaviour. In addition these studies included only children that were hospitalised or seen in emergency departments while children with mild TBI are usually seen in primary care [10]. It is therefore possible that children with mild TBI presenting at hospital and included in these studies of hospitalised children were simply ones with overly concerned parents. These children therefore may not be an accurate reflection of the general population.

Another way to obtain accurate pre-injury information would be with well-designed prospective studies like birth cohorts. In a large birth cohort study that included 1265 children, McKinlay et al [185] challenged the hypothesis that children who have behavioural, cognitive problems or learning disabilities are more likely to experience

TBI and found that pre-injury behavioural problems were not a significant factor of TBI. This result was in agreement with other studies using prospectively collected data [186, 187]. Although the study identified three main risk factors of TBI (male sex, punitive parental style and number of adverse life events) the level of predictive efficacy of the statistical model suggested a number of unaccounted risk factors. It is hypothesised that children from stressed families are likely to be less supervised and therefore their exposure to TBI events is higher.

There is however agreement regarding the behavioural outcome following TBI. Changes of both internalizing (anxiety/depression [188, 189], withdrawal [190], somatic complaints) and externalizing behaviours (aggression [175, 191], rule breaking and intrusive) have been reported. Whether pre-existing or not, inattention is among the most commonly reported disorder post TBI as almost half of children developed symptoms of ADHD after TBI [192, 193]. Interestingly in children with pre-injury ADHD, their symptoms persist without the fluctuations described in TBI children without a pre-injury diagnosis of ADHD [194]. Children with TBI are at a much greater risk to develop new onset mood or and/or anxiety disorders as they are often left with residual cognitive, physical, behavioural and emotional deficits, all of which interfere with reintegration into the community (almost half of them at 6 months post TBI compared to 14% in children with orthopaedic injuries [195]). One year post injury, 10% of TBI children fulfil criteria for posttraumatic stress disorder (PTSD) which include avoidance, re-experiencing and hyperarousal [196]. Age at injury may affect the outcome in different ways. Younger children with TBI appear more vulnerable to anxiety disorders [197] but older ones are more vulnerable to depression [198].

Aggression and rule breaking behaviour are prevalent in children with TBI both before (explaining the higher TBI event risk described in some studies but not in prospective birth cohorts) and after their injury. In a prospective study of fifty children hospitalized after TBI, injury severity predicted oppositional defiant disorder (ODD) symptomatology at 2 years after injury but psychosocial factors appeared to play a greater role in the development of ODD symptomatology in the first year after TBI [199]. Children with ODD are also more likely to use aggression to resolve social conflicts rather than behaviours that preserve relationships with both peers and family [200]. All of the above attributable to TBI disorders can still be present several years post-injury [172, 182] and predispose these individuals to violent crime [201] or further mental health disorders [202].

Depressive symptoms shortly after TBI are not more common or different to the symptoms experienced by other children hospitalized for other trauma (orthopaedic)

reasons. This suggests that the hospitalization experience rather than the type of injury determines the emergence of depressive symptoms [188]. At follow-up however, TBI children are more likely to report more depressive symptoms with socioeconomic status being a significant outcome moderator. Most studies suggest that TBI increases the risk of depressive symptoms (10-25% up to 2 years post TBI), especially among more socially disadvantaged children.

The relationship between GH secretion and depression is multifactorial. Considering that GH secretion occurs mainly during sleep and patients with depression have problems with sleep quality (falling asleep and sleep maintenance), it has been hypothesised that GH secretion is reduced in these patients. Although some studies in adult patients with depression support this hypothesis [203, 204] other studies have failed to find blunted GH secretion in depressed adults [205, 206]. Studies in children and adolescents measuring spontaneous GH secretion are also inconclusive. De Bellis et al [207] in a study of 38 medically fit children with prepubertal major depression and 28 healthy control children, found that depressed children had lower cortisol secretion during sleep but GH secretion did not differ between groups. Subgroup analysis showed lower GH secretion in depressed females when compared to depressed males. These findings were not replicated by Kutcher et al [208] in a smaller group of depressed adolescents who demonstrated higher overnight GH secretion compared to a matched (age, gender, pubertal stage, weight) control group. Using pharmacological stimuli (insulin induced hypoglycaemia, clonidine and GHRH) Ryan et al [209] demonstrated blunted GH secretion in 38 medically fit children with major depressive disorder. Although the study supported existing evidence demonstrating a relation between childhood depression and GH dysregulation, there was no clear explanation for the blunted GH response to GHRH suggestive of impaired pituitary responsiveness which was also described by Dahl et al [210].

Studies in adults with GHD have not found a relationship between measures of GH and psychometric tests [211]. The observed improvement of psychosomatic complaints and depression following GH treatment is therefore possible to be secondary to the associated improvement in somatic effects with GH replacement. Most studies overall suggest that TBI increases the risk of depressive symptoms (10-25% up to 2 years post TBI), especially among more socially disadvantaged children.

1.6 TBI AND NEUROIMAGING

Studies of paediatric TBI using conventional neuroimaging techniques have explored the location and volume of focal lesions or volumetric changes in total brain volume,

grey or white matter. It is not clear however that the methodology of cerebral localization [212] (the concept that specific areas of the brain control or mediate certain behaviours) which is based on adult based models can be extrapolated to the developing brain, which has less fixed functional organisation [213].

CT is widely available and is still regarded as the first line neuroimaging modality in TBI. Short acquisition time and high sensitivity in detecting life-threatening conditions requiring immediate intervention while allowing easy access to medical staff during the scan are important advantages over MRI (magnetic resonance imaging).

Studies where CT brain scans were performed in children with inflicted or non-inflicted TBI suggest that the modified GCS, duration of impaired consciousness, number of intracranial lesions are predictive factors of Glasgow Outcome Scale and cognitive outcome after one year with pupillary abnormalities being associated with poorer motor outcomes [214]. However, other studies, using more advanced techniques [voxel-based morphometry (VBM)], did not demonstrate persisting morphometric changes of the pituitary gland and hypothalamus in the long-term (minimum of 7 years post TBI) in paediatric TBI survivors without endocrine dysfunction requiring intensive care treatment [215].

Although CT provides important and timely information, MRI is able to identify significantly more intraparenchymal lesions [216, 217] but acquisition time is longer and will usually require the paediatric patient to be sedated. The identification however of more TBI related lesions has significant implications in regards to outcome measures and prediction models. Centrally located lesions appear to be directly associated to severity of acute impairment of consciousness and inversely related to functional outcome measures (Glasgow Outcome Scale and the Vineland Adaptive Behaviour Scale) [217]. Children with thalamic and/or basal ganglia injury are three times more likely to develop secondary ADHD when compared to children without injuries in those regions [218].

The frontal brain region, which is the most frequent – not centrally located – area of damage following TBI is believed to be associated with executive function, attention, and affections [219-221]. Although research in adults has clearly linked frontal lobe region integrity (prefrontal cortex) to measures of executive function [222], this functional specificity for executive processes has not been convincingly demonstrated in children where the volume of extrafrontal (rather than frontal) lesions and total number of lesions was predictive of executive functioning [223]. Frontal white matter damage correlated with novel depression 6 months after injury [198]. Diffuse cerebral

atrophy following paediatric TBI was associated with worse global outcomes (Glasgow Outcome Scale). Brain tissue preservation correlated positively with recovery outcomes [178].

These findings suggest that in paediatric TBI, diffuse/extensive rather than localized brain injury may be more important with regard to predicting outcomes. Consequently, the focus has been in developing neuroimaging techniques that generate not only more detailed images of grey and white matter but also provide measures of function integrity.

Diffusion Weighted Imaging (DWI) by using differences in diffusion/mobility of protons within the brain can reveal a greater extent and degree of abnormality than that seen with T2-weighted and FLAIR sequences [224]. The mapping of the diffusion process in biological tissues has been shown to be more sensitive to the diffuse white matter damage that often occurs in TBI [225-228]. DWI also allows differentiation between cytotoxic and vasogenic oedema (both associated with DAI which is seen following TBI), the latter or which may be reversible with appropriate treatment [229].

Following from DWI, Diffusion Tensor Imaging (DTI) measures the directionality of proton diffusion within the brain, providing information about white matter integrity through the calculation of Fractional Anisotropy (FA). High FA (range 0-1) corresponds to maximal anisotropic diffusion, as seen for example in intact, tightly packed fibre tracts [226]. FA across a number of white matter tracts was found to correlate with severity of TBI in children at least 1 year after TBI [230].

1.7 INTRODUCTION SUMMARY

TBI remains the leading cause of acquired neurological morbidity in children. Data collected from registers of all children admitted to hospitals with TBI represents a very conservative estimate of the true incidence of TBI in childhood and an overestimate of the proportion of severe TBI, as most cases of mild TBI are not seen in hospital. Large, population-based studies estimate the incidence of TBI to be 550-800 per 100,000 people per year with almost a third of the general population having sustained TBI by 25 years of age.

Although severe TBI has been reported to be associated with poor health-related quality of life, depression, fatigue, behavioural changes and sexual disturbances not much is known about the long term (10 year) prevalence of neuroendocrine dysfunction in children. Studies in adults report long-term prevalence of post traumatic hypopituitarism ranging from 10% to 70% which reflects the significant differences in

methodology and interpretation of tests used to measure outcomes (cognitive, behaviour, quality of life and endocrine). Similar variability is seen in the small number of available paediatric studies which in addition are limited by the small number of participants. Overall, post-TBI hypopituitarism was less prevalent in studies that included patients with mild TBI but indicators of trauma severity (GCS, skull fracture, DAI) have not been consistently reported to be associated with TBI related hypopituitarism. As there is a considerable overlap between post-TBI symptoms and chronic hypopituitarism, it has been hypothesised that TBI related morbidity could be secondary to undiagnosed and subsequently untreated hypopituitarism stressing the need for common diagnostic criteria to facilitate comparisons between studies and treatment outcomes.

For practical reasons dynamic, non-physiological tests are being used to diagnose GH deficiency. These were initially developed for the endocrine assessment of specific patient groups (short children, cancer survivors etc.) but it is not known if these tests are appropriate for TBI patients where the “true” risk of chronic hypopituitarism is unknown. Measurement of spontaneous GH hormone secretion facilitates analysis of hormone pulsatility which could be used for assessing GH status in TBI survivors as it has already proven helpful in clinical settings such as oncology patients treated with cranial irradiation or slow growing children with normal GH response to dynamic testing (neurosecretory dysfunction).

The clinical manifestations of hormone deficiencies due to post-traumatic hypopituitarism can be obvious or very subtle especially with mild TBI. In addition any symptoms or signs of hypopituitarism may be masked by the cognitive, physical, and/or behavioural sequelae secondary to damage to other regions of the cortex or diffuse axonal injury. Advances in neuroimaging techniques suggest that in paediatric TBI, diffuse/extensive rather than localized brain injury may be more important with regard to predicting outcomes.

2 CHAPTER 2 (STUDY DESIGN)

HYPOTHESES

- Potentially treatable long-term endocrine dysfunction occurs in a significant proportion of children following severe/moderate TBI, and possibly mild TBI.
- There is an association between neuro-endocrine dysfunction in TBI children and impaired physical, cognitive and psychological functioning and reduced health related quality of life (HRQL).

2.1 AIM

The aim of KHINES was to assess the long-term prevalence of endocrine dysfunction and the impact on health, cognition, emotional/behavioural status and health related quality of life in an established, well-characterised cohort of severe/moderate and mild TBI children and adolescents when compared to non-injured controls.

2.2 OBJECTIVES

a. Endocrine assessment of:
    a. GH (Growth Hormone) status in the severe/moderate TBI group using:
        i. provocation tests
        ii. overnight venous sampling
    b. HPA (Hypothalamus-Pituitary-Adrenal) axis function with:
        i. provocation tests
        ii. overnight venous sampling (in severe/moderate TBI group only)
        iii. Non-invasive (salivary) sampling for cortisol (all groups).
b. The relationship of neuro-endocrine function post-TBI to detailed measures of injury severity, including neuroradiological imaging.
c. The relationship of neuro-endocrine function post-TBI to detailed and longitudinal measures of outcome including cognition, psychological status and Health-Related Quality of Life (HRQL).

2.3 PROJECT APPROVAL

Sponsorship was obtained from the University Hospitals Bristol National Health (NHS) Foundation Trust in October 2009 (Appendix 6.1.1)

- The study was approved by the South West Research Committee 4 on 13 April 2010 (appendix 6.1.2) and University Hospitals Bristol Research and Development Department on 18 June 2010 (appendix 6.1.3).

2.4 DATA PROTECTION

Data were collected and retained in accordance with the Data Protection Act 1998. Identifiable personal information was anonymised, with codes accessible only to the investigators of the study, to ensure confidentiality. All study data have been stored according to local research policies and standards from Good Clinical Practice Guidelines on the University Hospitals Bristol, NHS server and accessible only from Trust intranet. Data were collected with the help of a Case Record File and transferred to an Access Database. Non-patient identifiable data were also backed up in an encrypted USB flash drive registered to ND. Data was entered in a Microsoft Access Database and exported for analysis with SPSS.

2.5 STUDY PARTICIPANTS

All individuals who had participated to the **K**ids' **H**ead **I**njury **S**tudy (KHIS, 2002-2004) and had agreed to participate in any further TBI related studies were eligible for recruitment. KHIS was a prospective study of outcome in children with severe, moderate and mild TBI for whom detailed data were available concerning the severity and mechanism of TBI and outcome. A research psychologist who had been a member of the original KHIS team made initial contact with participants. Failing initial contact by telephone, participant details were verified and updated if necessary by contacting their primary care physician followed by an invitation letter to their last known address if they could not be contacted by phone. Informed consent/assent was obtained from participants and parent/legal guardian as appropriate for age.

Participants were divided into 3 groups.

- Group 1 (control group)

  Control participants in KHIS were non-injured school children matched for age, sex, socio-economic status based on the Income Deprivation Affecting Children Index (IDACI) and pre-TBI academic attainment using the "best friend model". In this model KHIS participants were asked to nominate a friend

or acquaintance of the same sex and age who were at the same school. Friend controls are presumed to be more motivated, inadvertently matched on characteristics not intended as matching factors and have higher response rates compared to general population controls [231, 232].

- Group 2 (mild TBI)

  Participants with admission Glasgow Coma Score [2] (GCS) 13-15 *and* normal neuroimaging)

- Group 3 (moderate/severe TBI)

  Participants with Moderate/Severe TBI (admission GCS 3-8 for severe and 9-12 for moderate TBI) *or* injury related abnormalities on initial neuroimaging even with GCS 13-15 .

Exclusion criteria were failure to obtain informed consent, pregnancy, history of cranial irradiation, significant pre-TBI endocrine dysfunction (excluding transient endocrinopathy e.g. transient hypothyroidism, recovered adrenal suppression from steroids), medical or psychological problems not related to TBI (including alcohol and drug abuse) that could disturb interpretation of results and history of further TBI. In addition, participants on any steroid preparation (topical or systemic) were asked to stop this before the tests where clinically possible. If this was not clinically acceptable the endocrine tests were either postponed (if a short course of treatment was involved) or were not performed. Shift workers were tested at least a week after their last night shift and alcohol/drug intake was not permitted for a minimum of 24 hours before any assessment. Participants who had travelled across more than three time zones were seen at least 3 months after their return in the UK.

Power calculations for the endocrine assessments were based on a conservative, estimated prevalence of GH deficiency in the general population of 1:10,000 (1:3,000 – 1:17,000 from large population studies) [233, 234] and 10% in the moderate/severe TBI group. The number of participants needed to demonstrate with a power of 80% a statistically significant difference (5% level of significance) was 16.

For salivary cortisol assessment, where a control group would be available, at least 17 patients were needed per group in order to demonstrate with 80% power, a difference of one standard deviation (SD) between morning salivary cortisol levels in TBI patients and controls.

The study design is shown in Figure 9.

	Control group	Mild TBI	Mod/Sev TBI
Clinical examination	✓	✓	✓
Stimulation tests & overnight profiles			✓
Salivary Cortisol & Cortisone	✓	✓	✓
Neuroimaging	✓	✓	✓
Cognitive assessment	✓	✓	✓
QoL & Health assessment	✓	✓	✓

Figure 9 KHINES study design

2.6 ENDOCRINE INVESTIGATIONS

2.6.1 Clinical Assessment

Participants were studied at the Bristol Royal Hospital for Children and had a baseline clinical assessment, including pubertal staging according to Tanner method [235], and symptom review with questionnaires for measures of HRQL and health status. All assessments were done by the same experienced research doctor (ND).

2.6.2 Anthropometry

Height

Measurements included morning height using a daily-calibrated Harpenden® wall mounted stadiometer. Shoes, hair clips and braid were removed. Participants were positioned with feet together, flat on the ground, heels touching the back plate of the measuring instrument, straight legs, buttocks and scapulae against the backboard and arms loosely at their side. Head was positioned so that the lower margins of the orbit and external auditory meati were in the same horizontal plane. While applying gentle pressure and holding their mastoid processes to stabilise the head in the correct position, participants were asked to breathe normally and the headboard was placed on the top of their head. Height was recorded to the nearest mm after full exhalation. The measurement was repeated three times and the average rounded to the nearest mm.

Weight

Weight was measured to the nearest 0.1 kg with participant wearing light indoor clothing after removing shoes/slippers and pocket contents. The scales used were regularly calibrated with a traction weight.

Body Mass Index (BMI)

BMI was calculated as weight (kg) divided by the square of height. Weight, height, and BMI were corrected for age and gender using SDS from the Cole's LMS method [236, 237].

Waist circumference was taken during expiration at the narrowest waist level, or if this was not apparent, at the mid-point between the lowest rib and iliac crest using a cloth tape. Three measurements were taken at different levels and the lowest measurement recorded.

Body composition

Methods to measure body fat can be divided in two categories: “reference” (body density, total body water, DEXA [dual-energy X-Ray absorptiometry), MRI] that measure a physical property of the body or “prediction” methods (skinfold thickness [238] and bioelectrical impedance analysis [BIA]) that use regression analysis to give an estimate of the “reference” method outcome [239]. Skinfold thickness has been found to provide an accurate estimate of total body fat when compared to DEXA including in GH deficient patients [240].

BIA estimates total body fat indirectly by using electrical current tissue resistance to measure total body water and by extrapolation fat free mass. Total body fat is then calculated as the difference between body weight and fat free mass [241]. BIA, especially when using recently developed analysers, provides a quick, safe and accurate method of measuring total body fat. Limitations are mainly dependent on hydration state including fluid shifts between different areas of the body throughout the day [241]. In healthy, normal weight, young adults this is not an issue and bioelectrical impedance is an accurate tool to estimate total body fat regardless of their activity level [242].

Skinfold thickness was measured over three sites (biceps, triceps and abdomen) using a Holtain® skinfold calliper. Measurements were taken on the right side for consistency. If for any reason measurements needed to be taken on the left side (injuries, amputation, deformities) this was recorded. All measures were performed by the same researcher. The mean of two measurements were taken and if they differed greatly (over 20%), a third was done and the median value was recorded.

For bioelectrical impedance analysis, a Tanita® Body Composition Analyser (Model BC418, Tanita, Arlington Heights, Illinois) was used. Measurement of body composition is done using a constant, high frequency current (50kHz, 500μA). The electric current is supplied from electrodes on the toes and fingertips of both extremities. To calculate body fat percentage, the Body Composition Analyser uses normative data acquired from a representative Western population using DEXA and regression formula using height, weight, age, and impedance between right hand and foot as variables. Body fat percentage, fat mass, and fat free mass for individual parts and for the entire body calculated with this method, correlate strongly with DEXA, hydrostatic weighing and total body potassium results [243, 244]. Body composition measurements attained by bioelectrical impedance are therefore highly reproducible. Factors that can affect readings include hydration status (readings are usually highest in the morning). For consistency all body fat percentage readings were taken before

noon. In participants admitted for overnight studies measurements were taken the following morning before the stimulation test. Readings were taken with light clothing after socks or stockings were removed. Soles were inspected to ensure there were clean before stepping on the analyser.

2.6.2.1 Statistical analysis of anthropometry

Standard deviation scores (SDS) according to British references [236, 245] were used for comparisons. Differences between participant and non- participant characteristics were assessed by a χ^2 test (for categorical variables) or t test (for continuous variables). A level of $p<.05$ was considered statistically significant.

When analyses included comparisons between the 3 study groups (control group versus mild TBI versus moderate/severe TBI) these were performed by analysis of variance followed with pairwise comparisons (two tailed t-test with Bonferroni adjustment) when a significant difference was indicated. Non-parametric tests were used (Mann Whitney U for 2 groups and Kruskal-Wallis for 3 or more groups) to compare for dependent variables that were continuous but did not meet requirements of parametric equivalents. These were followed by pairwise comparisons (adjusted for the number of comparisons made) when the overall test showed significant differences across samples.

2.6.3 Endocrinology

2.6.3.1 Baseline Endocrine

Baseline samples were drawn before the start of the ITT for analyses of free thyroxine (fT4), TSH, testosterone (men), oestradiol (E2, women), LH, FSH, prolactin (PRL), IGF1 and IGF-BP3. Three of the tested female participants were on oral contraceptives.

2.6.3.2 Stimulation test

The hypothalamic–pituitary–adrenal and GH–IGF1 axes were evaluated by an insulin tolerance test (ITT) if there were no contraindications (epilepsy or history of seizures, heart disease, pregnancy). The ITT was performed after completion of overnight sampling by administering soluble insulin i.v. (Actrapid, Novo Nordisk, Denmark) at a dose of 0.15 U/kg or 0.10 U/kg if there were concerns of multiple pituitary hormone deficiency. Participants were supervised constantly during the test by the research doctor and specialist endocrine nurse and hypoglycaemia was treated with oral glucose followed by a carbohydrate rich snack (biscuit or bread) or i.v. (2ml/kg of 10% Dextrose). Serum cortisol and GH were measured at the following time points: -15, 0,

30, 60, 90 and 120 min (insulin was given at time point 0). Between 5 and 30 min after insulin administration, serum glucose was determined every 5-10 min and every 15 min thereafter. A successful test was defined by a blood glucose nadir lower than 2.2 mmol/l and clinical symptoms of hypoglycaemia. Peak GH values used for diagnosing GHD differed depending on age group. A cut-off of <3 mcg/l was used in adults [246], <7 mcg/l in children and <5 mcg/l [77] in study participants in the transition phase – the 5-7 year period from late puberty to full adult somatic maturation [247]. Cortisol levels >500 nmol/l were considered to reflect normal GH and ACTH function [248].

2.6.4 Spontaneous GH and cortisol secretion

For assessing spontaneous GH and cortisol secretion participants were admitted for a 12-hour overnight venous profile in a special investigation unit, detached from clinical wards. An intravenous cannula was placed after application of local anaesthetic in a forearm vein. Blood sampling started at least 1 h after catheter insertion to avoid artefactual effects related to the venepuncture stress and continued for every 15 minutes. Sampling was performed with every effort not to disturb the participant and the IV line was kept patent with 2ml flushes of normal saline after sampling. All participants were ambulatory and encouraged to continue with their normal activities until their usual bedtime and had an age-appropriate meal. They were asked not to eat or drink anything after midnight, except water in preparation for the following mornings GH stimulation test. Participants were free to turn off the lights at their usual sleep times. During bedtime hours, the lights were turned off. Sleep status was recorded during sampling. Participants were allowed to wake up spontaneously in the morning. Total volume of sampled blood was less than 100 ml. Blood samples were kept at 5^{o}C until completion of the overnight sampling and following centrifugation all samples were stored at -70^{o}C until analysis (within 3 months). Assays were performed in duplicate, and each subject's sample was run in one batch to minimize inter-assay variance.

2.6.4.1 Statistical analysis for hormone profiles

Integrated GH and cortisol concentration was calculated as the area under the GH curve using the trapezoidal rule. Parameters of GH and cortisol pulsatility were analysed with regard to concentration and secretion. The absolute GH nadir was assumed to reflect basal GH secretion. Wave form–dependent deconvolution analysis of the 12-h GH and cortisol profiles was used to calculate pulse frequency and secretion profile. Non-parametric tests were used (Mann Whitney U for 2 groups and Kruskal-Wallis for 3 or more groups) to compare dependent variables that were continuous but did not meet requirements of parametric equivalents. These were

followed by pairwise comparisons (adjusted for the number of comparisons made) when the overall test showed significant differences across samples.

In the absence of a control group for the overnight GH profiles, normative data from other studies using the same GH assay were used as a guide. The study from Nindl et al [249] that included participants with physical characteristics (age, BMI, body fat) similar to the KHINES mod/sev TBI group was selected.

2.6.5 Salivary cortisol

Several research studies have determined that saliva can be used to measure reliably cortisol levels [250]. More recently salivary cortisone has been shown to closely reflect free serum cortisol levels with the added advantage of being unaffected by cortisol binding globulin changes and being able to identify the rare patient with contamination of the salivary sample with topical steroids [251] [252]. Salivary samples were collected using Salivette® Cortisol (Sarstedt AG & Co) synthetic swabs. These are designed for measuring free cortisol and require a small volume of saliva (less than 1ml). Written instructions on how to collect the samples were handed to the participant on their first appointment followed by a demonstration [Appendix page **Error! Bookmark not defined.**]. Participants kept the swabs in their mouth for a 2-min period to collect saliva passively and then the swab was returned to the salivette tube, sealed and preserved at the participants' domestic fridge. After all samples were collected they were posted or delivered in person to our laboratory in insulated packs and stored at -20oC until time of assay. Participants were instructed not to collect saliva when they were ill, had a cold, headache, or taking any medication.

In order to assess the diurnal rhythm of cortisol, 4 samples were collected each day for 3 days at waking, 4-6 hours, 8-10 hours, and at bedtime (all samples synchronised to the first morning sample). For assessing the response to physiological stress (cortisol awakening response [CAR]), cortisol samples were collected 30 minutes after awakening. Although the CAR is not an orthostatic response and is not influenced by morning activities [131], for uniformity we advised all participants to collect the first two samples while still in bed. Absence of diurnal variation was defined when the ratio of the first morning sample to the fourth or fifth sample (8-10 hours post awakening) was less than 0.5 [253]. HPA axis feedback was assessed with a dexamethasone suppression test. Dexamethasone 0.3 mg/m^2 was administered orally at bedtime and three more salivary samples were collected on the final day at 8am, 12am and 4pm. Participants were given no direction as to when to wake up – this was left to their convenience. Samples were taken before or at least 30 minutes after drinking or

brushing teeth in order to prevent damage to the oral mucosa and contamination with blood. Eighteen samples were collected over 4 days.

2.6.5.1 Statistical analysis for salivary cortisol

Repeated measures ANOVA was performed to compare the salivary cortisol profiles of severe/moderate and mild TBI groups with the control group. Analyses were done with and without participants having a negative CAR on all three days of sampling.

2.6.6 Assays

All samples were assayed by biochemistry laboratories, which had full Clinical Pathology Accreditation (CPA). All analysis was done at the Bristol Royal Infirmary, University Hospitals Bristol, UK except from IGF-1 and IGFBP-3 that were assayed at Guildford biochemistry laboratory.

Serum GH concentrations were measured using a commercial immunoassay ("hGH", Roche Diagnostics GmbH) for in vitro quantitative determination of human GH forms with molecular masses of 20 kDa and 22 kDa. The electrochemiluminescence immunoassay (ECLIA) was used on a Cobas e601 immunoassay analysers. Intra- and inter-assay coefficients of variation (CV) were 1.9% and 3.0% at GH concentrations of 0.163mcg/L; 2.0% and 3.0% at GH concentrations of 8.23 mcg/L. The limit of detectability was 0.03mcg/L with a limit of quantitation of 0.05mcg/L (lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20%).

Salivary cortisol and cortisone were measured with ultra-performance liquid chromatography-tandem mass spectrometry (Waters Acquity UPLC, Waters Quattro premier XE tandem mass spectrometer) with intra- and inter-assay CV of less than 10% at 5-48 nmol/L of cortisol and less than 7% at 10-102 nmol/L of cortisone.

Serum IGF-1 and IGF-BP3 measurements levels were measured by solid-phase, enzyme-labelled chemiluminescent immunometric assay (IMMULITE/IMMULITE 1000 IGF-I). For IGF-1, intra-assay and inter-assay CV's were under 4% and 8% respectively for concentrations between 6-120nmol/L. IGF-BP3 intra- and inter-assay CV's were 5.6% and 9.9%; 3.5% and 7.5% at IGF-BP3 concentrations of 1.6 and 6.8mg/L respectively. IGF1 and IGF-BP3 SDS were calculated from the reference range values.

Glucose concentration was measured on a COBAS analyser (Roche Professional Diagnostics' Products, Burgess Hill, West Sussex, UK) by Hexokinase method with intra- and inter-assay CV of under 1% over the whole measuring range.

TSH, FT4, LH, FSH, prolactin, oestradiol and testosterone were all measured by ECLIA with intra- and inter-assay CV of less than 5% over the whole measuring range except from testosterone intra- and inter-assay CV were less than 5% at the range between 2.4-45 nmol/L but 15% at 0.3 nmol/L.

2.6.7 Deconvolution

Pulse analysis methods were introduced to assist in identifying hormone pulses in an objective way. Early methods such as Pulsar [254] and Cluster [121] did not provide detailed information about the secretory events or the substrate/hormone elimination and were still subject to operator measurement error. AutoDecon [255] automates the procedure and introduces statistical verification of secretory episodes.

2.6.7.1 AutoDecon

The pattern of GH and cortisol secretion was analysed using validated Deconvolution software (AutoDecon). The automatic algorithm identifies statistically significant secretory peaks using the assay as a scale factor rather than arbitrary defining a pulse as an increase in growth hormone exceeding the preceding nadir by 20% [256]. It also discriminates (by applying a multiple-parameter deconvolution model) the amplitudes, durations, interpulse intervals of all statistically significant underlying secretory bursts from the plasma hormone concentrations. The program does not make any assumptions on timing, size or duration of secretion events but assumes that secretory events follow a Gaussian distribution and elimination follows a single compartment pharmacokinetic model

$$E(t-z) = e^{-\frac{ln2}{HL}(t-z)}$$

where HL is the elimination Half Life.

The mathematical model for the time course of the hormone concentration is:

$$C(t) = \int_0^t S(\tau)E(t-\tau)d\tau + C(0)E(t)$$

where C(t) is the hormone concentration at time t, S(t) is the secretion at time t and E(t-τ) the elimination from serum as a function of time.

Using a weighted, non-linear, least-squares method the software fits the above equation to experimental data by adjusting parameters of secretion and elimination and reporting the values of determined parameters that are statistically significant – i.e. having the highest probability of being correct. When one or more these parameters

are known in advance, they can be fixed and the remaining ones will be altered automatically in order for the mass of each peak to remain the same.

The triage module of Autodecon applies the above deconvolution model in cycles and recursively assigns the locations of secretion events (i.e. peaks) after performing a series of statistical tests using a probability level of 0.05. Peaks are added one at a time, starting at the optimal location to lower variance-of-fit remains low. All peaks are then tested and removed if not statistically significant and the process is repeated until no additional secretion event can be added.

AutoDecon uses the following settings when initialized:

1. basal secretion set equal to zero
2. concentration at time zero set equal to zero
3. elimination half-life (HL) set to any physiologically reasonable value (approximately 10-20 minutes for GH)
4. standard deviation of the secretion events (Secretion SD) set to one-half of the data sampling interval, and
5. zero secretion events

After all secretion events are added and the parameters above are estimated, AutoDecon will examine differences (residuals) between the data points and the fitted curve for fitting problems – usually failure to identify a partial secretion event at the beginning or end of the times series. When this is observed, that data point is removed and the AutoDecon is repeated without the offending data but with current initialization values. AutoDecon will highlight outliers as data points being statistically different from the expected. These are data points with a Z score of the particular residual greater than 4. Outliers may be the result of incorrect data entry or simply a reflection of inherent measurement uncertainty within experimental data. In any case, the cause for the outlier has to be investigated before removing from the data series.

In order to estimate the precision of the measured hormone concentrations (as typically most studies use only a small number of replicate samples – usually 2), AutoDecon uses a weighting factor based on a variance model that takes into account parameters of the hormone assay which are well known as they are part of the quality control and performance characteristics of the assay.

$$\sigma^2(Concentration) = \left(\frac{\%CV}{100} * Concentration\right)^2 + \left(\frac{MDC}{2}\right)^2$$

where CV is the % coefficient of variation at the optimal range for the assay, MDC is the minimal detectable concentration.

A typical output of the use of the AutoDecon algorithm is seen on Figure 10.

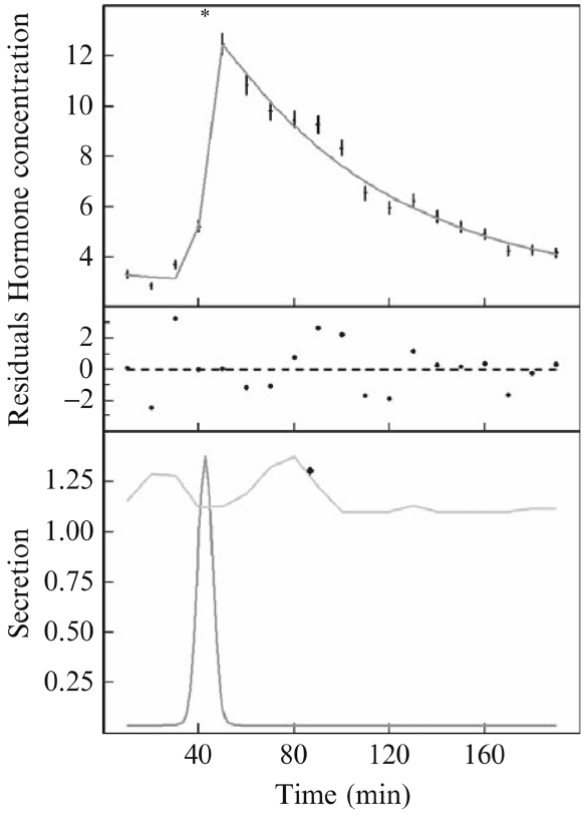


Figure 10 Sample of AutoDecon output

At the top (Hormone Concentration) a time series of data points with vertical error bars, with the solid curve corresponding to the predicted concentration is plotted. The asterisk at the top of the panel marks the location of the single secretion event. In the middle panel weighted differences (residuals) between the data points ones calculated by AutoDecon. At the bottom (Secretion) is a representation of the calculated secretion pattern. The diamond in this panel marks the location of the next presumed secretory event, which was discarded because it was not statistically significant.

The AutoDecon algorithm allows for non-equal data spacing and missing values. The area under the curve (AUC) was estimated from the level of the calculated baseline. The duration of a secretory pulse was defined as the time interval separating the preceding and following troughs and in each individual profile, the level of baseline secretion was estimated as the secretory rate necessary to maintain the baseline GH concentrations during the inter pulse intervals. For each significant pulse, pulsatile GH secretion was calculated by subtracting the baseline secretion from the total secretion. The amount of pulsatile GH secretion over a given time interval was determined by summing the amounts of pulsatile secretion in each of the significant pulses occurring during that time interval.

2.6.7.2 Approximate Entropy (ApEn)

ApEn is a reflection of the regulation of a feedback network. It was developed as a single statistic to quantify the amount of regularity in time-series data which could not be measured with descriptive statistics like mean, median and variance. For example, the data series (70, 80, 70, 80, 70, 80, 70, 80, ….alternating 70 and 80) and (70, 70, 80, 70, 80, 80, 80, 70, 80 ... randomly 70 or 80) have the same mean, median and standard deviation but are “different” in that the first series is "regular" and knowing one value gives insight into what the next value will be.

Most biological processes have temporal irregularity and ApEn is a measure of how these processes are controlled [257]. Low temporal irregularity implies tight control and too strong control makes adaptation to changes in the environment difficult. For example a patient with complete heart block and fixed heart rate has not temporal irregularity and cannot adapt to changes, for example physical activity as he cannot increase his heart rate. The ApEn in this example would be close to 0. [258, 259].

In endocrinology ApEn has been used to distinguish acromegalic from normal pulsatile growth hormone release. Low irregularity implies tight control and high irregularity implies no control. For hormone time series, ApEn computed from *secretion* time series (for example after applying deconvolution methods to *plasma* concentration time series) enhances the discriminating ability of ApEn to identify endocrine states characterized by enhanced secretion irregularity [260].

2.7 NEURO-PSYCHOLOGICAL AND HRQL ASSESSMENT

Cognition and psychological assessments were undertaken by a team of student research psychologists under the supervision of an experienced clinical psychologist (HM). Measures of HRQL and health status were assessed by ND. All tests are listed in Table 2.

		Age	Self	Proxy
Cognition	WASI-II (Wechsler Abbreviated Scale of Intelligence)	7+	✓	
	TEACh (Test of Everyday Attention for Children)	17-	✓	
	TEA (Test of Everyday Attention)	17+	✓	
	CMS (Children's Memory Scales)	17-	✓	
	WMS (Wechsler Memory Scales)	17+	✓	
	Wechsler Quicktest	17-	✓	
	WRAT-4 (Wide Range Achievement Test)	17+	✓	
Psychology	Youth Self report (11-18y)	18-	✓	
	Child Behaviour Checklist (CBCL)	18-		✓
	Adult Self Report (18-59y)	18+	✓	
	Adult Behaviour Checklist (ABCL)	18+		✓
	Beck Depression Inventory (BDI-II)	13+	✓	✓
	Beck Anxiety Inventory (BAI)	13+	✓	✓
	Birleson Depression Scale	11+	✓	
	Children's Impact of Event Scale	8+	✓	
	BRIEF (Behaviour Rating Inventory of Executive Function)	18+	✓	
	BRIEF for under 18	18-		✓
Health	PedsQL	All	✓	✓
	Participation	All		✓
	Fatigue/Sleep	All	✓	
	Health Utilities Index (HUI)	All	✓	✓

Table 2 List of tests used in KHINES for assessing cognition, psychological status, fatigue and health related quality of life.

2.7.1 Cognitive outcome

In children aged <16 years, verbal and non-verbal reasoning ability, executive functioning and attention skills was assessed using the Wechsler Intelligence Scales for Children (WISC-III-UK) [261] and the full range of subsets from the Test of Everyday Attention in Children (TEACh) [262]. Objective assessment was

supplemented with behavioural ratings of executive function using parent and child-completed Behaviour Rating Inventory of Executive Function (BRIEF) questionnaire [263]. Memory functioning was assessed using the Children's Memory Scales (CMS); academic achievement using the Wechsler Quicktest, incorporating Basic Reading, Spelling, and Mathematical Reasoning subtests. In young people aged ≥16 years, assessment, where necessary, employed analogues of the above tests utilising adult normative data (i.e. WASI-II, WMS, TEA, WRAT-4).

2.7.2 Psychological & behavioural outcome

Long-term psychological status in participants aged years was assessed using the following tools:

- Youth self-report and parent-report forms of the Child Behaviour Checklist (CBCL) [264]
- Beck Depression Scale [265] (or Birleson Depression Scale [266] if under 13 years) and Beck Anxiety Scale [267], both of which can be used in adolescents [268], as well as adults
- Children's Impact of Event Scale [269, 270].

In young people aged ≥ 19 years at the time of the study, assessment employed analogues of the above tests utilising adult normative data (e.g. Impact of Events Scale) and comparison with previous test scores made using statistical algorithms accounting for association between test scores across adult and child versions.

2.7.2.1 Behaviour checklist and self-report

The Achenbach System of Empirically Based Assessment (ASEBA) comprises a family of forms for assessing adaptive functioning and problems. Originating in research on child and adolescent problems ASEBA forms were extended to adults in the 1980s and 1990s [271].

ASEBA items are designed to tap strengths and problems that are potentially relevant to a person's need for help. They include Self-Report (children CSR, adult ASR) and Behaviour Checklist (children CBCL, adult ABCL). These parallel forms facilitate comparisons between people's perceptions of their own functioning and other people's perceptions of their functioning. Behaviours are broadly categorized as internalizing or externalizing. The Internalizing grouping consists of three syndromes (anxious/depressed, withdrawal, somatic complains) that are mainly within the self. Externalizing grouping consists of three other behaviour syndromes (aggressive, rule

breaking and intrusive) that mainly involve conflicts with other people and with social mores [272, 273].

Each item is intended to provide useful information in its own right, in addition to contributing to scale scores. Some adaptive functioning scales comprise items that are relevant to most people, such as relationships with friends and family. Other scales comprise items that are scored only for people for whom they have been relevant at some time in the preceding 6 months, such as relationships with spouse or partner and job functioning.

The respondent rates each item as 0 = not true, 1 = somewhat or sometimes true, and 2 = very true or often true, based on the preceding 6 months. Raw and T scores are calculated. T scores facilitate comparisons between respondents using different instruments (adult vs child). T scores of 70 for example corresponds to a score on the 98th centile of the normative sample for participants of that age and sex. T scores for competence scales are truncated at the nondeviant end, therefore comparisons between respondents that are “healthy” should be done using raw rather than T scores. Internalizing, Externalizing and total competence T scores are *not* truncated and can be used for statistical analyses and comparisons without losing any of the differentiation.

Finally, ASEBA items have been used to construct DSM-oriented scales for behavioural, emotional, and social problems. Although not diagnostic per se, a high score on a particular DSM-oriented scale should prompt a check to see whether the client meets criteria (impairment, age of onset or duration of problems) for any DSM diagnoses corresponding to that scale. Validity testing of these scales has shown that scores on the CBCL and YSR anxiety problems scale predicted DSM-IV disorders only moderately but scores on the affective problems scale corresponded closely to DSM-IV major depressive disorder and dysthymia [274].

2.7.2.2 Depression

The Birleson Depression Scale was developed as a clinical instrument to assess the degree of depressive feelings in children and adolescents [275]. The questionnaire consists of 18 items and children are asked to judge the extent to which a particular item has applied to them in the course of the previous week. Items are scored 0, 1, or 2 and a total score of 17 or above is seen only in children with a diagnosis of clinical depression.

The Beck Depression Inventory – Second Edition (BDI-II) is a 21-item self-report instrument for measuring the degree of depression in adults and adolescents aged 13

years and older. The second edition includes additional items such as agitation, worthlessness, concentration difficulty and loss of energy that address DSM-IV depression criteria, which were not included in the first edition. Respondents are asked to endorse the most characteristic statement over the previous 2 weeks.

BDI-II is scored by summing the ratings for the 21 items (4-point scale ranging from 0 to 3). Cut-off scores depend on the characteristics of the sample and the clinical considerations for which the instrument is administered. A cut-off score of 17 or over yielded a 93% true-positive rate in a clinical sample from the University of Pennsylvania [276] but lower cut-off scores can been used if the purpose is to screen for possible cases of depression. Total score ranges of 14-19, 20-28 and 29-63 for mild, moderate and severe depression respectively were obtained from the same clinical sample.

2.7.2.3 Anxiety

The Beck Anxiety Inventory (BAI) was developed as a clinical instrument for measuring the severity of an individual's anxiety [267]. It consists of 21 items describing common symptoms of anxiety and uses a 4-point scoring scale (range 0 to 3). There is an overlap with the depression scale as measured by the BDI-II (even when encompassing anxiety and depression specific items only in “purified” versions of the BAI and BDI-II) suggesting that anxiety and depression seem to be inherently linked [277]. BAI is scored by summing the ratings for the 21 items (4-point scale ranging from 0 to 3). Total scores in the 8-21 range are suggestive of mild, 21-42 of moderate and over 42 severe anxiety.

2.7.2.4 Impact of events scale (IES)

Children and adolescents can react to single acute stressors but also to chronic stressful situations with a particular form of anxiety called Post-Traumatic Stress Disorder (PTSD). The IES was developed to monitor the main phenomena of re-experiencing the traumatic event and of avoidance and feelings associated with them. The original 15-item, four-point scale was reduced to 8 items as some of them were misinterpreted by children. The remaining 8 items still contain intrusion and avoidance items and the total score correlates highly with the total on the 15-item version ($r>0.95$, $p<0.001$). The IES is self-completed and the eight items are scored on a four-point scale with values of 0,1,3 or 5. The equivalent cut-off score indicating a high risk of PTSD for the shorter 8-item version is 17.

2.7.2.5 Behaviour Rating Inventory of Executive Function (BRIEF)

BRIEF is an 86 item instrument designed to assess impairment of executive function. It has been shown to be useful in evaluating children and adults with a wide spectrum of developmental and acquired neurological conditions including TBI [278, 279]. Self and proxy rating forms are available. Two groups are assessed: behavioural regulation and metacognition.

Clinical scales assessed in Behavioural regulation include inhibition (impulses control, stop behaviour), shift (ability to move freely from one activity/situation to another; transition; problem-solve flexibly) and emotional control (modulation of emotional responses).

Clinical scales assessed in Metacognition include initiation (starting activity; generate ideas), working memory (holding information in mind for purpose of completing a task), organisation of materials (anticipating future events; setting goals; develop steps) and monitor (checking work; assessing own performance).

Once raw scores for all scales are obtained, they can be converted to standard scores (T scores) with a mean of 50 and SD of 10. These scores are age and gender specific. Higher scores suggest a higher level of dysfunction.

2.7.3 HRQL and health status

Health related quality of life (HRQL) is the value assigned to duration of life as modified by the impairments, functional states, perceptions, and social opportunities that are influenced by disease, injury, treatment or policy [280]. In assessing HRQL, dimension or items (questions) are components of a domain of health. Instruments can be generic or disease specific.

2.7.3.1 Paediatric Quality of Life Inventory

HRQL was assessed using the generic Paediatric Quality of Life Inventory (PedsQL 4.0) [281] a brief, standardized, modular instrument applicable for both children and adults. Generic core scales were designed to measure the core items of health as delineated by the World Health Organization and encompass: 1) physical functioning, 2) emotional functioning, 3) social functioning and 4) school functioning. Although the PedsQL was derived from data collected on paediatric cancer patients, its reliability and validity was subsequently demonstrated on healthy and patient populations. It has been widely applied to children with a variety of acute and chronic conditions including TBI in childhood. In particular, the cognitive function scale of the PedsQL detected the largest differences among groups of children with varying severities of TBI [282]. Scores can be transformed to a 0-100 scale, so that higher scores indicate better

HRQL. An overall value of <69.7 (equivalent to one SD below the population mean) has been shown to represent poorer HRQL. PedsQL has been shown to be reliable: Total Scale Score (α=0.88 child, 0.90 parent report), Physical Health Summary Score (α=0.80 child, 0.88 parent) and Psychosocial Health Summary Score (α=0.83 child, 0.86 parent). Both self and parent-report questionnaires were used in KHINES.

For disease specific assessment the QoL-AGDHA, a one-dimensional, patient needs-based HRQL instrument developed specifically to detect deficits in needs achievement in areas that are affected in adults with GHD was used. It consists of 25 'yes' or 'no' questions [283]. The number of 'yes' responses constitute a score, with a high score denoting a poor HRQL.

2.7.3.2 Chalder Fatigue Scale

The Fatigue Scale devised by Chalder et al (1993) is a short, self-report measure of the severity of tiredness [284]. It produces a total score, with sub scores reflecting mental fatigue (4 items), and physical fatigue (7 items). The 11 items are rated on a four-option continuum, from "better than usual", to "much worse than usual". The responses are either assigned scores from 0 to 3 (Likert method) giving a maximum of 33, or 0, 0, 1, 1 (bimodal method), giving a maximum of 11. A bimodal score of 3/4 was initially recommended for identifying significant fatigue [284] with subsequent studies estimating bimodal fatigue scores in community samples being 3.27±3.21 or 14.2±4.6 using Likert method [285]. The scale has been validated in a general population study [286]. The validity of the questionnaire in assessing fatigue in the general population suggests that it is a useful tool for assessing fatigue in a variety of medical disorders. The limitations of the Fatigue Scale include its inability to distinguish between CFS and primary physical or cognitive dysfunction, which may confound interpretations of the responses [284].

2.7.3.3 Health Utilities Index (HUI)

The term utility corresponds to the value attached to a particular health state and is derived from judgments made by panels of the public, professionals or patients about relative values of different health states. The Health Utilities Index (HUI) is a system of measuring health status and is recognised to be a useful tool for reporting HRQL [287, 288]. It was evolved in response to the need for a standardised system to describe a) the experience of patients undergoing therapy; b) long-term outcomes of disease or therapy; c) efficacy, effectiveness and efficiency of healthcare interventions and; d) the health status of general populations. HUI health-state attributes include vision, hearing, speech, ambulation, pain, dexterity, health-care, emotion and cognition. The major

criterion for selecting these particular attributes was the importance placed on each of them by members of the public. They are clearly distinguishable from one another and cover an extensive range of possible disabilities as each attribute has 3-6 levels that together describe almost one million unique health states. HUI attributes can be translated into a health utilities score by applying the utility formula of Torrance and colleagues [289]. HUI is scored using single- and multi-attribute utility functions and will allow for negative scores of HRQL that represent health states considered worse than dead. Utility scores have interval-scale measurement properties to support the use of HUI in constructing single summary indexes and to enable the use of parametric statistical techniques for making comparisons among clinical groups. The HRQL scoring systems provide utility scores on a generic scale where dead = 0 and perfect health = 1. Differences of 0.03 or greater in mean HUI overall HRQL scores have been reported to be definitely important, and differences as little as 0.01 may be meaningful and important in some contexts [290]. The overall HRQL score is determined using the HUI3 multi-attribute utility function (MAUF) is:

=1.371*(lvl1*lvl2*lvl3*…lv8)-0.371

Descriptive levels within HUI attributes were defined to be meaningfully different from each other when difference in utility scores between levels of an HUI attribute was 0.05 or more. The lowest possible HUI3 multi-attribute utility is defined for the interval -0.36 to 1.00. Single-attribute utility functions provide scores that describe the morbidity attribute by attribute. Each single-attribute utility function is defined on a scale from 0.00 to 1.00 (0 for most disabled and 1 for no disability) and has interval scale properties allowing for comparisons between groups or to assess changes within groups over time. In brief, the overall health status of each person can be described using an 8-element vector (one from each of the eight attributes) and a single overall score of HRQL.

The HUI-Mark 3 has been shown to discriminate between various child populations [291]. The health status measure provides information on the type and extent of disabilities whilst the utility scoring measures relative importance of disabilities, which is necessary for any health economic evaluations as outcomes, can be measured in quality-adjusted life years (QALYs). QALYs are estimated by multiplying the number of life years with a utility score that has the value 1.00 for “perfect health” and 0.00 for “a health state comparable to the value of death” as described previously. QALYs are particular useful to provide a common metric of burden of disease across the entire spectrum of diseases.

In addition, HUI measures have been shown to be responsive to changes of health status over time, which is an important property in detecting effects of recovery or treatment over time [292]. The HUI-3 can be used in all people age 5 and older in both clinical and general populations. Respondents were the participants and people other than the participant (usually parent or spouse) referred as “proxy”. Participants were asked to focus on their “usual” health without defining a specified period and explaining that short-time, self-limiting illnesses or injuries were not the point of interest.

2.7.4 Child and Adolescent Scale of Participation (CASP)

The Child and Adolescent Scale of Participation (CASP) has been developed to assess the extent to which children participate in movement-related, communication related and school-based social activities compared to children of the same age as reported by the carer. It was initially designed as part of the Child and Family Follow-up Survey (CFFS) to monitor outcomes of children with acquired brain injury [293] and subsequently, has been used alone or as part of the CFFS to examine participation of children other diagnoses [294].

The CASP consists of 20 ordinal-scaled items (applicable to children who are 5 years or older) in four subsections: 1) Home Participation (6 items), 2) Community Participation (4 items), 3) School Participation (5 items), and 4) Home and Community Living Activities (5 items). The 20 items are rated on a four-point scale: (4=Age-expected, 3=somewhat limited; 2=Very limited; 1=Unable).

CASP summary scores are created by summing all applicable item responses (maximum score 80), then dividing this number from the total possible score from all applicable items and then multiplying this number by 100 to conform to a 100-point scale. Higher scores indicate greater extent of age-expected participation. Item-level scores can be used to assess specific situations where for example mobility related participation is likely to be more affected.

2.7.4.1 Statistical analysis of neuropsychological and HRQL data

When analyses included comparisons between the 3 study groups (control group versus mild TBI versus moderate/severe TBI) these were performed by analysis of variance followed with pairwise comparisons (two tailed t-test with Bonferroni adjustment) when a significant difference was indicated. Non-parametric tests were used (Mann Whitney U for 2 groups and Kruskal-Wallis for 3 or more groups) to compare for dependent variables that were continuous but did not meet requirements of parametric equivalents. These were followed by pairwise comparisons adjusted for

the number of comparisons made) when the overall test showed significant differences across samples.

2.8 NEURO-IMAGING

CT brain scans previously performed as part of KHIS in severe/moderate TBI children during the acute post-injury phase were classified according to criteria/patterns described by Lobato et al [295]. Eight anatomical patterns emerged in a series of CT scans in 277 patients with severe TBI which provided stronger prognostic information when compared to the following four patterns of severe head injury: epidural haematoma, subdural haematoma, brain contusion and diffuse brain damage. The eight patterns included the following:

Pattern 1: Pure extracerebral hematoma

Pattern 2: Extracerebral hematoma plus acute hemispheric swelling

Pattern 3: Single brain contusion, whether or not associated with a neighboring extracerebral hematoma

Pattern 4: Multiple unilateral brain contusion whether or not associated with subdural hematoma

Pattern 5: Multiple bilateral brain contusion

Pattern 6: General brain swelling whether or not associated with small extracerebral hematoma

Pattern 7: Diffuse axonal injury

Pattern 8: Normal CT scans.

Patients with acute hemispheric swelling after operation for a large extracerebral hematoma, multiple brain contusion (unilateral or bilateral) and diffuse axonal injury had worse outcomes. Conversely patients with normal CT scans, uncomplicated extracerebral hematoma, single brain contusion and general brain swelling had better prognosis [295, 296].

Unless contraindicated, all study participants were examined on a 3T MRI scanner (Magnetom Skyra, Siemens Medical Systems, Clinical Research and Imaging Centre, Bristol).

Sagittal and coronal T2 TSE (TR/TE 4430/89ms; thickness 2mm; FOV 190; resolution 0.5x0.5mm) and T1 weighted images (TR/TE 500/2.93; thickness 2mm; FOV 190) were obtained. These data sets were assessed visually by the same consultant neuro-radiologist blinded to clinical details to rule out artefacts, structural abnormalities or other pathologies.

Assessment of the hypothalamic-pituitary axis included pituitary volume (using the ellipsoid formula i.e. volume = 4/6 * π * height * length * width) and height and thickness of the pituitary stalk.

Volumetric and region of interest analysis (ROI) from obtained volumetric T1 data sets in participants who did not have any contraindication for having an MRI scan was performed using open source packages and toolboxes. The latest version of SPM [297] (SPM12, Statistical Parametric Mapping) developed at the University College London was used for most analyses in conjunction with CAT12 toolbox – an SPM12 extension which provides computational anatomy options using Voxel-based approach (VBA) including voxel-based morphometry (VBM) but also surface-based morphometry (SBM) and deformation-based morphometry (DBM).

VBA provide a voxel-based estimation of volume of a specific tissue compartment and as it “interrogates” the entire brain for abnormalities [298] it is particularly useful in evaluating TBI where brain abnormalities are not always fully delineated.

VBM requires a number of pre-processing steps before data are appropriate for statistical analyses. Firstly, images have to be converted to a compatible format for processing which can be done with SPM12. Study imaging files were converted from DICOM (Digital Imaging and Communications in Medicine) to NIfTI (Neuroimaging Informatics Technology Initiative) format, and imported for pre-processing.

Following conversion and in order to compare MRI data from different participants, all brain images were spatially registered in the same 3D space (normalization). This was done using a template defined by the Montreal Neurological Institute (MNI152 or MNI305). In general, the closer the images start out to the MNI template orientation, the better the outcome of the normalization. Alignment was further improved by using computational anatomy methods (DARTEL toolbox for SPM) [299] which can align fine structures across individuals in a way that takes into account anatomical constraints.

Once images were normalised, tissue classification (segmentation of brain tissue into separate tissue compartments) allowed estimation of CSF, grey and white matter volumes. This was followed by scaling modulation so the total amount of grey matter was scaled back to the volume it would be in the original image. A sample of the output report following image segmentation is show in Figure 11.

Version: Matlab / SPM12 / CAT12:	7.13 / 6906 / 1113
Tissue Probability Map:	..MATLAB\R2011b\toolbox\spm12\tpm\TPM.nii
Spatial Normalization Template:	..tes_1.50mm\Template_1_IXI555_MNI152.nii
Spatial Normalization Method / vox:	Dartel / 1.50 mm
Affine regularization:	mni
Noise reduction:	SANLM(0.06) + MRF(0.06)
LASstr / GCUTstr / CLEANUPstr:	0.50 / 0.50 / 0.50
Voxel resolution (original > intern):	0.94x0.94x0.94 mm³ > 0.94x0.94x0.94 mm³

Image and Preprocessing Quality:	
Resolution:	86.23% (B)
Noise:	90.30% (A-)
Bias:	83.69% (B)
Weighted average (IQR):	87.50% (B+)
Processing time:	14:06 min:s

Volumes:	CSF	GM	WM	
Absolute volume:	327	781	462	cm
Relative volume:	20.8	49.8	29.4	%
TIV:	1570 cm³			

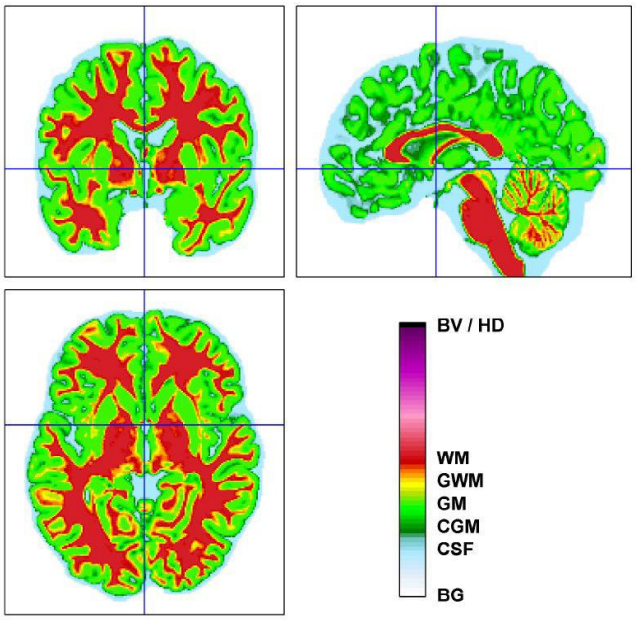


Figure 11 VBM output sample

2.8.1 Statistical analysis of neuro-imaging

When analyses included comparisons between the 3 study groups (control group versus mild TBI versus moderate/severe TBI) these were performed by analysis of variance followed with pair comparisons (two tailed t-test with Bonferroni adjustment). Non-parametric tests were used (Mann Whitney U for 2 groups and Kruskal-Wallis for 3 or more groups) to compare for dependent variables that were continuous but did not meet requirements of parametric equivalents. These were followed by pairwise comparisons (adjusted for the number of comparisons made) when the overall test showed significant differences across samples.

3 CHAPTER 3 – (RESULTS)

3.1 PARTICIPANTS

Exploration of the KHIS database identified 216 potential participants who were eligible for participation in this study. Forty-four participants were not contactable (11 moved outside the UK, one deceased in prison, five left home not registered with new GP, 27 with updated contact details not contactable). From the remaining 172, 93 (54%) were not willing to participate in the study (in decreasing frequency due to time constrictions related to school/studies/work/family commitments, relocation outside the area, participating in other studies and court proceedings related to TBI in 2 cases). Six were excluded (two further TBI, 2 pregnant, 2 with non-TBI related endocrinopathy). All of the remaining 73 consented and 72 completed the study (one participant withdrew for family reasons) [Figure 12].

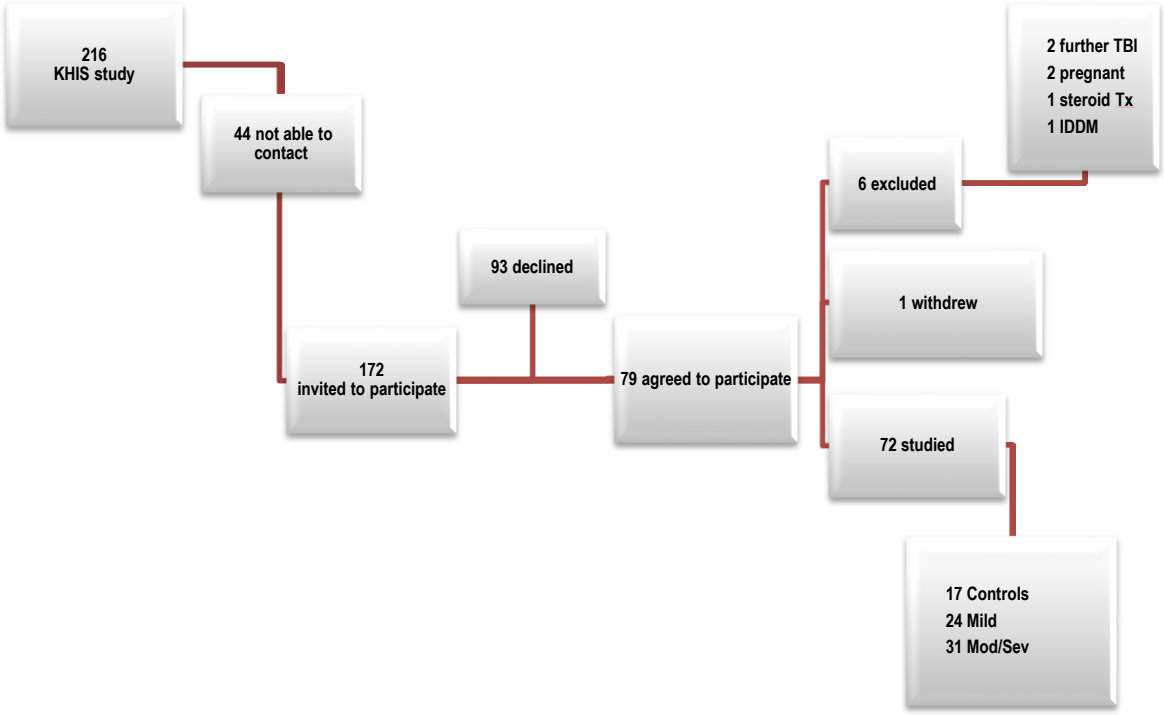


Figure 12. KHINES recruitment flow chart

There were no differences between participating and non-participating groups with regards to age, gender, time since TBI, duration of post-traumatic amnesia, days in intensive case or type of structural abnormalities on acute neuroimaging (extradural/subdural haemorrhage, diffuse axonal injury or skull fracture) as seen in Table 3

	KHINES (n=72)			Non – KHINES (n=93)			
	N (%)	median	range	N (%)	median	range	*p*
Male	48 (67)			57 (61)			0.52
TBI moderate/severe	31 (43)			31 (33)			0.42
TBI mild	24 (33)			23 (25)			0.55
Extradural haemorrhage	20 (28)			18 (19)			0.46
Subdural haemorrhage	9 (12)			18 (19)			0.65
Diffuse axonal injury	9 (12)			12 (13)			0.94
Skull fracture	34 (47)			36 (39)			0.50
Post traumatic amnesia days		10	1-99		10	1-77	0.81
Intensive care days		1.5	0-22		0.5	0-30	0.20
Age at TBI		11.7	0.2-16.7		11.1	0.1-17.5	0.45

Table 3. Comparison between participating and non-participating patient groups

The mechanism of injury in the 55 TBI participants is shown in Table 4. Two thirds of studied participants were male (n=49, 67%). Characteristics of study groups including age at injury and time from injury are shown in Table 5

Mechanism of injury	n=55
Fall	*23
Motor vehicle accident	23
pedestrian	21
passenger	2
Cyclist	9

*Possible Non-Accidental Injury in one fall.

Table 4. Injury mechanism in TBI children

Of the 72 participants 31 (43%) were categorised as moderate/severe TBI (GCS severe 3-8, moderate 9-12), 24 (33%) mild TBI (GCS 13-15) and 17 (24%) were healthy controls. Three female participants were on oral contraceptive. No participants were on treatment for epilepsy.

KHINES groups	Mean	SD	Range
Control			
Age at evaluation (years)	18.5	5.5	10.9 - 27.6
Mild TBI			
Age at evaluation (years)	17.8	5.0	9.4 - 26.3
Age at injury (years)	9.5	5.4	.2 - 16.6
Time from TBI (years)	8.3	1.3	5.8 - 10.7
Mod/Sev TBI			
Age at evaluation (years)	19.8	4.2	11.3 - 26.4
Age at injury (years)	10.7	4.1	.4 - 16.8
Time from TBI (years)	9.0	1.1	6.8 - 10.8

Table 5. KHINES Study group characteristics

3.2 CLINICAL ASSESSMENT AND ANTHROPOMETRY

The anthropometry of the three participant groups is shown in table 4. There was no statistical difference (independent samples Kruskal-Wallis test) between groups with regards to height, weight, BMI, skinfold thickness, abdominal circumference or body composition [Table 6].

N=72	Mild (n=24)	Mod/Sev (n=31)	Control (n=17)	p
Height (cm)	165.2 ± 14.1	172.3 ± 9.9	171.8 ± 14.0	p=.112
Weight (kg)	66.1 ± 22.6	69.4 ± 12.1	72.2 ± 21.8	p=.655
BMI	23.6 ± 6.1	23.3 ± 3.6	24.1 ± 5.3	p=.703
Skinfold Biceps (mm)	7.2 ± 5.9	7.7 ± 7.1	7.2 ± 5.6	p=.955
Skinfold Triceps (mm)	13.8 ± 9.2	13.5 ± 12.0	13.8 ± 8.7	p=.497
Body fat (%)	23 ± 12	20 ± 10	21 ± 7	p=.597
Abdominal circumference (cm)	77.0 ± 12.9	79.0 ± 8.1	80.5 ± 12.2	p=.753

Table 6 Auxology of participant groups

Comparison of SD scores did not show any statistical difference between groups either (independent samples Kruskal Wallis test) [Table 7].

N=72	Mild (n=24)	Mod/Sev (n=31)	Control (n=17)	p
Height SDS (range)	0.185 ± 1.17 (-2.0 – 2.43)	0.234 ± 0.92 (-1.74 – 2.45)	0.439 ± 0.92 (-1.01 – 2.8)	p=.750
Weight SDS (range)	0.775 ± 1.49 (-2.56 – 3.88)	0.636 ± 1.02 (-1.36 – 2.85)	1.095 ± 1.03 (-0.37 – 3.85)	p=.446
BMI SDS (range)	0.732 ± 1.29 (-1.81 – 3.17)	0.528 ± 0.95 (-1.08 – 2.81)	0.953 ± 1.14 (-0.65 – 3.44)	p=.554

Table 7 Participant SDS scores for height, weight and BMI

3.3 ENDOCRINE RESULTS

3.3.1 Baseline endocrine function

All participants were clinically euthyroid and had normal thyroid and posterior pituitary function. Testosterone levels in pubertal/post-pubertal males were within the normal range [Table 8].

One post pubertal female with primary amenorrhea and history of eating disorder 3 years ago had low levels of both oestradiol (<50 pmol/L) and gonadotropins. IGF1 SDS and IGF-BP3 SDS for this participant were -2.39 and -2.14 respectively.

N=25	Mean	Minimum	Maximum	Reference range
TSH (mIU/L) (n=25)	2.6	0.6	4.9	0.5-5
FT4 (pmol/L) (n=25)	15.8	11.5	20.2	9-20
Testosterone (nmol/L) (n=15)	18.5	9.3	28.1	9-30
Oestradiol (pmol/L) (n=10)	194.1	50.0	572.0	>73
IGF1 (nmol/L) (n=23)	37.5	21.6	60.1	Age dependent
IGF-BP3 (mg/L) (n=23)	3.2	2.3	4.6	Age dependent
IGF1 (SDS)	-0.56	-2.39	1.53	Age dependent
IGF1BP3 (SDS)	-0.58	-2.14	1.13	Age dependent

Table 8 Baseline endocrine function in mod/sev TBI participants

3.3.2 Stimulated growth hormone and cortisol

Twenty-five out of 31 mod/sev TBI participants had a stimulation test. In 7/25 (4 male and 3 female including the one with secondary amenorrhea and low gonadotropin levels), GH response to ITT using age/pubertal stage appropriate cut-off values was abnormal [Table 9].

	Gender	Age (yr)	Tanner stage	Height SDS	BMI SDS	Injury type	Injury mechanism	Stimulated GH peak (mcg/l)	IGF1 SDS	IGF-BP3 SDS	Stimulated cortisol peak (nmol/l)
1	F	12	3	1.42	2.58	Mod	Fall	4.4	0.21	1.13	524
2	M	22	5	1.10	1.30	Mod	Pedestrian	1.8	-0.49	-0.86	586
3	F	16	4	0.76	0.16	Mod	Pedestrian	3.7	-2.39	-2.14	584
4	M	24	5	0.23	-1.04	Sev	Fall	0.1	-0.46	-0.74	501
5	M	25	5	-0.33	0.59	Mod	Pedestrian	0.1	-0.30	-0.51	526
6	M	14	3	0.35	-0.36	Mod	Cyclist	1.2	-1.06	-1.17	483
7	F	17	5	0.35	0.73	Sev	Pedestrian	4.1	-0.71	0.76	511

Table 9 Mod/sev TBI participants with abnormal GH response following ITT

IGF1 SDS was not different between participants with normal and abnormal stimulated GH response (Mann-Whitney p=.86) [Figure 13]. The peak GH response to ITT did not correlate with IGF1 (ρ = .21, p =.35), IGF-BP3 (ρ = -.04, p =.87), IGF1 SDS (ρ = .07, p =.77) or IGF-BP3 SDS (ρ =-.06, p =.79) even when controlling for weight.

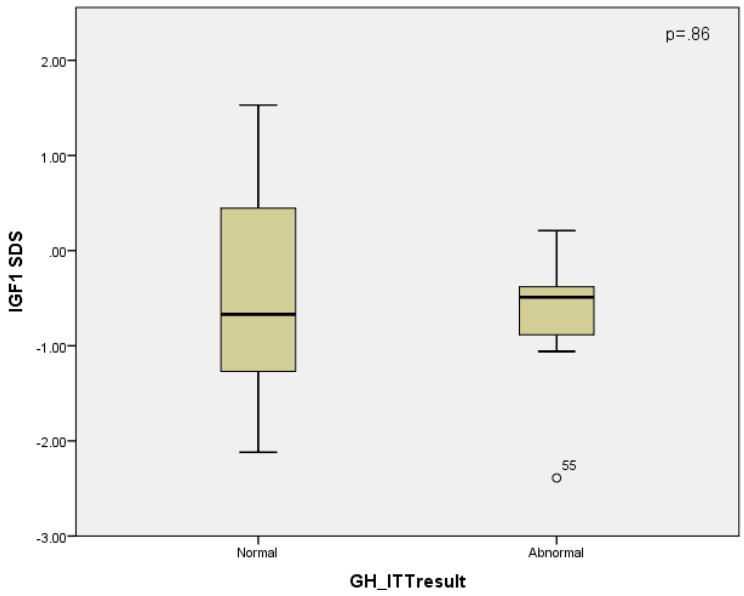


Figure 13 IGF1 SDS in TBI patients depending on result of GH stimulation test

In three ITT's, intravenous glucose had to be administered to correct symptomatic hypoglycaemia that did not respond to oral treatment.

In two participants, the cortisol response was suboptimal (peak cortisol 392 and 483nmol/l). One of them had also abnormal stimulated GH response (peak GH 1.2 mcg/L) but normal height for his age (Height SDS 0.35).

3.3.3 Spontaneous growth hormone and cortisol

Twenty-two of 31 participants completed an overnight GH profile (eight did not consent to the overnight stay and one GH profile was incomplete due to difficult venous access).

3.3.3.1 Growth hormone

The following pulsatility parameters of GH were studied: half-life, area under the curve, mean secretion, peak secretion, number of secretory peaks, mean secretion pulse amplitude, mean secretion pulse mass and approximate entropy (ApEn).

Mean 12-hour GH secretion was under 3.3 mcg/l in 18/22, under 2 mcg/l in 12/22 and under 1 mcg/l in 4/22 profiles. In the four participants with mean 12-hour GH secretion <1 mcg/l ("x" in Figure 14), peak stimulated GH was 9.7, 4.4, 3.7 and 0.1 mcg/l (two abnormal ITT's using age appropriate cut-offs). There was no significant correlation between peak stimulated and peak spontaneous GH levels (ρ=.17, p=0.45). Although spontaneous peak GH was higher compared to the stimulated one (11.1±7 vs 8.1±6.9 mcg/L) the difference was not statistically significant (paired t-test, p=.129).

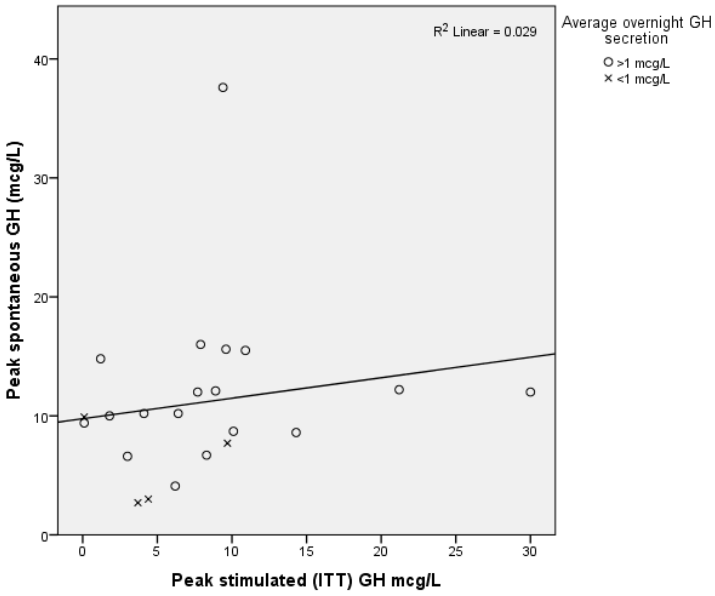
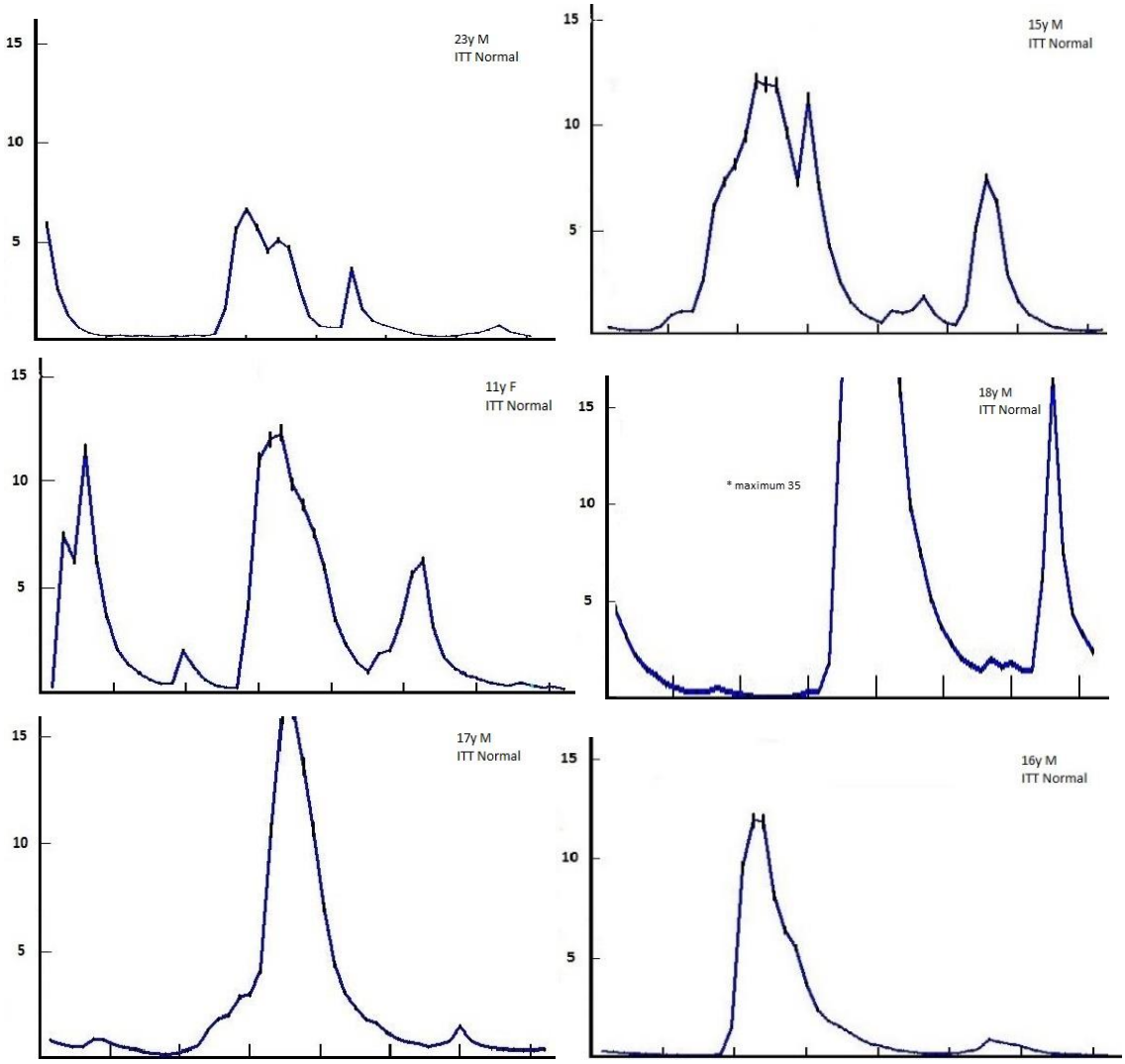


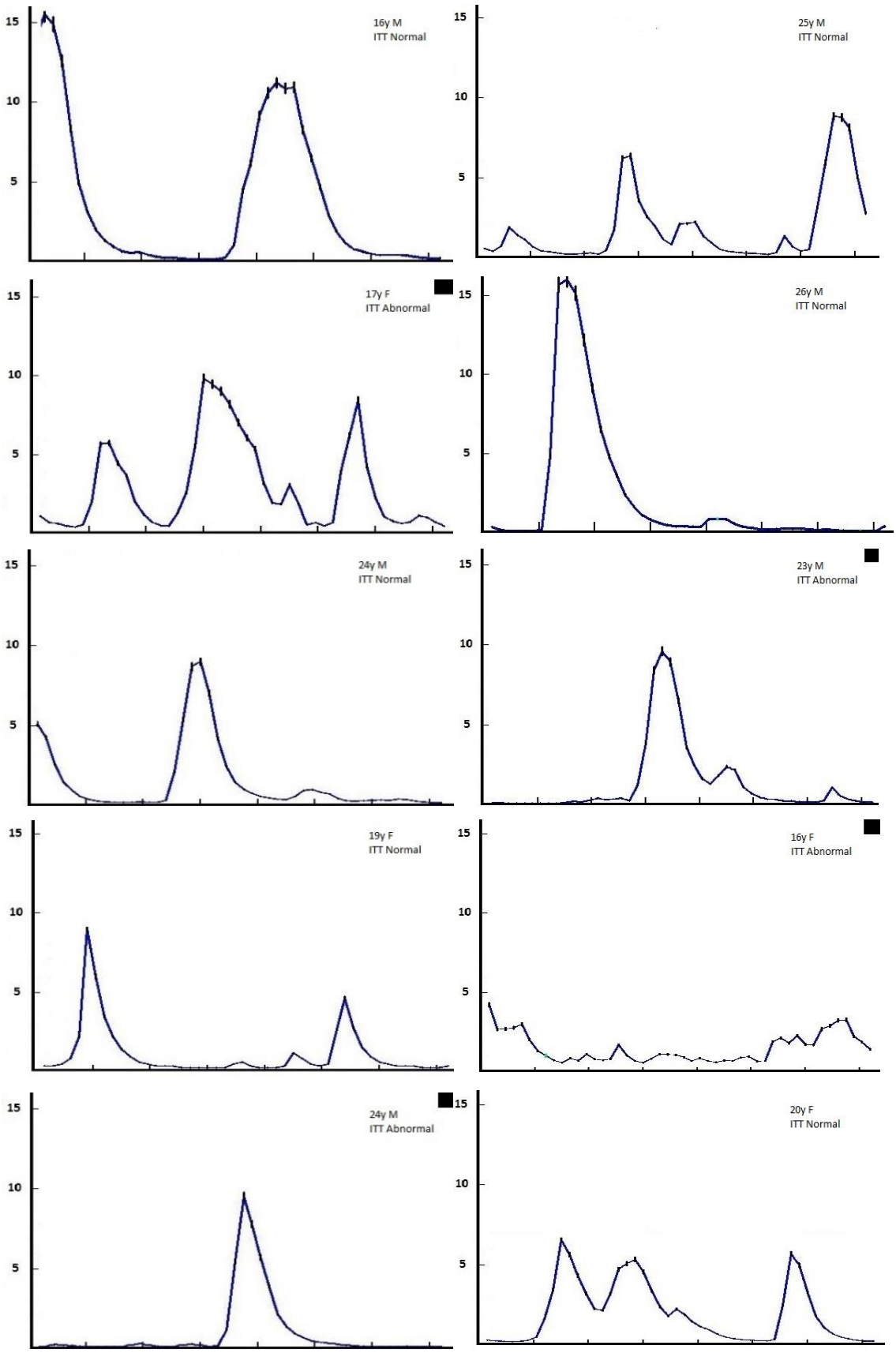
Figure 14 Peak stimulated and spontaneous overnight GH secretion in survivors of childhood mod/sev TBI.

Agreement between spontaneous ("normal"/"abnormal" profile using cut-off of <1mcg/L, <2mcg/L or <3.3<mcg/L) and "normal"/"abnormal" stimulated GH secretion (ITT) was less than .20 in all comparisons. This indicates poor agreement.

Using the GH cut-off from the stimulation test as the differentiating factor to categorise a profile as “normal” or “abnormal” [84], only one profile was “abnormal”. In this participant, both stimulated (3.7 mcg/L) and spontaneous peak GH secretion (2.7mcg/L) were low. This 16y old (TBI at 7y of age) female participant had also low IGF1 (-2.39 SD), but normal height (0.76 SD), weight (0.39 SD) and did not have any symptoms of fatigue.

All individual GH concentration profiles are shown in the following pages [Figure 15].




16y M
ITT Normal
25y M
ITT Normal
17y F
ITT Abnormal
26y M
ITT Normal
24y M
ITT Normal
23y M
ITT Abnormal
19y F
ITT Normal
16y F
ITT Abnormal
24y M
ITT Abnormal
20y F
ITT Normal
15
10
5

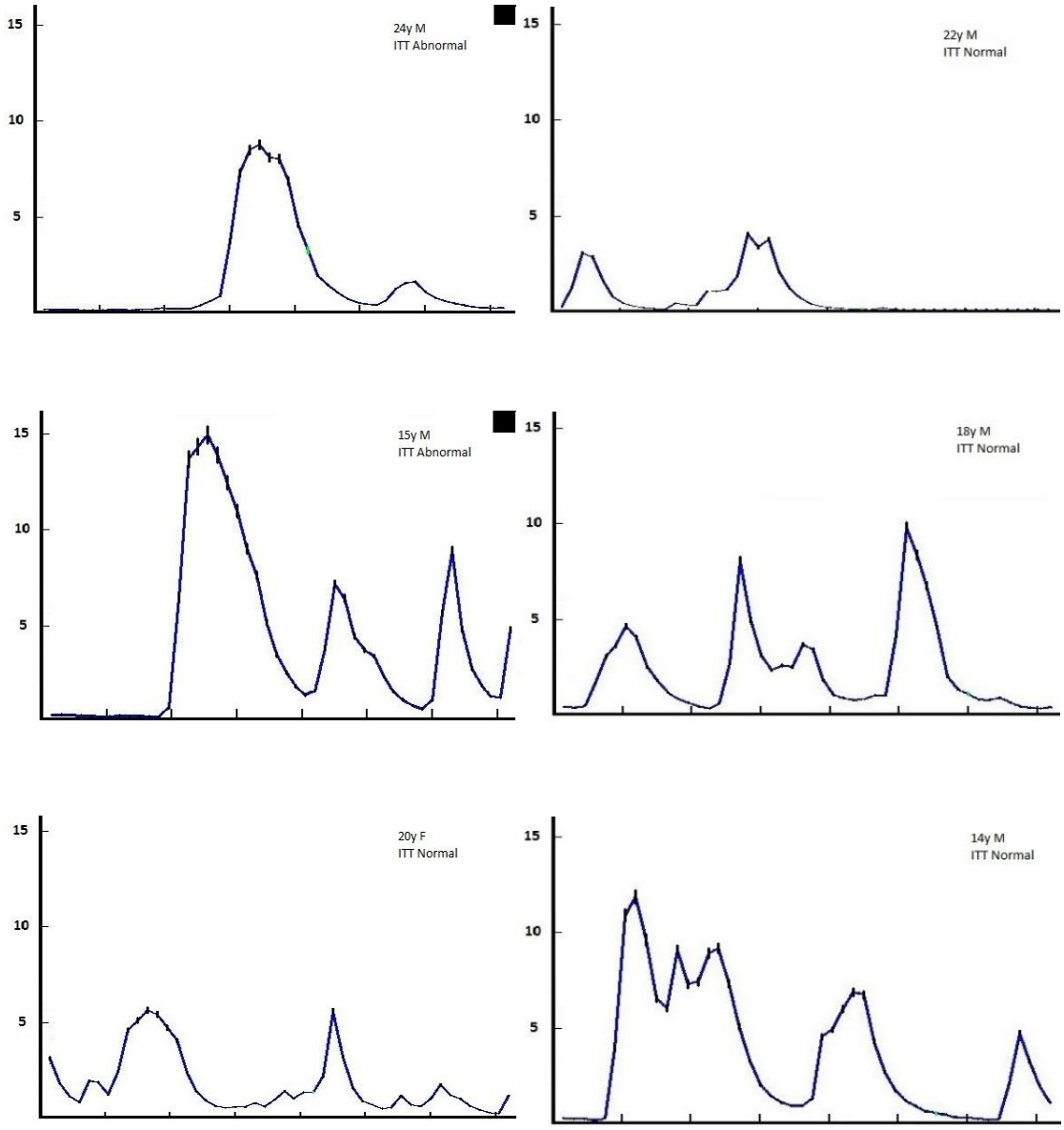


Figure 15 Individual overnight GH profiles of children/young adults with TBI (black square at the top right corner indicates patients with abnormal ITT). X axis: time 12 hours (8pm to 8am), Y axis: GH (mcg/L)

Characteristics of GH profiles after deconvolution analysis in mod/sev TBI participants are shown in Table 10.

	Mean ± SD	Minimum	Maximum	*Mean ± SD
GH Basal Secretion (mcg/L/min)	0.0051 ± 0.0048	0.0002	0.0182	0.0011±0.0003
GH Half-life (min)	20.21 ± 5.32	11.5	29.5	19.32±0.57
GH AUC (mcg/L/min)	1600 ± 978	367	4585	1064.3±147.2
GH Average (mcg/L)	2.22 ± 1.35	0.52	6.37	0.87±0.12
GH Max Measured (mcg/L)	11.2 ± 7.0	2.7	37.6	3.11±0.4
GH Secretory Peaks	8 ± 2	3	14	5.5±0.3
GH Mean Secretory Pulse Height	0.39 ± 0.30	0.12	1.39	0.17±0.03
GH Mean Secretion Pulse Mass	6.98 ± 5.06	1.80	24.68	4.39±0.69
GH ApEn	0.51 ± 0.23	0.18	0.87	

*Table 10 Parameters of GH profiles in mod/sev TBI patient using deconvolution tools (AutoDecon) and representative values from *Nindl et al [249] (Cluster)*

Average GH secretion correlated negatively with age in our relatively age-homogenous study group (ρ=-.516, p=0.007) [Figure 16].

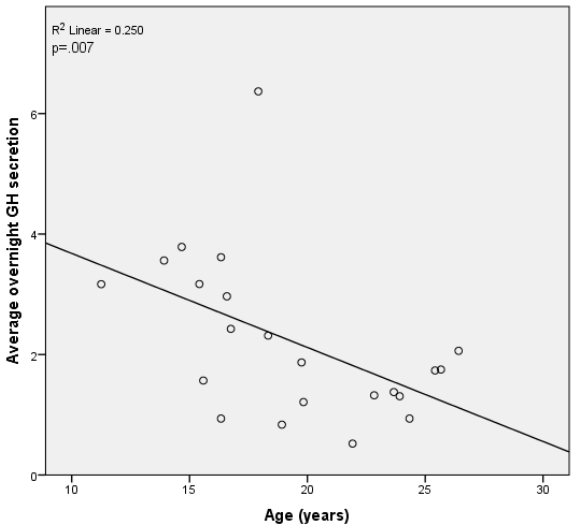


Figure 16 Average overnight GH secretion in mod/sev TBI group

In contrast to stimulated GH levels, which did not correlate with IGF1/IGF-BP3 and their standard deviation scores (SDS), parameters of spontaneous GH secretion (average, maximum, mean secretion pulse mass) correlated with IGF1/IGF-BP3 [Figure 17].

SDS for IGF1/IGF-BP3, also correlated with basal GH secretion and mean secretory pulse height (r=.495, p=.022 and r=.535, p=.013 respectively) but not with other measures of spontaneous GH secretion (average, maximum).

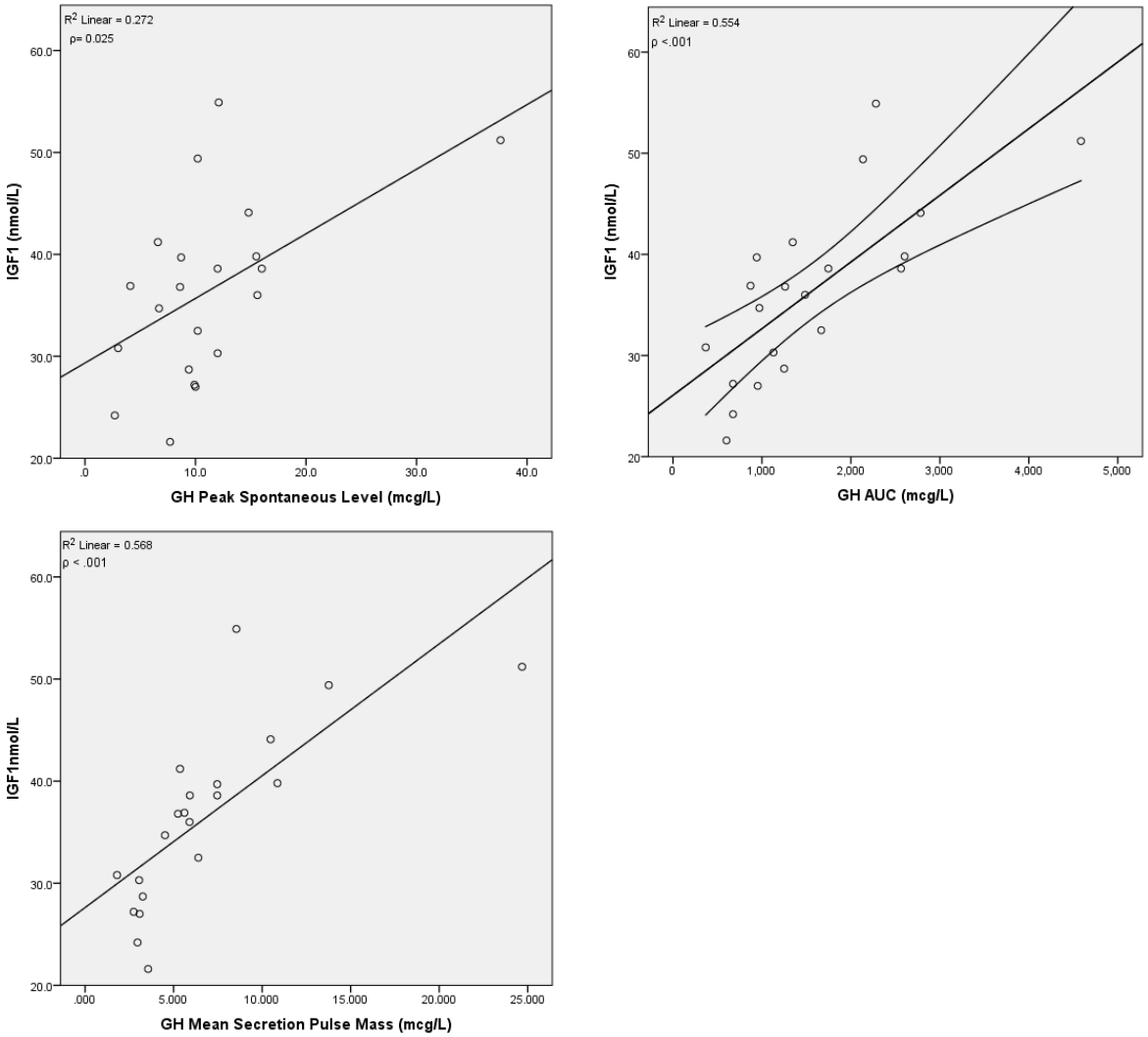


Figure 17 Correlation between IGF1 and measures of spontaneous GH secretion

Participants with average overnight GH secretion <1mcg/L had significantly lower IGF1 (t-test for equal variances, p=.001) [Figure 18] but not IGF-BP3, IGF1 SD or IGF-BP3 SD.

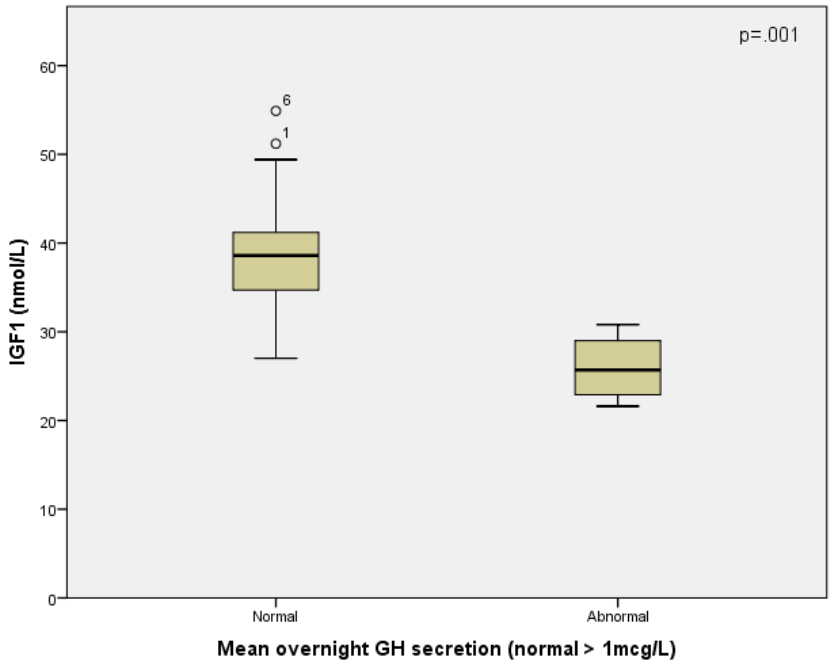


Figure 18 IGF1 levels in TBI patients with mean overnight GH secretion <1mcg/L

3.3.3.2 *Cortisol*

Mean, overnight, 12-hour cortisol concentration was 170 nmol/l and the mean of peak overnight levels 540 nmol/L. Cortisol levels from 00:00-02:00 were significantly lower than those between 06:00-08:00 (paired samples t-test, p<.001) [Table 11] and [Figure 19].

	Mean	SD	Minimum	Maximum
Maximum overnight cortisol (nmol/L)	540	127	227	811
Mean overnight cortisol (nmol/L)	170	63	33	310
Mean cortisol 00:00 – 02:00 (nmol/L)	59	35	13	127
Mean cortisol 06:00 – 08:00 (nmol/L)	411	136	132	715

Table 11 Summary data of overnight cortisol secretion in mod/sev TBI patients

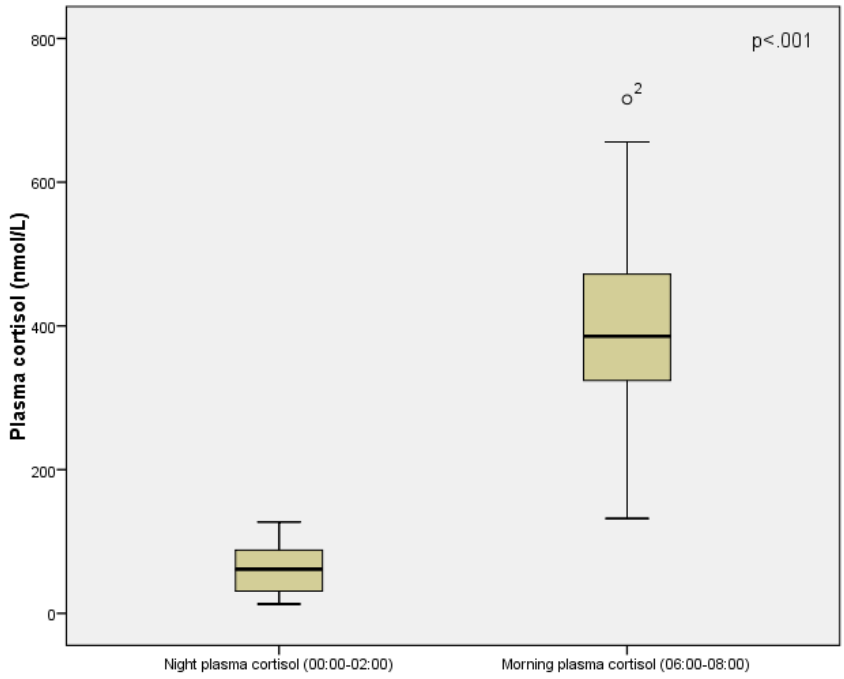


Figure 19 Midnight and morning plasma cortisol levels in mod/sev TBI patients

Maximum measured cortisol was under 500 nmol/l in 9/22 profiles. Stimulated cortisol (ITT) was normal in seven of these. In one of the two participants with abnormal stimulated and spontaneous cortisol secretion, GH response to ITT was also abnormal (peak GH 1.2 mcg/L). In the other participant (20y female), stimulated GH response was normal (peak GH 6.2 mcg/L) but ApEn for cortisol was very low (0.009, Z score -3.15) as was the average cortisol secretion which was the lowest in the study group

(minimum values in Table 11 are all from this participant). Fatigue and depression scores in this participant were high.

Peak spontaneous and stimulated cortisol levels correlated strongly (r=.788, p<.001) [Figure 20].

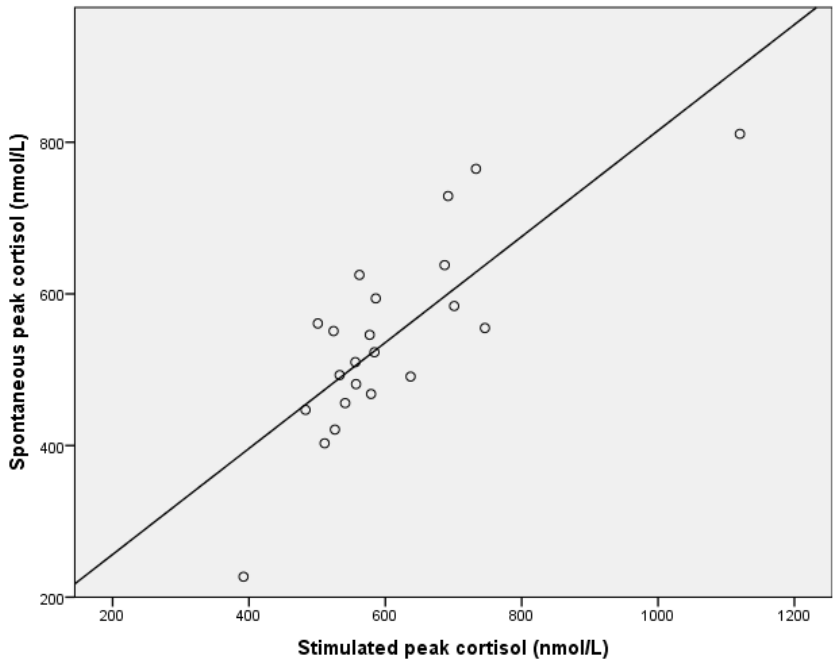


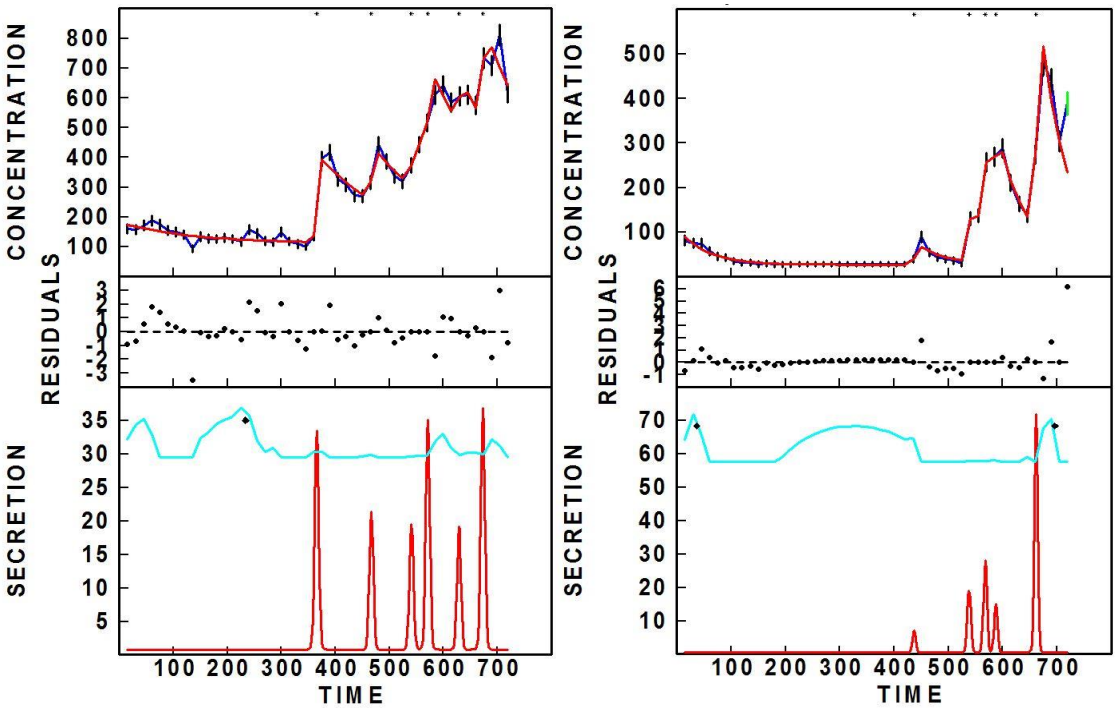
Figure 20 Stimulated and spontaneous plasma cortisol in mod/sev TBI patients

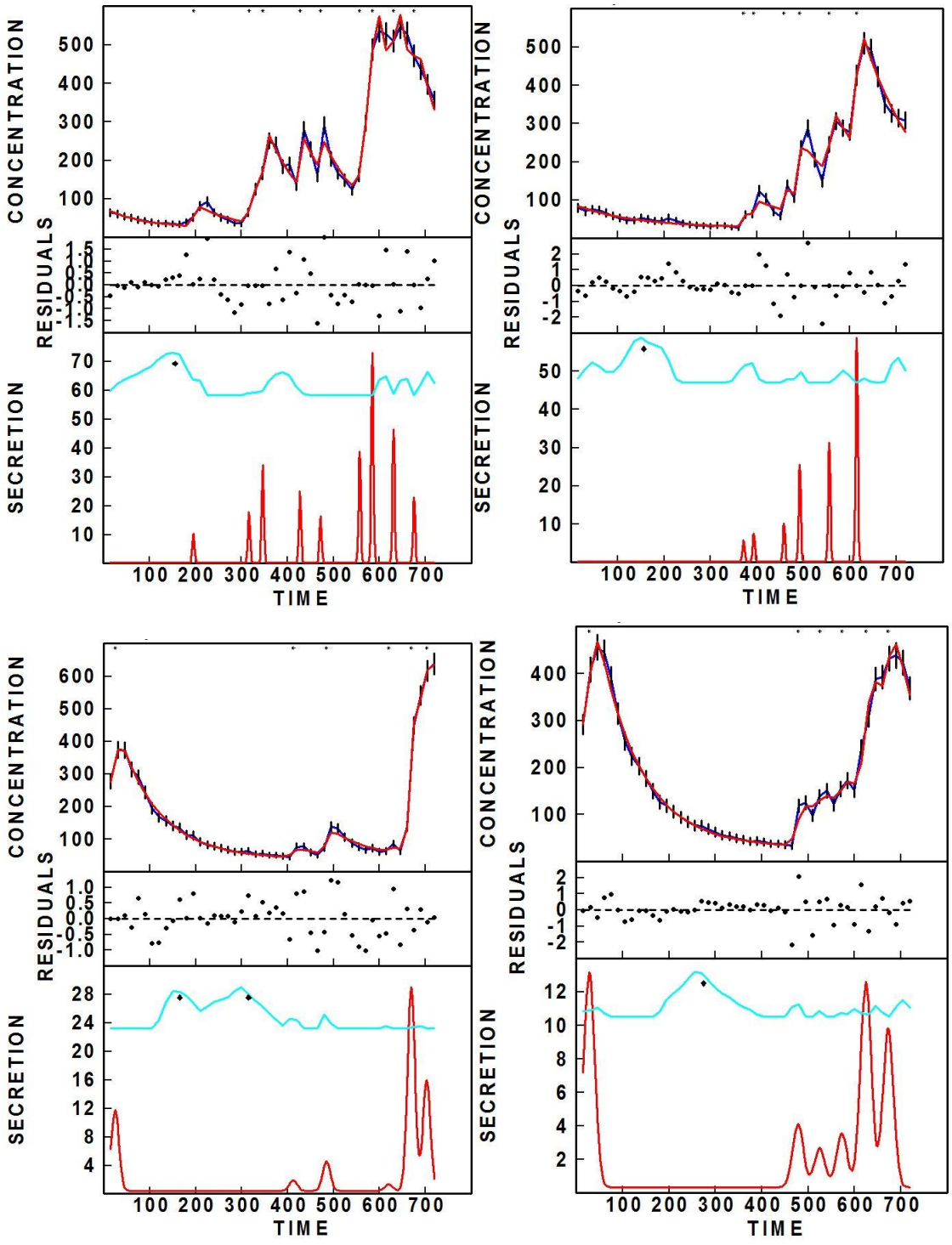
The correlation between stimulated and measures of spontaneous cortisol secretion was also significant for 00:00-02:00 am cortisol (r=.675, p<.001), 06:00-08:00 cortisol (r=.677, p<.001) and mean overnight cortisol secretion (r=.746, p<.001). The correlation with pulse height, pulse mass and basal secretion was not significant.

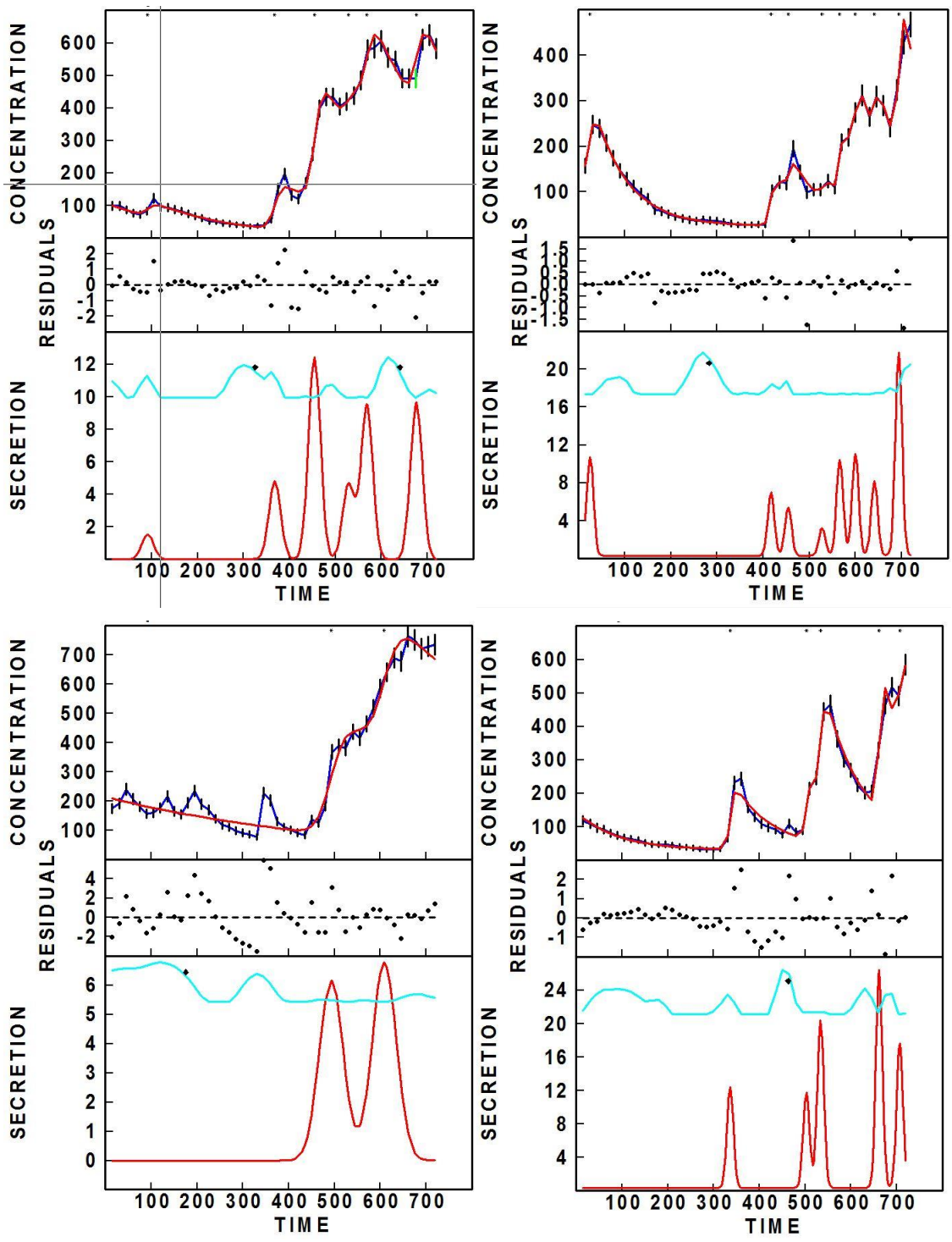
The average number of cortisol secretory episodes was 7 (range 2-9) and almost all occurred in the second half of the 12-hour overnight profile [Table 12 and Figure 21].

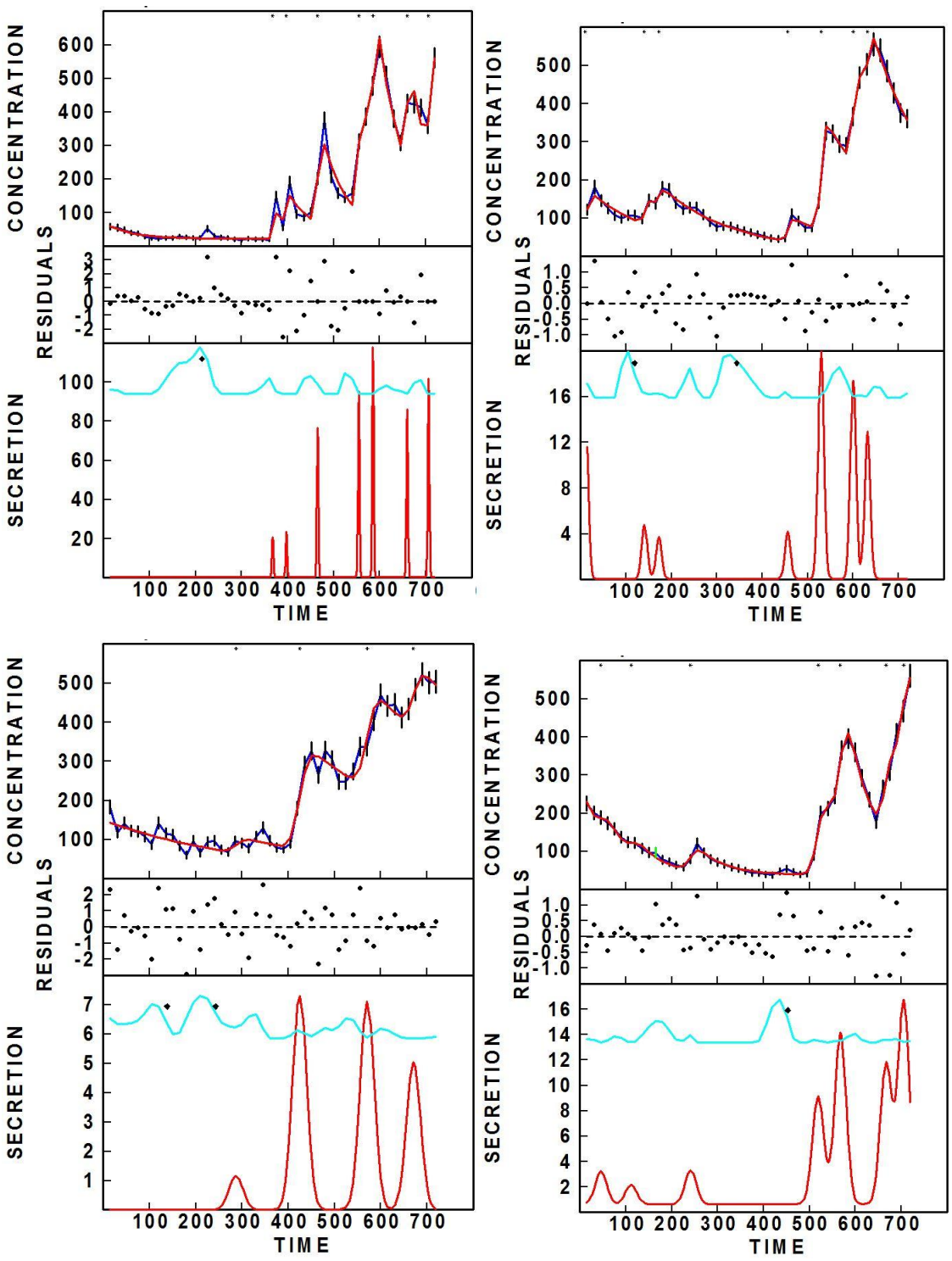
COR Basal Secretion (nmol/L)	0.50 ± 0.42
COR Half-life (min)	58.1 ± 22.0
COR AUC (nmol/L)	122562 ± 45685
COR Average (nmol/L)	170 ± 63
COR Max Measured (nmol/L)	539 ± 127
COR Peaks	7 ± 2
COR Mean Secretory Pulse Height (nmol/L)	23.3 ± 18.1
COR Mean Secretion Pulse Mass (nmol/L)	251.5 ± 112.7
COR ApEn	0.45 ± 0.14

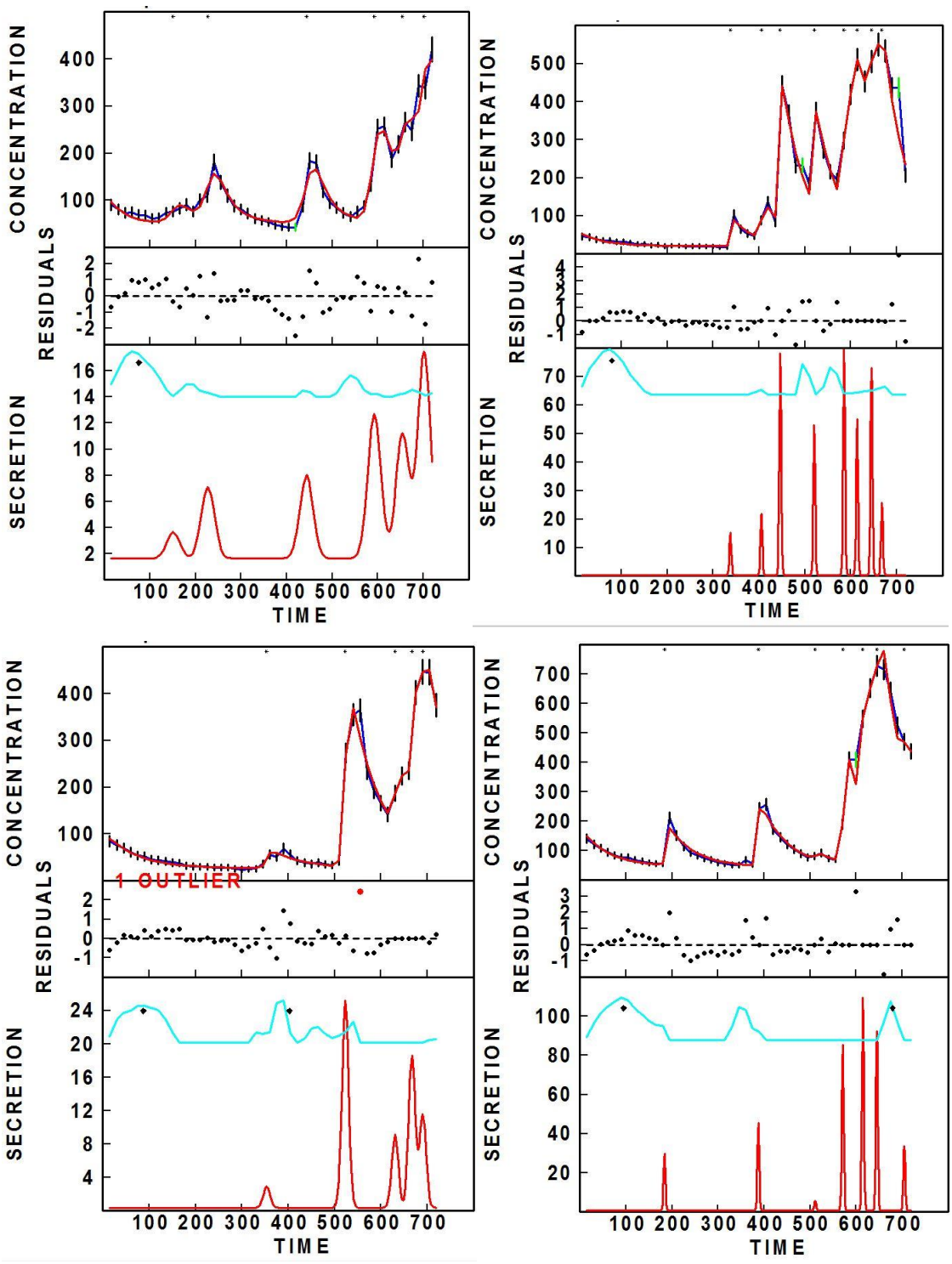
Table 12 Summary data of overnight cortisol profiles in mod/sev TBI patients (mean ± SD)




CONCENTRATION
RESIDUALS
SECRETION
TIME
100 200 300 400 500 600 700


CONCENTRATION
RESIDUALS
SECRETION
TIME
100 200 300 400 500 600 700


CONCENTRATION
RESIDUALS
SECRETION
TIME
100 200 300 400 500 600 700


CONCENTRATION
RESIDUALS
SECRETION
TIME
1 OUTLIER

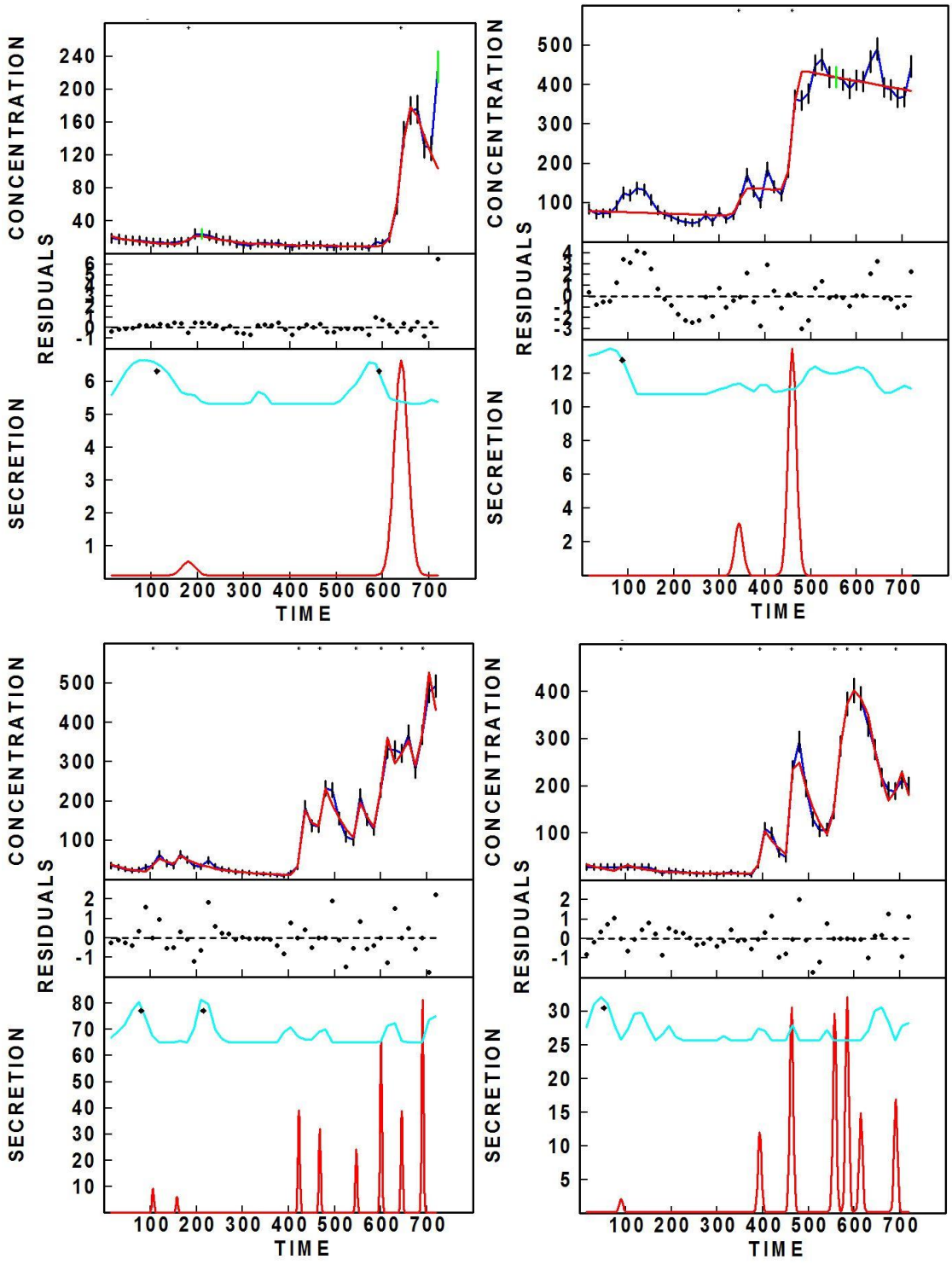


Figure 21 Individual overnight cortisol profiles of children/young adults with mod/sev TBI. X axis: time (minutes), Y axis: cortisol (nmol/L)

3.3.4 Salivary cortisol

Salivary cortisol and cortisone levels were averaged for each of the five samples collected over three days. CAR was calculated for each one of three sampling days (before Dexamethasone suppression). Participants with negative CAR were excluded

only if CAR was negative on all three sampling days. Complete absence of a post-awakening increase is likely to occur only if the delay and initiation of sampling is long enough so the first cortisol level exceeds the peak of the underlying CAR but can rarely be seen in patients with brain lesions (particularly in the hippocampus) [254]. A negative CAR over all three sampling days occurred in two participants (one control and one mild TBI).

Two way mixed ANOVA for the CAR over three consecutive sampling days did not demonstrate an effect of TBI [F(1,39)=22.25, p=.445], depression [F(1,39)=.002, p=.963] or fatigue [F(1,39)=.058, p=.811] on CAR over time.

When comparing the average CAR over three days there was no difference between groups (Kruskal-Wallis independent samples test, p=.053) [Figure 19] or when comparing TBI vs controls (Mann-Whitney U test, p=.828) [Figure 20].

Analyses were done excluding the two participants having a negative CAR over three days. Results were not different when those were included in the analysis.

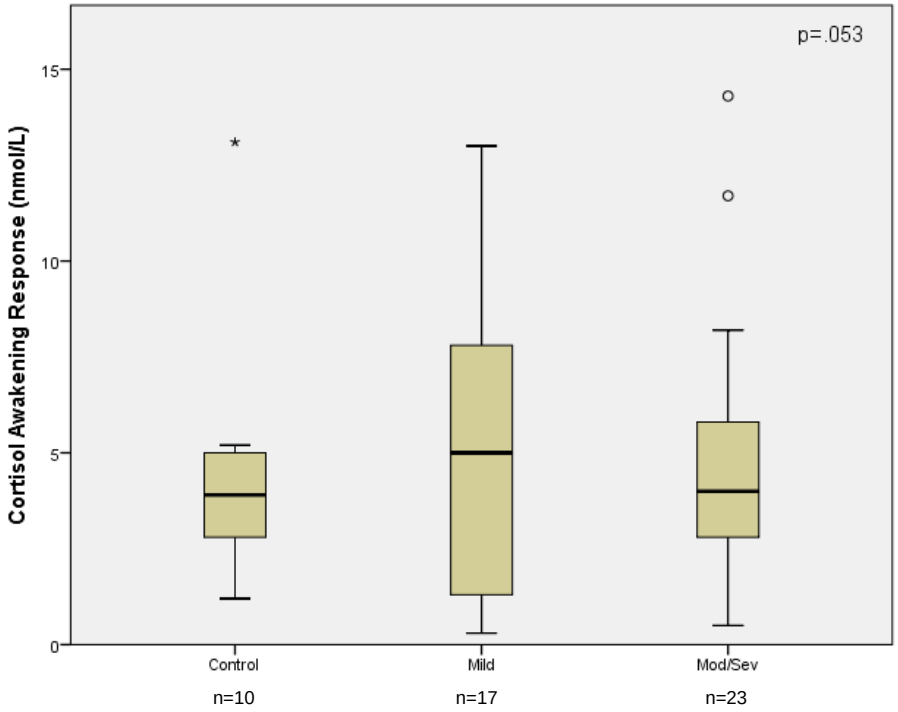


Figure 22 Comparison of Cortisol Awakening Response between TBI and control group

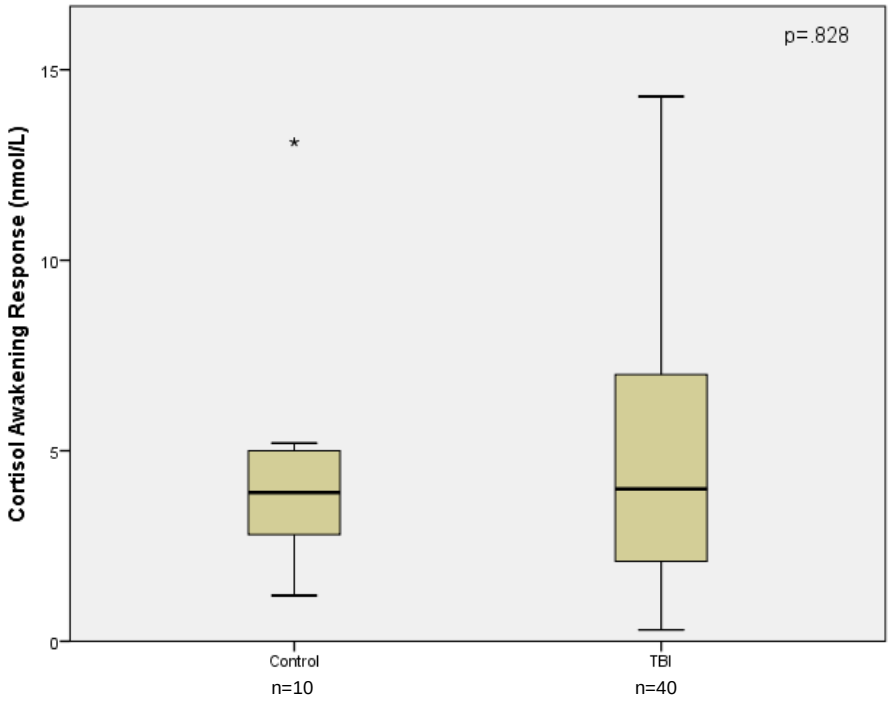


Figure 23 Comparison of Cortisol Awakening Response between TBI and non-TBI/control group

Subgroup analysis did not show any difference in CAR between male and female participants (Kruskal-Wallis independent samples test, p=.848) or between participants with fatigue (Mann-Whitney U test, p=.110) or depression (Mann-Whitney U test, p=.138).

Descriptive statistics of salivary cortisone samples before suppression with Dexamethasone are shown in Table 13. ANOVA with Bonferroni adjustment for each sampling time point did not show any differences between groups.

	Groups	Mean Cortisone (nmol/L)	SD	p
Waking	Control	16.3	6.2	.083
	Mild	19.8	4.4	
	Mod/Sev	22.0	8.0	
30min post waking	Control	18.7	8.6	.083
	Mild	25.4	7.2	
	Mod/Sev	23.9	7.2	
4-5h post waking	Control	9.0	4.5	.178
	Mild	12.1	5.0	
	Mod/Sev	12.6	5.5	
8-10h post waking	Control	8.4	2.6	.541
	Mild	6.8	4.2	
	Mod/Sev	7.8	4.0	
Bedtime	Control	2.5	2.0	.628
	Mild	3.0	1.8	
	Mod/Sev	3.4	2.8	

Table 13 Salivary cortisone levels in TBI and control groups. Values represent the average over 3 days of consecutive sampling.

Circadian rhythm pattern was preserved in all three groups (mild, mod/sev TBI and control). The change over time in salivary cortisone levels between groups was statistically significant [$F(2,47)=3.23$, $p=.049$, partial $\eta^2=.121$] due to the cortisone difference drop (but not absolute levels) between mild TBI and control group from 4-5h post waking to 8-10h post waking below [Figure 25].

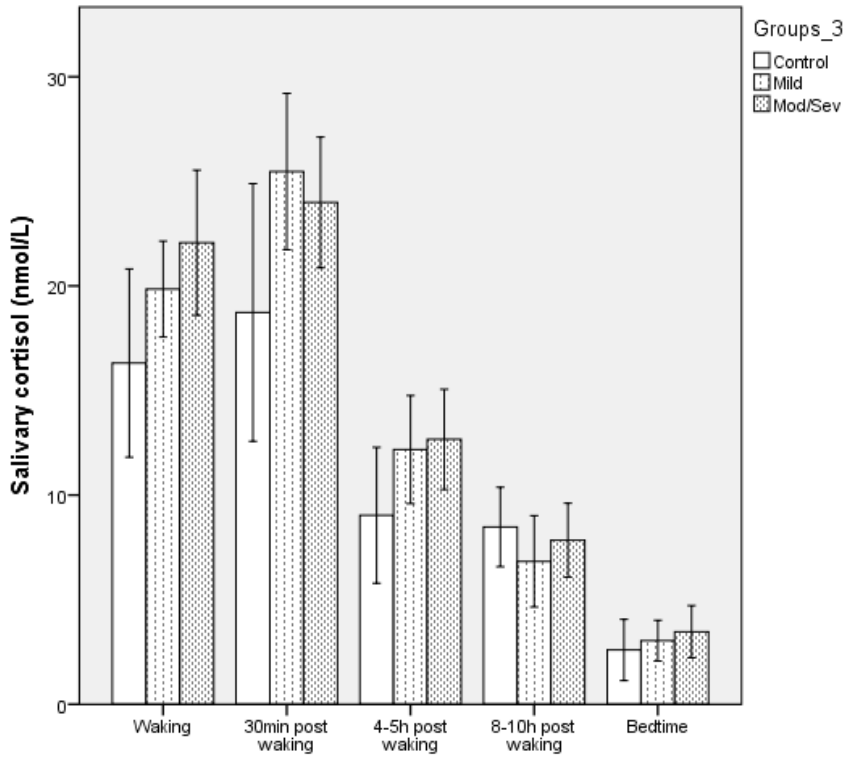


Figure 24 Diurnal salivary cortisone levels in TBI and control groups (mean with 95% CI)

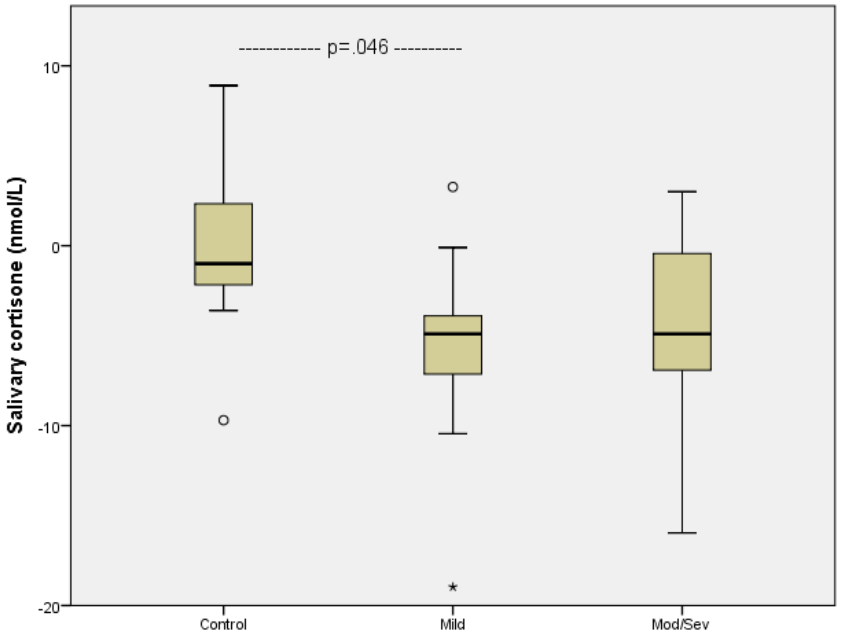


Figure 25 Salivary cortisone difference between 4-5h and 8-10h post waking in TBI and control groups.

There was no difference between groups in cortisone suppression with dexamethasone [F(2,46)=1.85, p=.169] [Figure 26]. In two participants (one mild and

one mod/sev TBI) there was no suppression of morning salivary cortisone. Both had normal auxology and no depressive symptomatology.

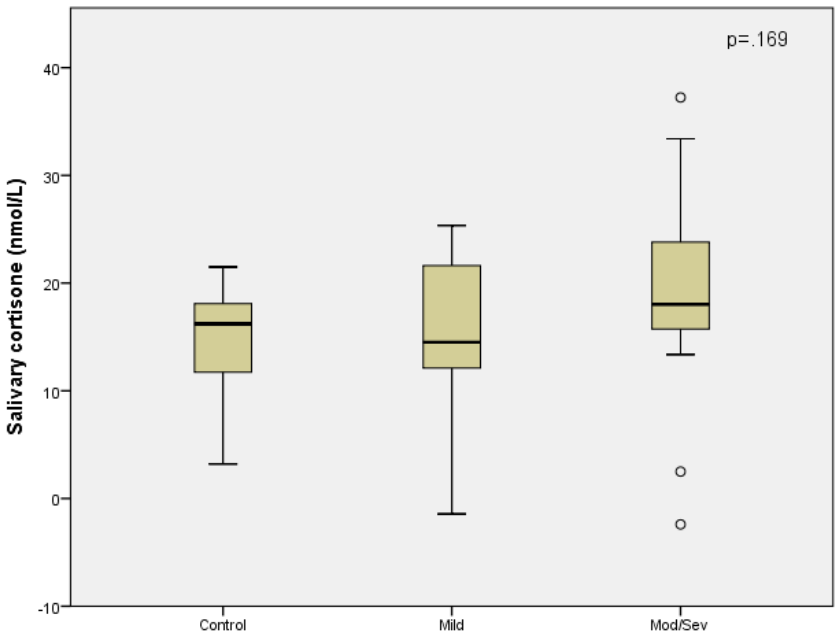


Figure 26 Difference in morning salivary cortisone pre- and post- suppression with Dexamethasone in TBI and control groups.

3.3.5 Endocrine results summary

Baseline endocrine function was normal in all but one female participant with primary amenorrhea. There was no clinical or biochemical indication of posterior pituitary dysfunction. GH response to standard stimulation testing (ITT) was abnormal in almost one third of mod/sev TBI participants. This did not correlate with IGF1/IGF-BP3 or auxology. Cortisol response to the same test was suboptimal in 10% of participants.

Spontaneous and stimulated GH levels did not correlate. In one participant, both stimulated and spontaneous peak GH secretion was low. Mean 12-hour GH secretion was low (under 1 mcg/l) in 20% of overnight profiles. Measures of spontaneous GH secretion (basal, average, maximum, mean secretion pulse mass) correlated with IGF1/IGF-BP3.

Spontaneous cortisol was under 500 nmol/l in 9/22 overnight profiles tests. Stimulated cortisol was normal in 7 of these. Spontaneous and stimulated cortisol correlated strongly. Cortisol pulsatility was preserved in all participants.

Salivary cortisol sample analysis did not show any clinically significant difference between groups in regards to CAR or suppression with dexamethasone. Circadian rhythm patterns were preserved overall with only a slightly more pronounced drop in

cortisone levels (between 4-5h and 8-10h post waking) in mild TBI participants when compared to controls.

3.4 NEUROPSYCHOLOGY RESULTS

3.4.1 Cognitive results

IQ scores were not different between groups (Kruskal-Wallis independent samples test, p=.068) [Figure 27] or when comparing TBI vs non-TBI (control) groups (Mann-Whitney U test, p=.200)

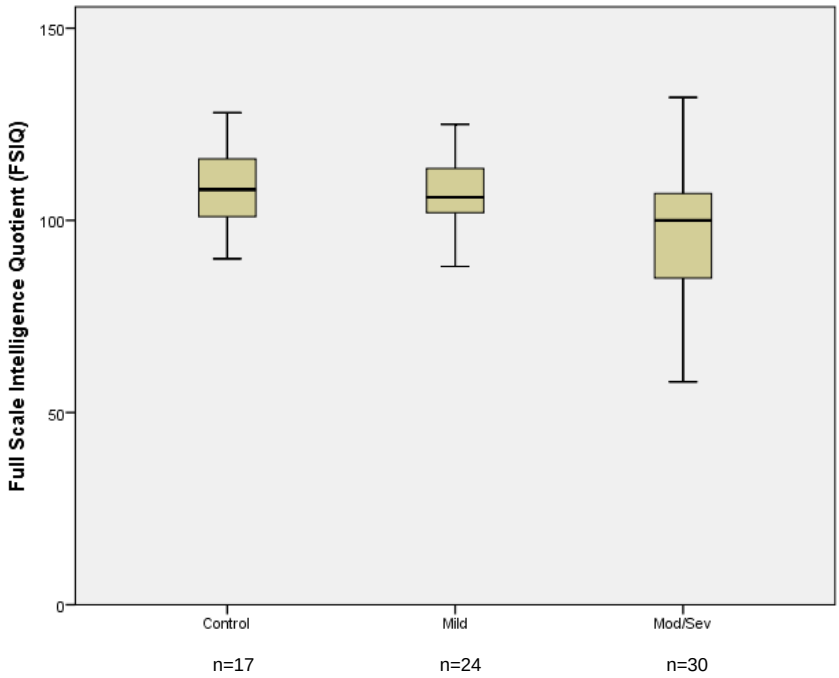


Figure 27 Comparison of summary IQ scores between TBI and control groups

Verbal IQ (VIQ) percentiles were lower in the Mod/Sev TBI group (39±33 centile) when compared to the control (62±25 centile) and mild TBI group (60±23 centile) (Kruskal-Wallis independent samples test, p=.029 and .022 respectively) [Figure 28]. There was no statistical difference in VIQ between mild TBI and control group participants.

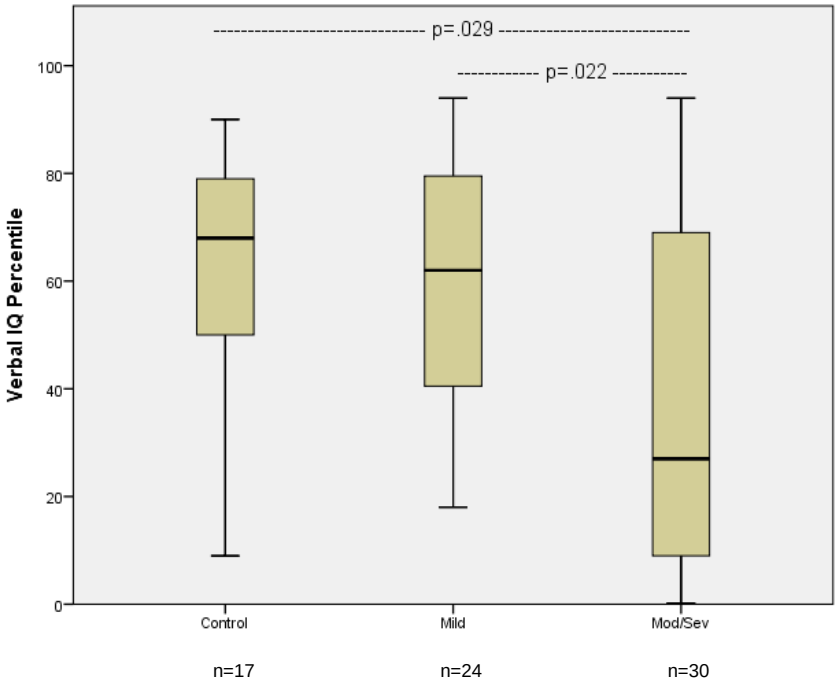


Figure 28 Comparison of Verbal IQ between TBI and control groups

Performance IQ (PIQ) percentiles between groups was not statistically significant (Kruskal-Wallis independent samples test, p=.415) [Figure 29] or when comparing TBI vs non-TBI (control) groups (Mann-Whitney U test, p=.304) [Figure 30].

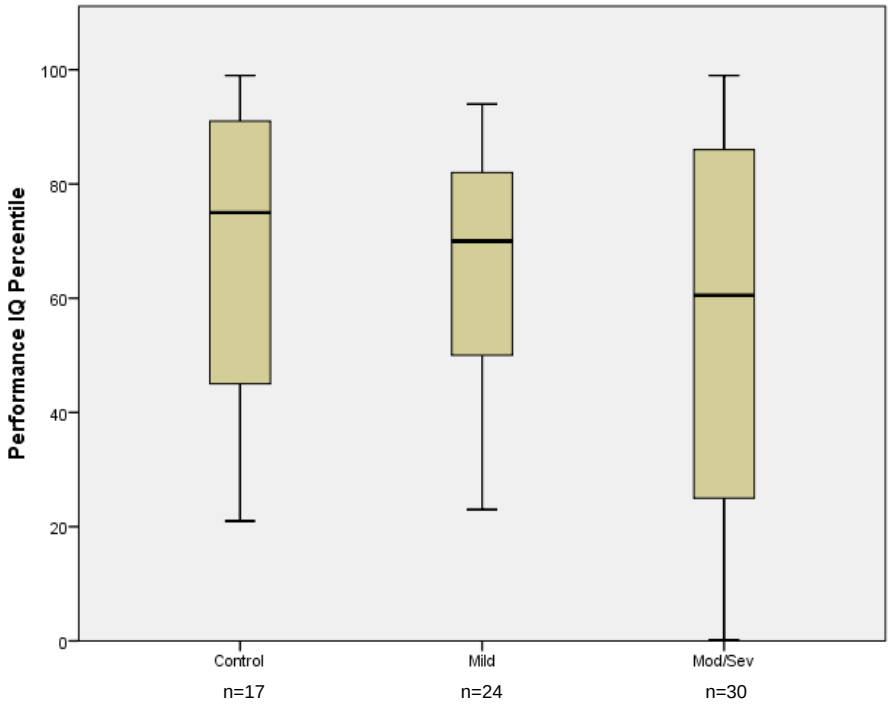


Figure 29 Comparison of Performance IQ between TBI and control groups

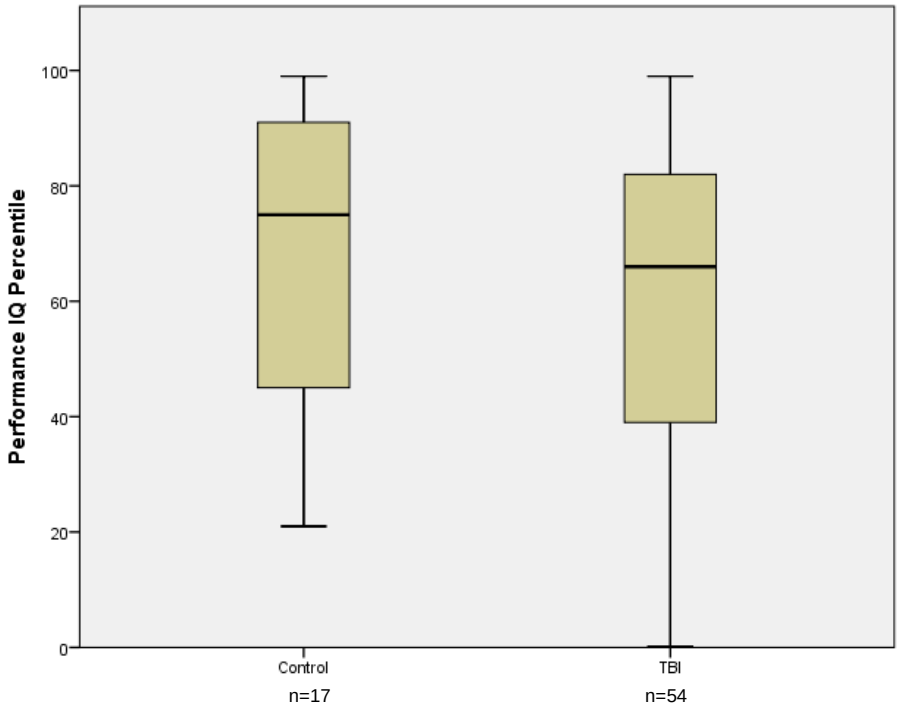


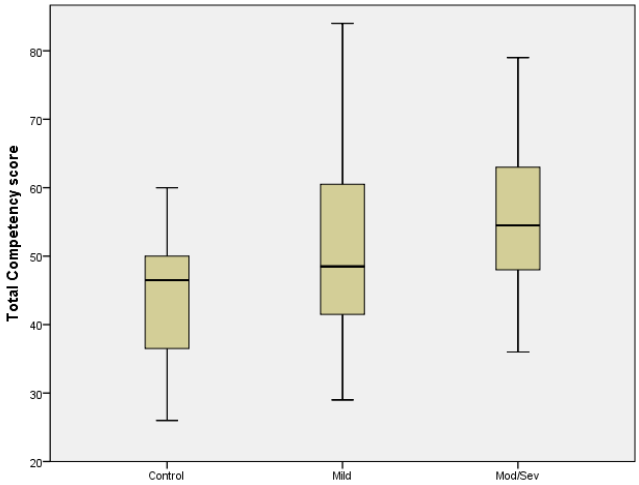
Figure 30 Comparison of Performance IQ between TBI and non-TBI/control groups

3.4.2 Psychology and Behaviour results

3.4.2.1 Behaviour checklist and self-report

The Self-Report (children CSR, adult ASR) and Behaviour Checklist (children CBCL, adult ABCL) is designed to obtain own and proxy reports of competencies and problems in a standardised format. As what individuals and especially adolescents report about themselves is subject to their recall at that moment, candour and self-awareness, reports by other informants (multiaxial assessment approach) were also analysed and compared.

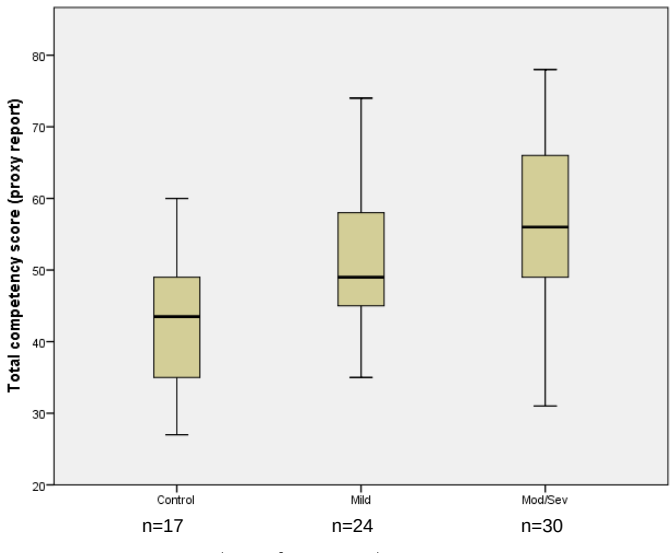
Total competency T scores were different between groups (Kruskal-Wallis independent samples test, p=.042). Pairwise comparisons showed a significant difference between mod/sev TBI and the control group [Figure 31]. T scores above 70 are in the clinical range.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0-1	-7.615	6.004	-1.268	.205	.614
0-2	-13.660	5.487	-2.490	.013	.038
1-2	-6.046	4.996	-1.210	.226	.679

Figure 31 Comparison of Total Competency scores between TBI and control groups (self-report)

The same was seen with proxy reporting (Kruskal-Wallis independent samples test, p=.014) [Figure 32.]



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0-1	-10.094	5.803	-1.740	.082	.246
0-2	-16.017	5.515	-2.904	.004	.011
1-2	-5.923	4.657	-1.272	.203	.610

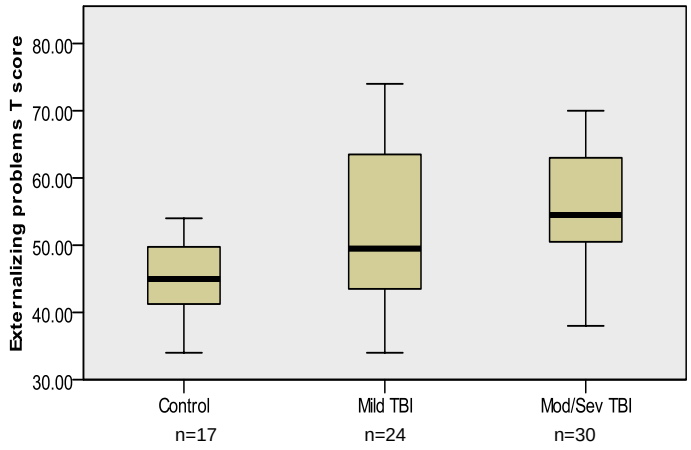
Figure 32 Comparison of Total Competency scores between TBI and control groups (proxy-report)

3.4.2.1.1 Externalising behaviour

Externalising grouping consists of behaviour syndromes that mainly involve conflict with other people and with social mores.

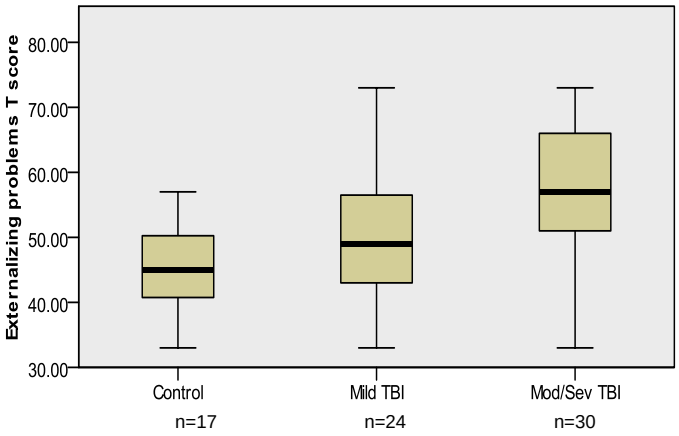
There were differences between groups (Kruskal-Wallis independent samples test, p=.006) with the mod/sev TBI group having higher T scores. There was no statistical difference between the mild TBI and control group [Figure 33].

The same difference was observed with proxy assessment (Kruskal-Wallis independent samples test, p=.007) [Figure 34].



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0-1	-10.990	6.003	-1.831	.067	.201
0-2	-17.554	5.486	-3.200	.001	.004
1-2	-6.565	4.995	-1.314	.189	.566

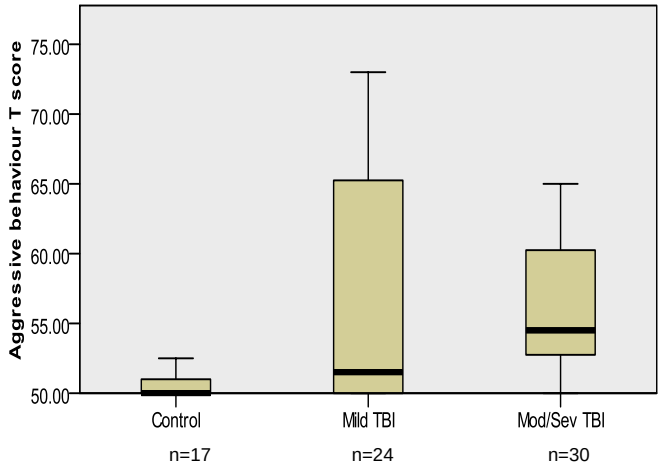
Figure 33 Comparison of Externalising behaviour between TBI and control groups (self-report)



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0-1	-7.503	5.803	-1.293	.196	.588
0-2	-16.520	5.515	-2.995	.003	.008
1-2	-9.017	4.657	-1.936	.053	.159

Figure 34 Comparison of Externalising behaviour between TBI and control groups (proxy-report)

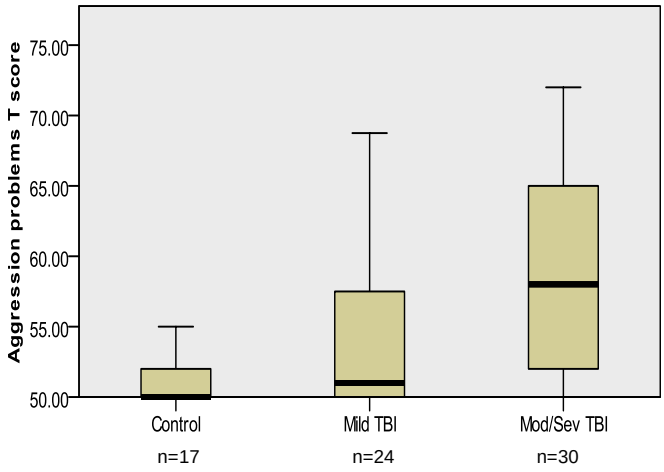
When analysing the components contributing to externalising behaviour problems, the mod/sev TBI group had higher T scores for aggressive behaviour (Kruskal-Wallis independent samples test, p=.003) [Figure 35] compared to the control group.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0-1	-13.844	5.903	-2.345	.019	.057
0-2	-18.221	5.395	-3.378	.001	.002
1-2	-4.377	4.912	-.891	.373	1.000

Figure 35 Comparison of Aggressive behaviour between TBI and control groups (self-report)

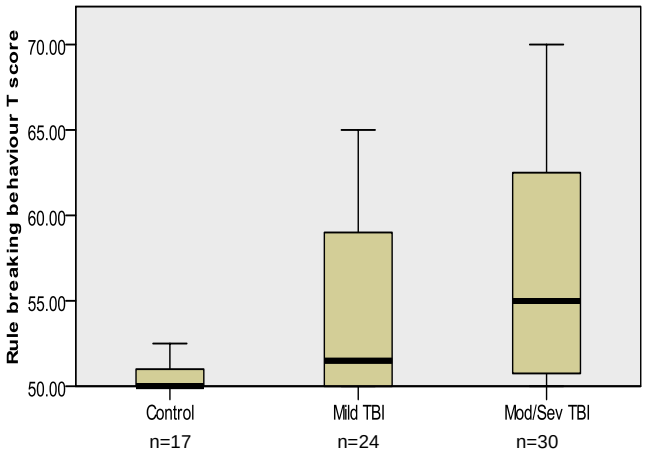
Proxy T scores for aggressive behaviour were also different between groups (Kruskal-Wallis independent samples test, p=.001) [Figure 36] and higher in the mod/sev TBI group.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0-1	-8.224	5.733	-1.435	.151	.454
0-2	-18.704	5.449	-3.433	.001	.002
1-2	-10.481	4.601	-2.278	.023	.068

Figure 36 Comparison of Aggressive behaviour between TBI and control groups (proxy-report)

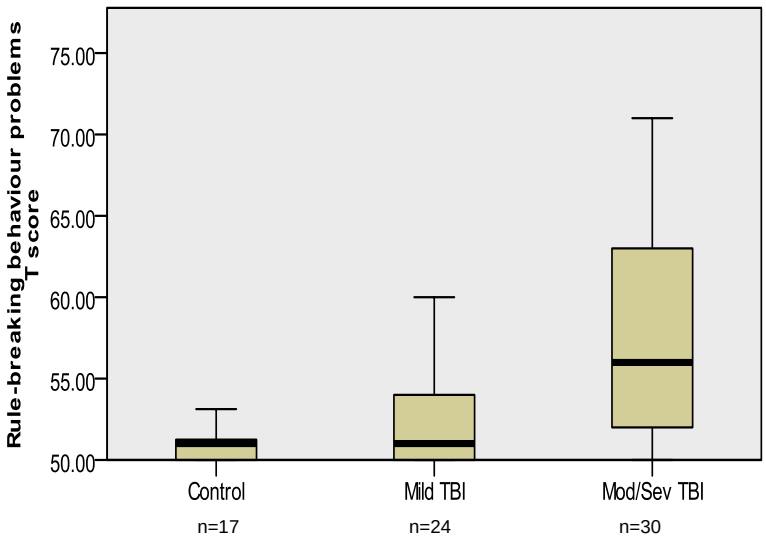
T scores were also higher in the mod/sev TBI group in regards to rule breaking behaviour (Kruskal-Wallis independent samples test, p=.014) [Figure 37].



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0-1	-9.615	5.844	-1.645	.100	.300
0-2	-15.545	5.340	-2.911	.004	.011
1-2	-5.930	4.862	-1.220	.223	.668

Figure 37 Comparison of Rule breaking behaviour between TBI and control groups (self-report)

A similar pattern was seen with proxy assessment (Kruskal-Wallis independent samples test, p=.004) [Figure 38] with the mod/sev TBI group having significantly higher scores for rule breaking behaviour when compared to either mild TBI or the control group.

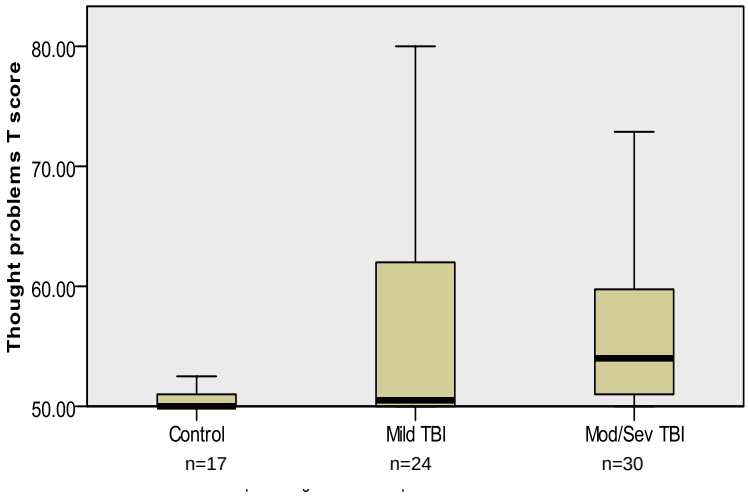


Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0-1	-5.391	5.730	-.941	.347	1.000
0-2	-16.559	5.447	-3.040	.002	.007
1-2	-11.168	4.599	-2.428	.015	.046

Figure 38 Comparison of Rule breaking behaviour between TBI and control groups (proxy-report)

The Thought problems scale is mainly comprised of low prevalence items and has low internal consistency unless sample sizes are large. It measures symptoms common in several mental disorders (hallucinations, OCD-symptoms, self-harm and suicide attempts).

Self-reporting showed differences between groups (Kruskal-Wallis independent samples test, p=.008) [Figure 39] with T scores for the mod/sev TBI group being higher when compared to the control group but not when compared to the mild TBI group.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0-1	-8.708	5.864	-1.485	.138	.413
0-2	-16.362	5.359	-3.053	.002	.007
1-2	-7.654	4.879	-1.569	.117	.350

Figure 39 Comparison of Thought problems between TBI and control groups (self-report)

Proxy assessment did not demonstrate a difference between groups (Kruskal-Wallis independent samples test, p=.079) [Figure 40].

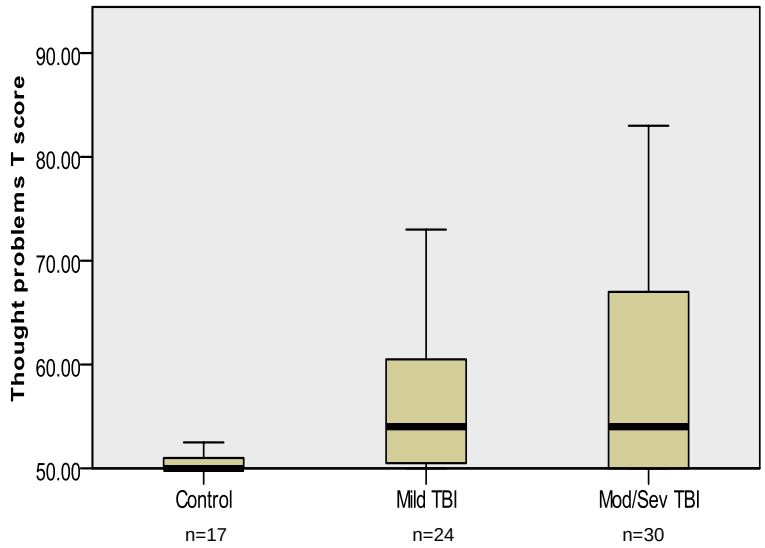
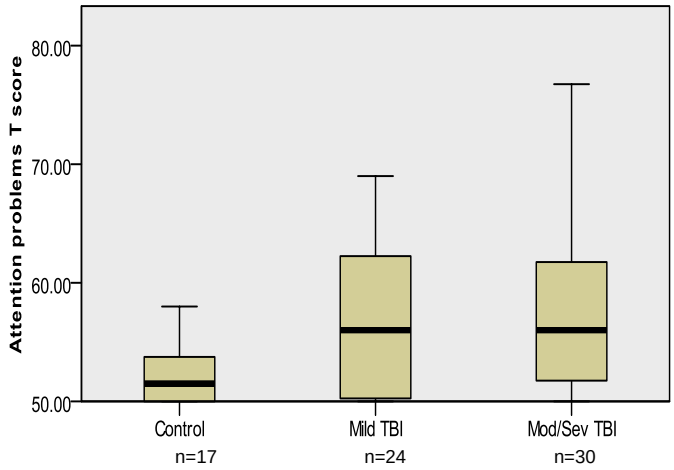


Figure 40 Comparison of Thought problems between TBI and control groups (proxy-report)

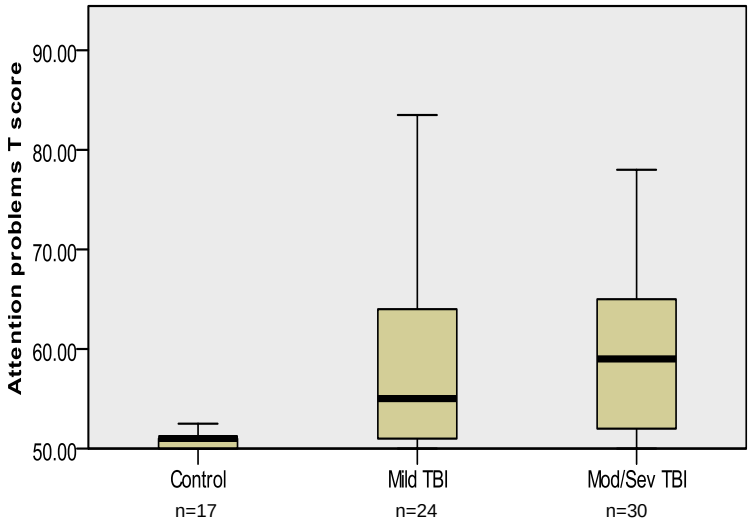
In the Attention problems scale mod/sev TBI participants demonstrated higher T scores (Kruskal-Wallis independent samples test, p=.042) [Figure 41].



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0-1	-8.917	6.006	-1.485	.138	.413
0-2	-14.849	5.488	-2.706	.007	.020
1-2	-5.933	4.997	-1.187	.235	.705

Figure 41 Comparison of Attention problems between TBI and control groups (self-report)

Proxy scores were similar with the mod/sev TBI group demonstrating higher T scores (Kruskal-Wallis independent samples test, p=.019) [Figure 42].



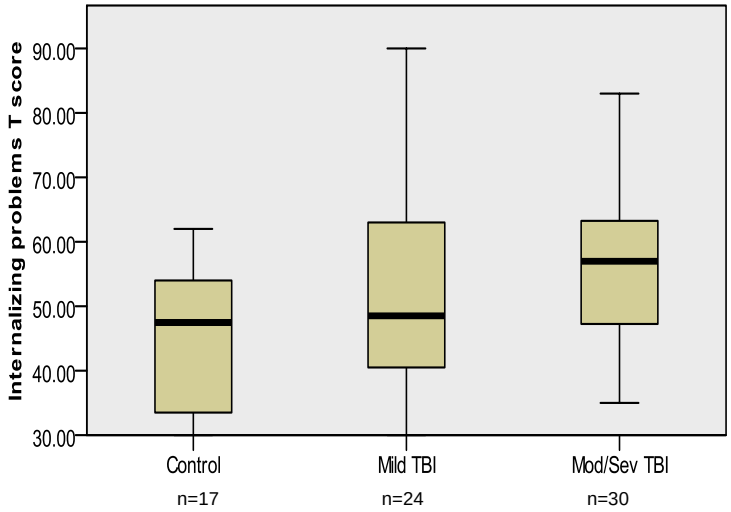
Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0-1	-12.879	5.752	-2.239	.025	.075
0-2	-15.154	5.467	-2.772	.006	.017
1-2	-2.275	4.616	-.493	.622	1.000

Figure 42 Comparison of Attention problems between TBI and control groups (proxy-report)

3.4.2.1.2 Internalising behaviour

The Internalising grouping consists of three syndromes (anxious/depressed, withdrawal, somatic complains) that are mainly within the self.

There were differences between groups (Kruskal-Wallis independent samples test, p=.046) [Figure 43] with Internalizing T scores being higher in the mod/sev TBI group compared to the control group.



Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0-1	-8.312	6.003	-1.385	.166	.498
0-2	-13.577	5.486	-2.475	.013	.040
1-2	-5.264	4.995	-1.054	.292	.876

Figure 43 Comparison of Internalising problems between TBI and control groups (self-report)

Proxy assessment did not demonstrate differences between groups (Kruskal-Wallis independent samples test, p=.069) [Figure 44].

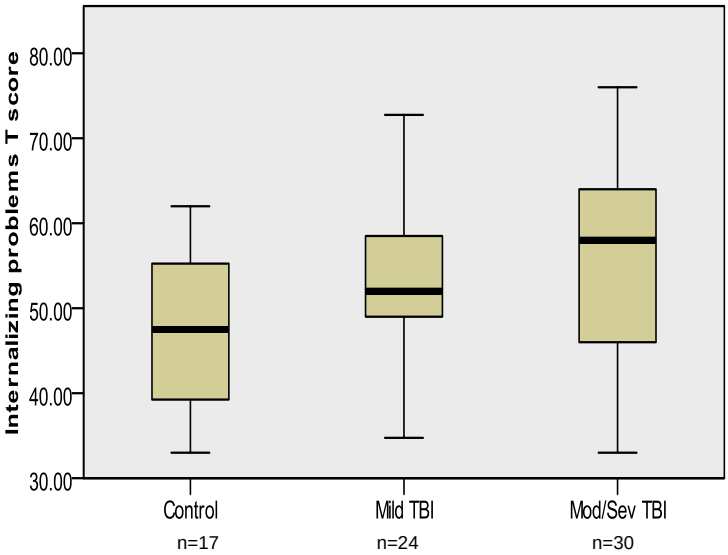


Figure 44 Comparison of Internalising problems between TBI and control groups (proxy-report)

Anxiety/depression and somatic problems T scores were not different between groups with either self or proxy assessment (Kruskal-Wallis independent samples test, p> .1) [Figure 45] & [Figure 46].

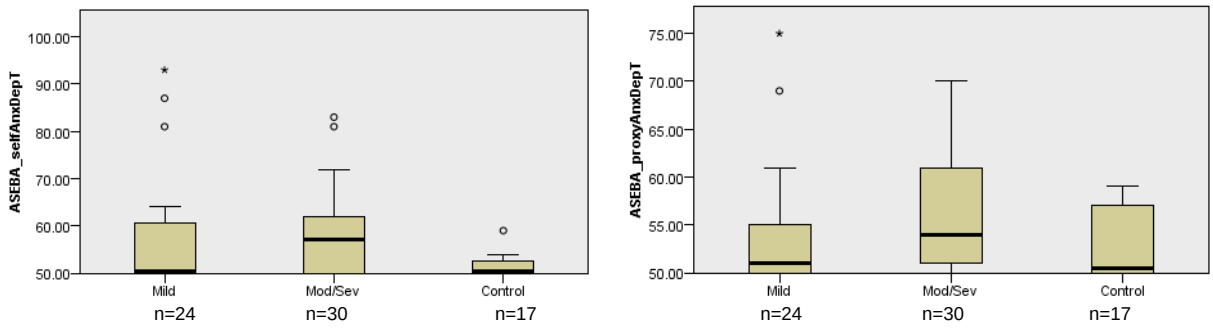


Figure 45 Comparison of Anxiety/Depression problems between TBI and control groups (self and proxy-report)

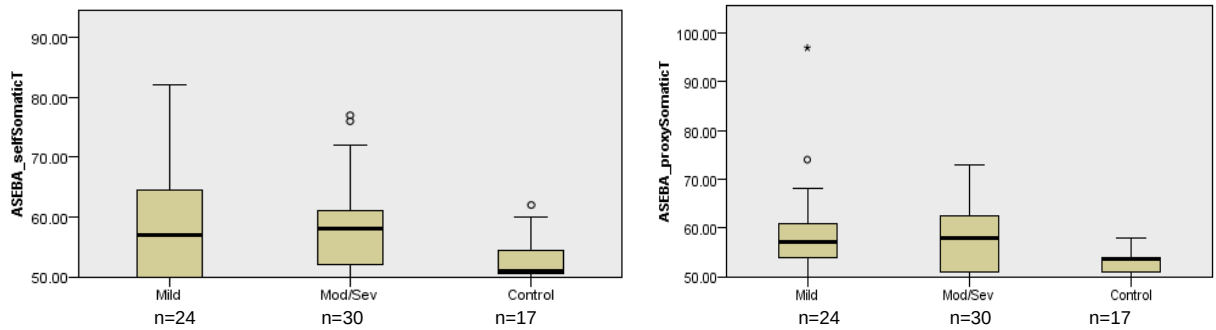


Figure 46 Comparison of Somatic problems between TBI and control groups (self and proxy-report)

Analysis of ASEBA derived DSM-oriented scales were also consistent with most of the ASEBA total and sub score results.

Correlation between self and proxy reports for ASEBA summary [Figure 47], externalising and internalising [Figure 48], anxiety/depression [Figure 49], withdrawal [Figure 50], somatic symptoms [Figure 51], attention [Figure 52] and aggressive [Figure 53] /rule breaking behaviour [Figure 54] scores was strong both using Raw and T scores.

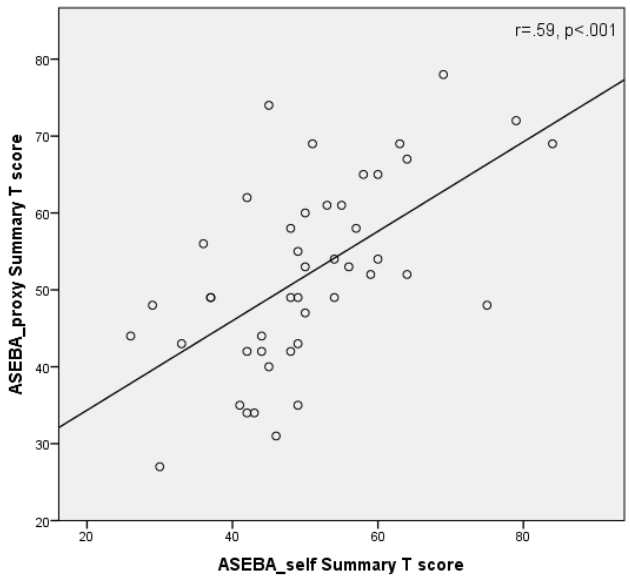


Figure 47 Correlation between self and proxy ASEBA Summary T scores

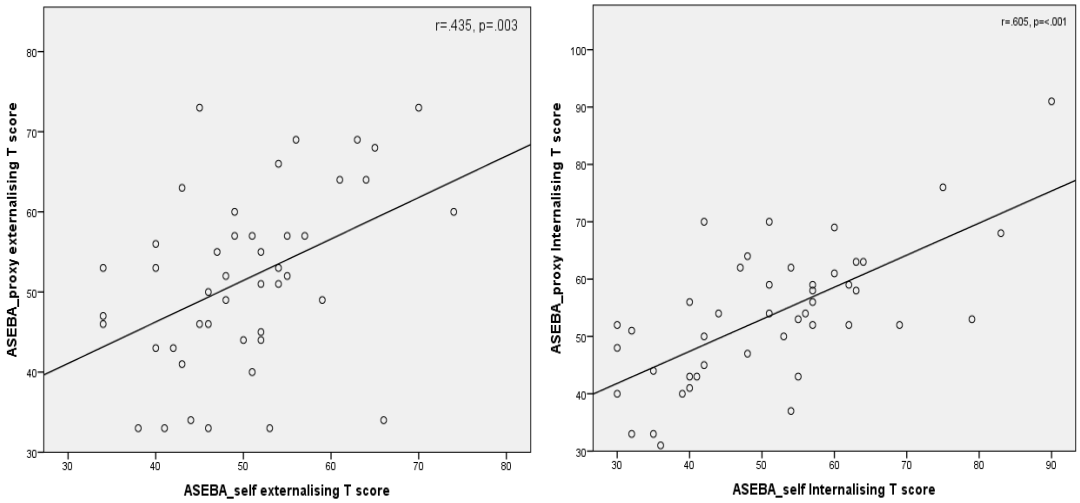


Figure 48 Correlation between self and proxy ASEBA Externalising and Internalising T scores

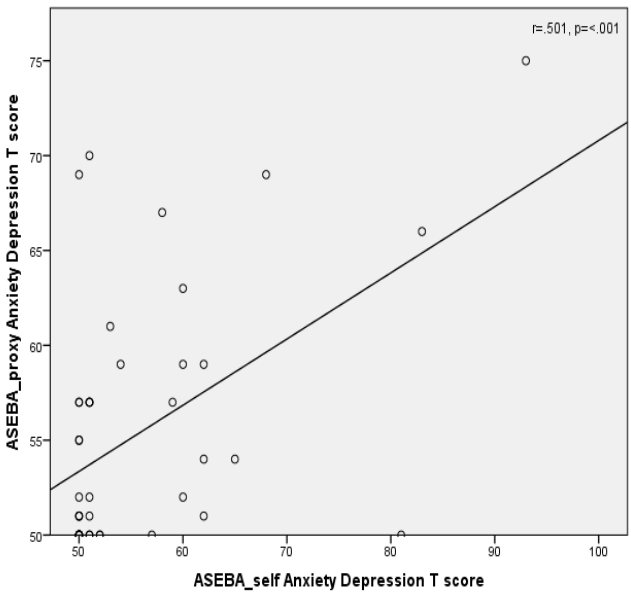


Figure 49 Correlation between self and proxy ASEBA Anxiety/Depression T scores

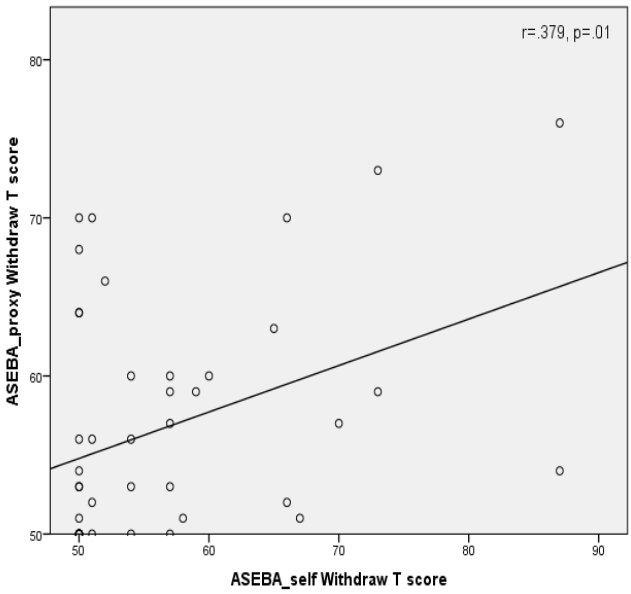


Figure 50 Correlation between self and proxy ASEBA Withdrawal T scores

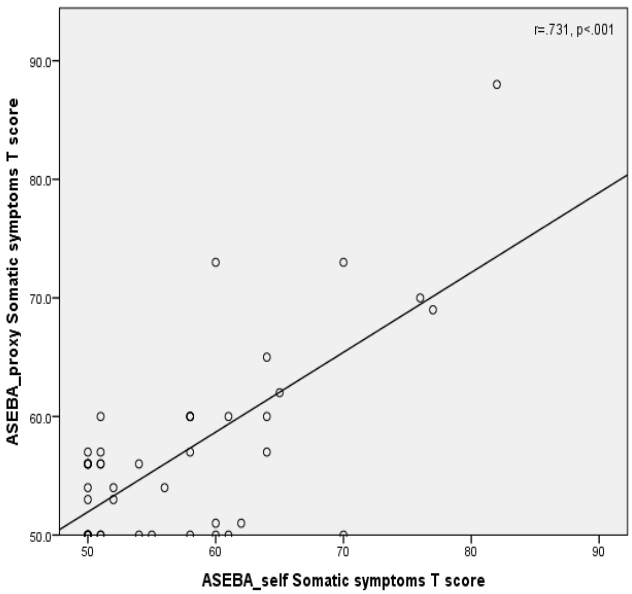


Figure 51 Correlation between self and proxy ASEBA Somatic symptoms T scores

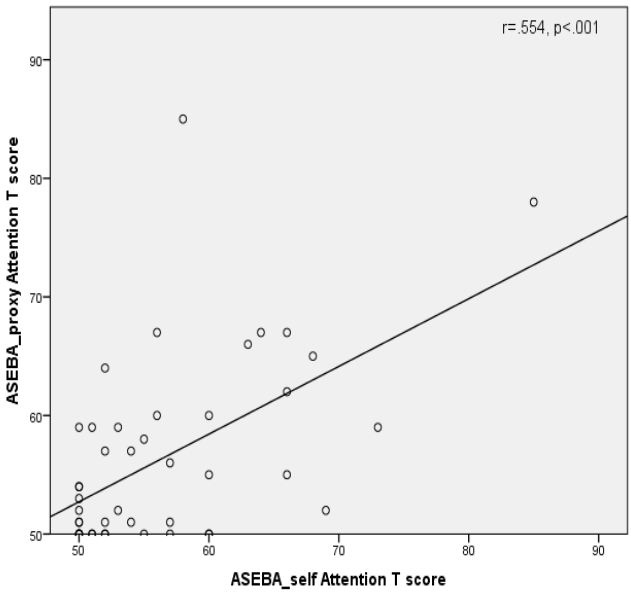


Figure 52 Correlation between self and proxy ASEBA Attention T scores

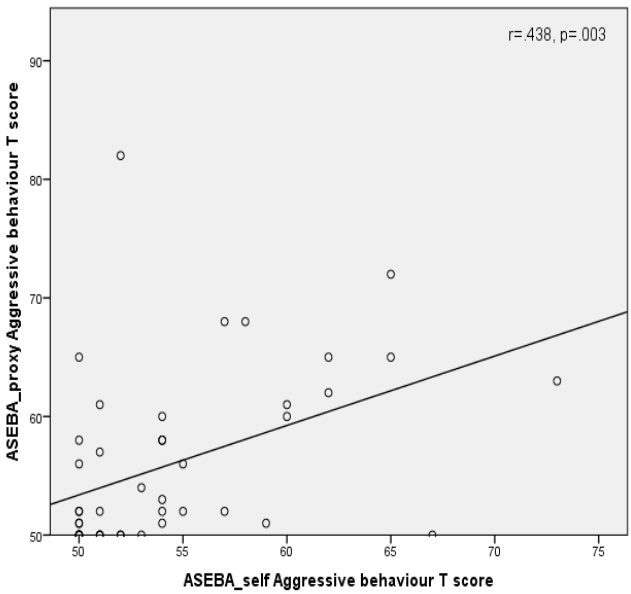


Figure 53 Correlation between self and proxy ASEBA Aggressive behaviour T scores

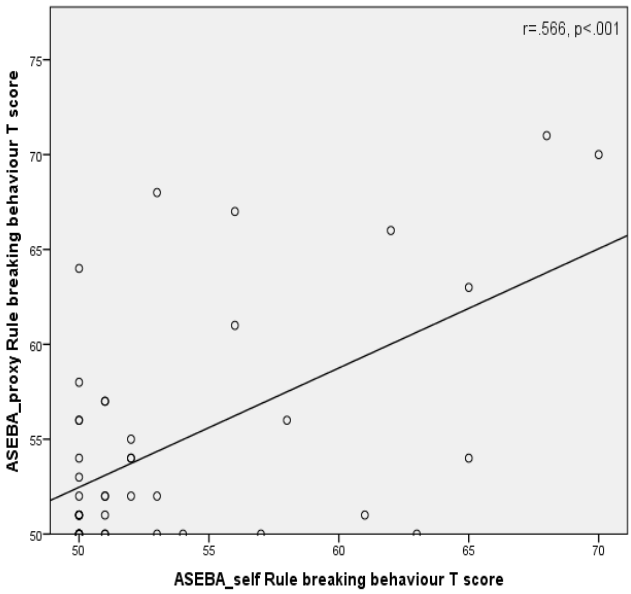
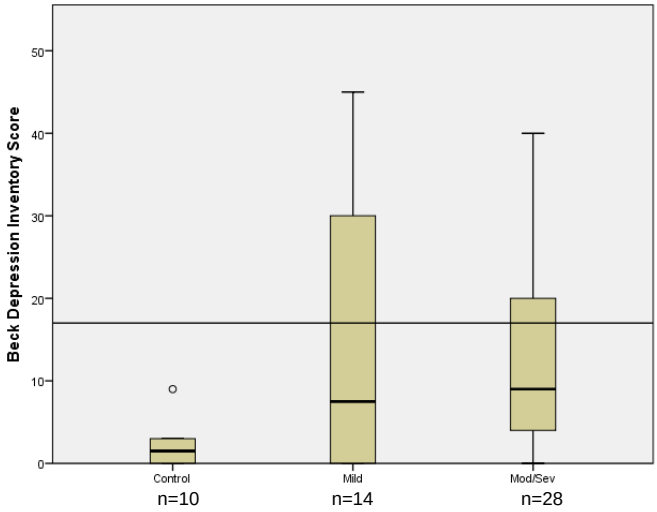


Figure 54 Correlation between self and proxy ASEBA Rule breaking behaviour T scores

3.4.2.2 Depression and Anxiety

In addition to ASEBA-derived DSM-oriented scales for behaviour and anxiety, assessments for depression and anxiety were also undertaken using specific clinical instruments. Beck Depression Inventory (BDI) was used for participants 13 years of age or older and the Birleson Depression Scale for younger children.



Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Mild-Mod/Sev	-2.585	4.813	-.537	.591	1.000
Control-Mod/Sev	16.092	5.403	2.979	.003	.009
Control-Mild	13.507	6.011	2.247	.025	.074

Figure 55 Comparison of Depression scores (BDI) in TBI and control groups

Depression scores using BDI were significantly higher in the mod/sev TBI group when compared to the control group (Kruskal-Wallis independent samples test, p=.011) [Figure 55]. Almost a third (9/28) of mod/sev TBI participants had a score of 17 or above which is seen only in patients with clinical depression. A similar proportion (5/18) of mild TBI participants scored above 17.

No differences were demonstrated between groups in the smaller group of younger participants (n=9) who were assessed using the Birleson depression scale.

Measures of spontaneous or stimulated GH or cortisol secretion were not different in participants with depression compared to the ones without.

Anxiety assessment using the Beck Anxiety Inventory (BAI) did not demonstrate any differences between groups (Kruskal-Wallis independent samples test, p=.798) [Figure 56]. Only one participant in the mild TBI group had anxiety levels in the severe range (BAI score over 42).

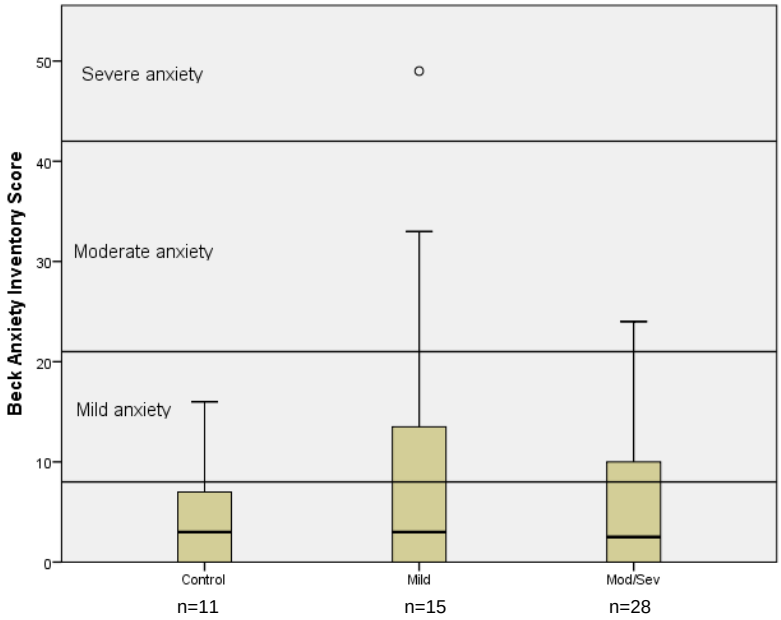


Figure 56 Comparison of Anxiety scores (BAI) in TBI and control groups

3.4.2.3 BRIEF (Behaviour Rating Inventory of Executive Function)

Global BRIEF scores were not different between groups (Kruskal-Wallis independent samples test, p=.207) [Figure 57].

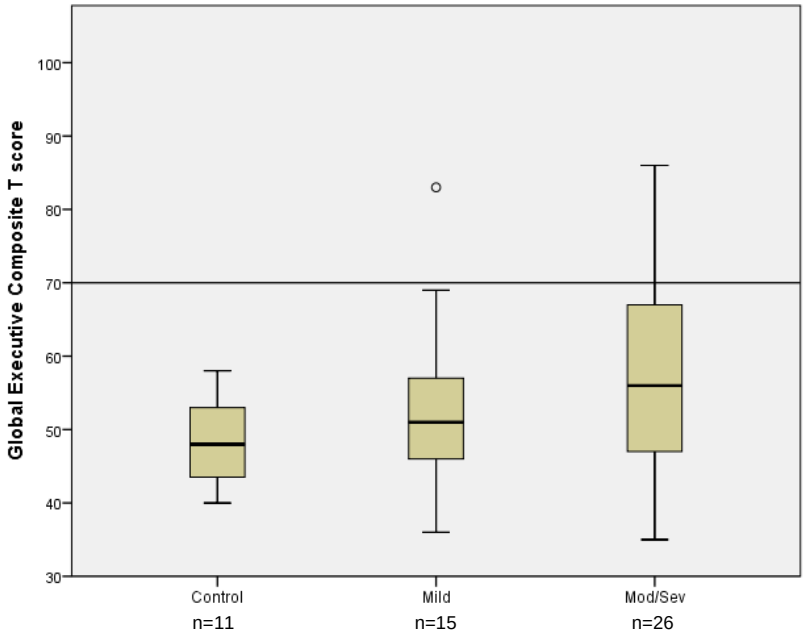
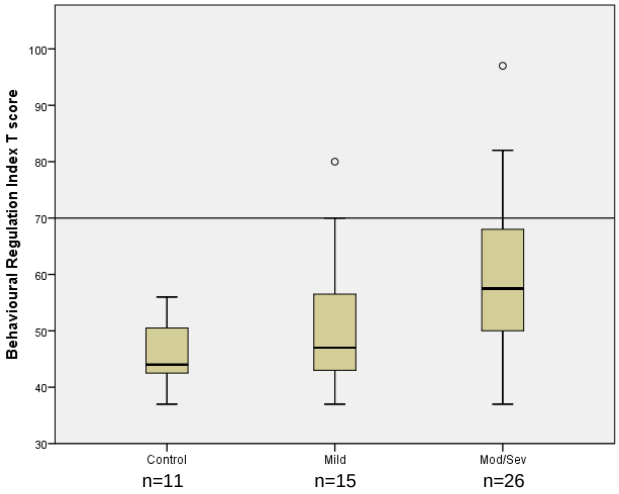


Figure 57 Comparison of Global T scores between TBI and control groups (BRIEF)

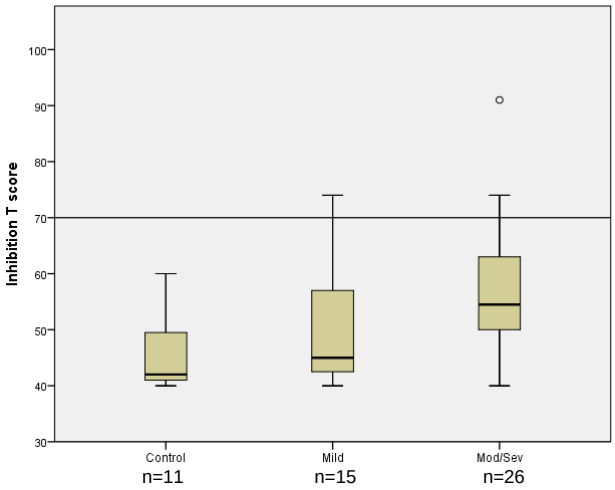
Behavioural regulation T scores were higher in mod/sev TBI group and statistically significant when compared to the control group (Kruskal-Wallis independent samples test, p=.02) [Figure 58].



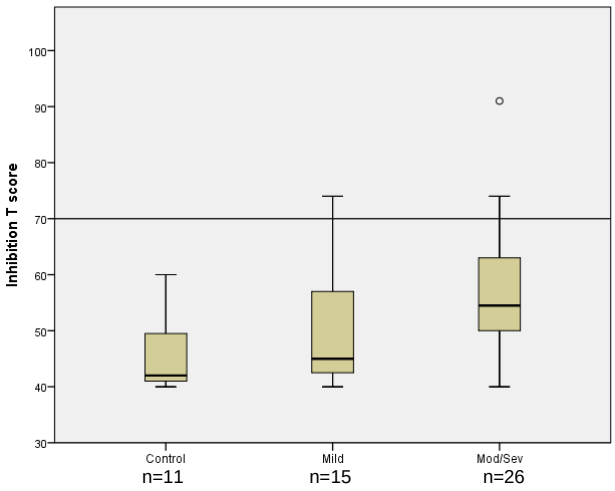
Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Control-Mild	5.606	6.010	.933	.351	1.000
Control-Mod/Sev	14.311	5.445	2.628	.009	.026
Mild-Mod/Sev	-8.705	4.909	-1.773	.076	.228

Figure 58 Comparison of Behavioural Regulation T scores between TBI and control groups (BRIEF)

When reviewing the clinical scales assessed with Behavioural Regulation, the main difference between groups was in the inhibition scale (impulsivity) (Kruskal-Wallis independent samples test, p=.009) [while there were no differences in the other two scales (shift and emotional control) [Figure 59].



Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Control-Mild	7.594	5.995	1.267	.205	.616
Control-Mod/Sev	16.073	5.432	2.959	.003	.009
Mild-Mod/Sev	-8.479	4.896	-1.732	.083	.250



Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Control-Mild	7.594	5.995	1.267	.205	.616
Control-Mod/Sev	16.073	5.432	2.959	.003	.009
Mild-Mod/Sev	-8.479	4.896	-1.732	.083	.250

Figure 59 Comparison of Inhibition T scores between TBI and control groups (BRIEF)

Metacognition summary T score was not different between groups (Kruskal-Wallis independent samples test, p=.315) [Figure 60].

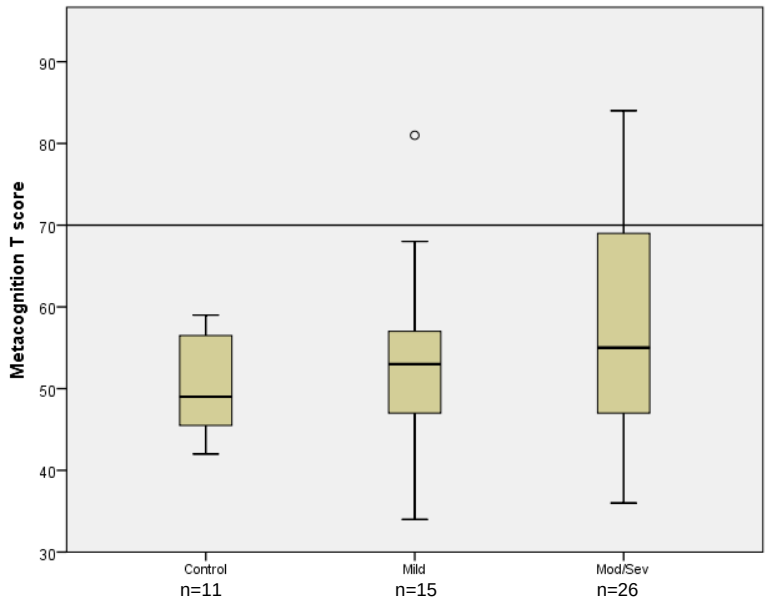
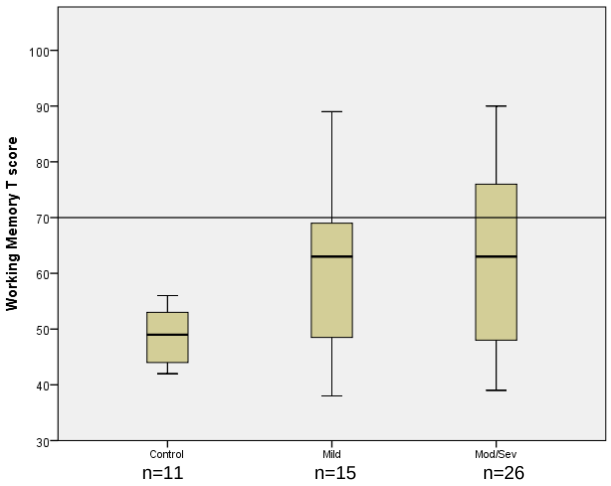


Figure 60 Comparison of Metacognition T scores between TBI and control groups (BRIEF)

Analysis of scales assessed in Metacognition (i.e. initiation, working memory, organisation of materials and monitor) were not different between groups except for working memory T scores. These were higher in the mod/sev TBI group suggesting a higher level of dysfunction in this domain, which involves holding information in mind for purpose of completing a task (Kruskal-Wallis independent samples test, p=.02) [Figure 61].



Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Control-Mild	12.503	6.008	2.081	.037	.112
Control-Mod/Sev	15.059	5.443	2.767	.006	.017
Mild-Mod/Sev	-2.556	4.907	-.521	.602	1.000

Figure 61 Comparison of Working memory T scores between TBI and control groups (BRIEF)

3.4.3 Neuropsychology results summary

Cognitive impairment was seen in the mod/sev TBI group. Verbal IQ was lower (-20% when compared to the control or mild TBI group) as was working memory which is important for holding information in mind in order to completing a task. The same group had higher scores for aggressive/rule breaking behaviour, attention problems, depression but not for anxiety.

3.5 QUALITY OF LIFE AND FATIGUE RESULTS

3.5.1 PedsQL

Total PedsQL score was not different between groups (Kruskal-Wallis independent samples test, p=.265) [Figure 62].

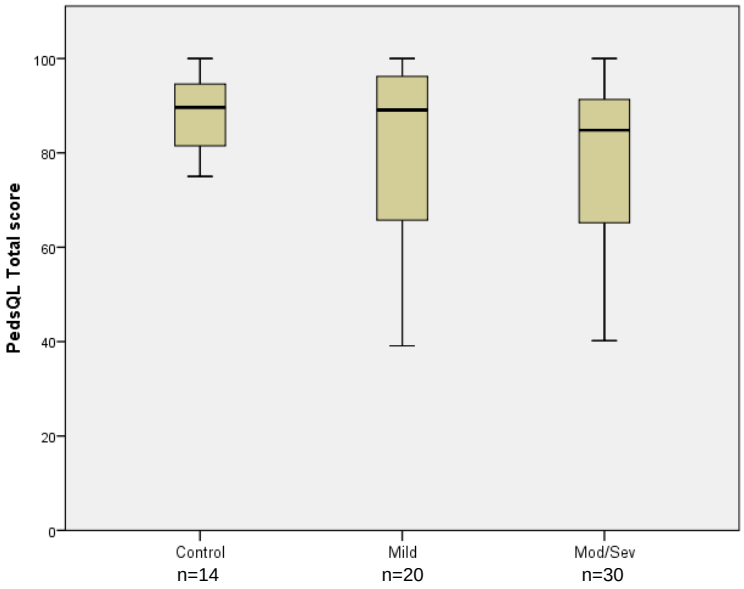


Figure 62 Comparison of Total QoL scores between TBI and control groups (PedsQL)

When comparing two groups (TBI vs non-TBI), the total PedsQL score in the TBI group was lower (80.3±16.8 vs 88.3±7.9, 95% confidence interval of the difference 1.5-14.4). The difference was statistically significant (t-test for unequal variances, p=.016) [Figure 63].

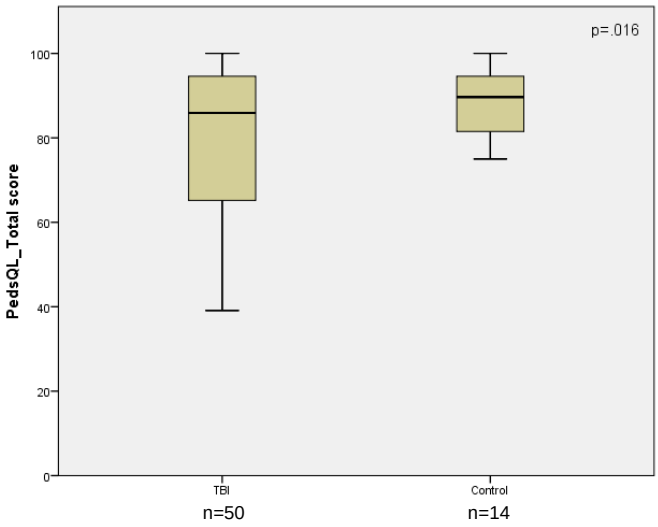


Figure 63 Comparison of Total QoL scores between TBI and non-TBI groups (PedsQL)

Physical and Psychosocial sub-scores were not significantly different between the three groups (Kruskal-Wallis independent samples test, p=.716 and p=.127 respectively) [Figure 64].

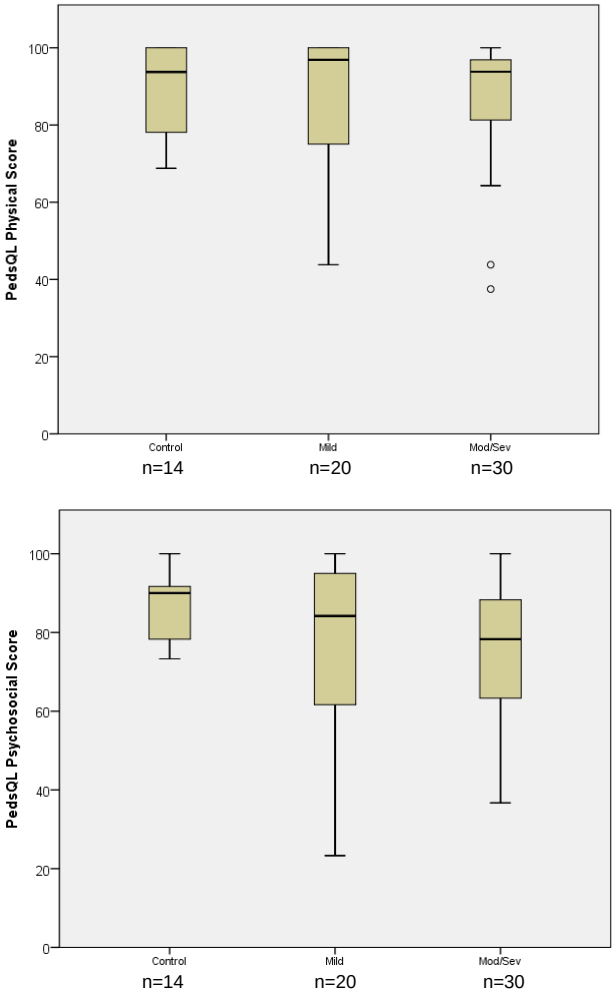


Figure 64 Comparison of Physical and Psychosocial QoL scores between TBI and control groups (PedsQL)

When comparing two groups (TBI vs non-TBI), the Psychosocial PedsQL score in the TBI group was lower by (76.6±19.1 vs 87.5±8.6, 95% confidence interval of difference 3.7-18.0). The difference was statistically significant (t-test for unequal variances, p=.003) [Figure 65].

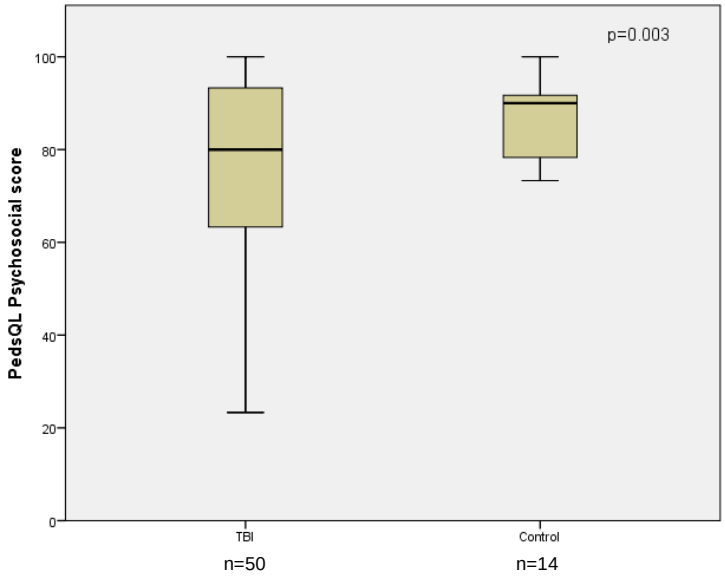


Figure 65 Comparison of Psychosocial QoL scores between TBI and non-TBI groups (PedsQL)

The school sub-score was not significantly different between the three groups (Kruskal-Wallis independent samples test, p=.154), but when comparing two groups (TBI vs non-TBI) it was lower in the TBI group (74.8±20 vs 84.4±9.3, 95% confidence interval of the difference 3.9-19.3). The difference was statistically significant (t-test for unequal variances, p=.004) [Figure 66].

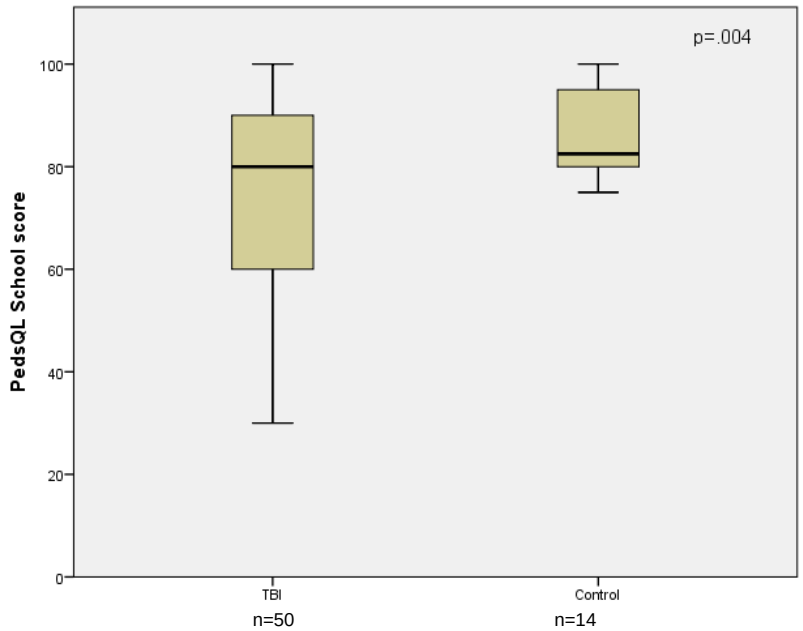


Figure 66 Comparison of School QoL sub-scores between TBI and non-TBI groups (PedsQL)

There was a strong correlation between self and proxy reports [Table 14].

	Pearson *r*	*p*
PedsQL Total score	.665	*<.001*
PedsQL Physical score	.555	*<.001*
PedsQL Psychosocial	.540	*<.001*
PedsQL Feelings	.655	*<.001*
PedsQL Social	.569	*<.001*
PedsQL School	.625	*<.001*

Table 14 Correlation between self and proxy report for PedsQL.

Overall, sixteen participants (7 mild, 9 mod/sev TBI) had a summary PedsQL score of less than 69.7%, which is the level for detecting children with chronic health conditions. Physical and psychosocial health summary sub-scores were less than 69.7% in 5 and 9 participants respectively.

3.5.2 Health utilities index (HUI)

Mean HUI scores were not significantly different between the three groups with either self or proxy report (Kruskal-Wallis independent samples test, p=.099 and .088 respectively) [Figure 67].

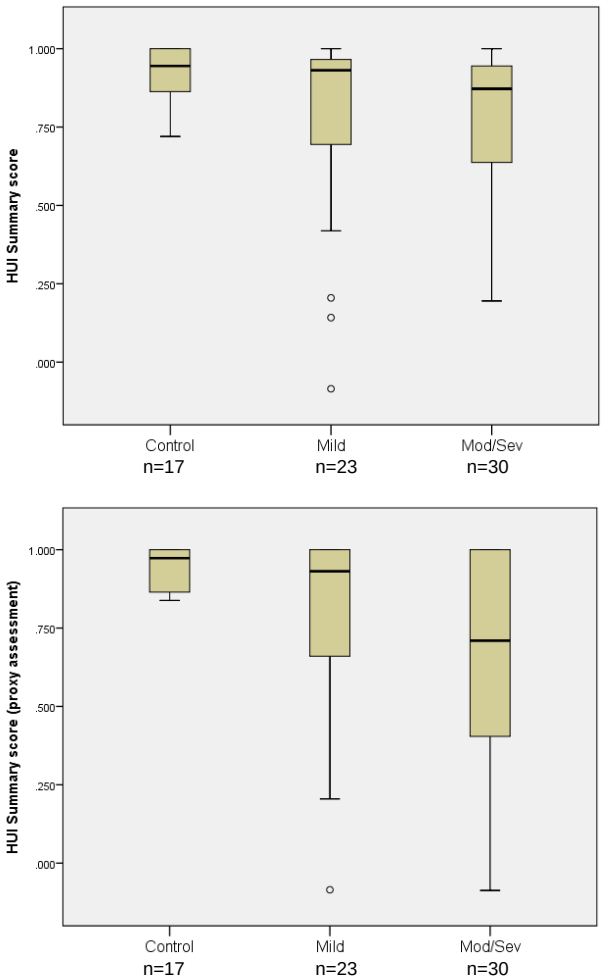


Figure 67 Comparison of HUI scores between TBI and control groups (self and proxy report)

Two group comparison (TBI vs non-TBI) showed that the mean HUI score in the TBI group was significantly lower in the control group [0.765 ± 0.28 vs 0.922 ± 0.09 (t-test for unequal variances, p=.001) with self-assessment and 0.706 ± 0.34 vs 0.942± 0.06 (t-test for unequal variances, p<.001), with proxy-assessment] [Figure 68].

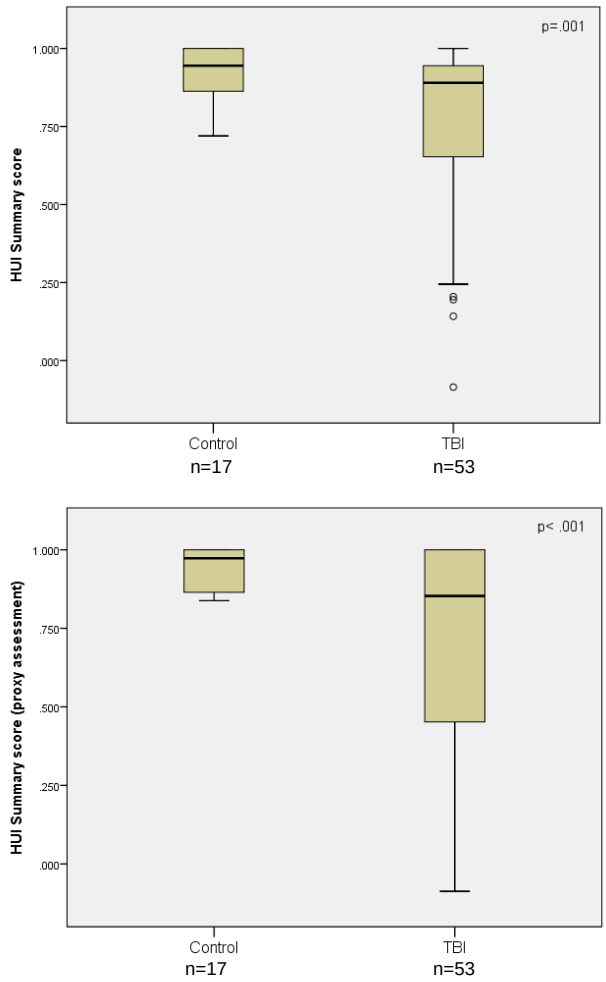
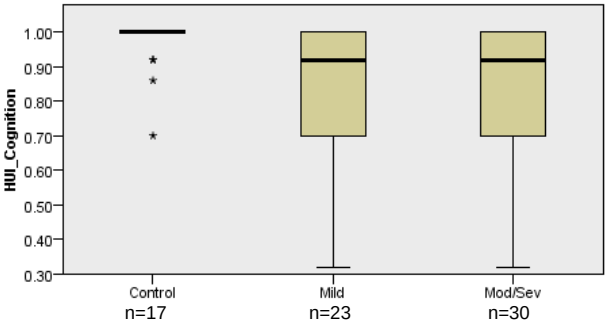


Figure 68 Comparison of HUI scores between TBI and non-TBI groups (self and proxy report)

Analysis of single attribute scores did not show any differences for Vision, Speech, Ambulation, Dexterity and Pain between groups with either self- or proxy- assessment.

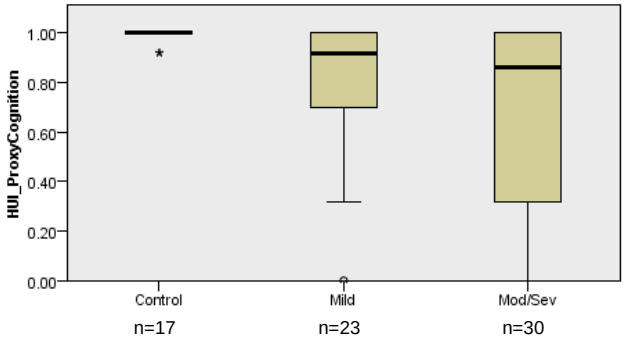
Cognition attribute scores were significantly lower in the TBI group (Kruskal-Wallis independent samples test, p=.006) for both mild TBI and mod/sev TBI groups compared to the control group [Figure 69].



Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Mod/Sev-Mild	.956	5.403	.177	.860	1.000
Mod/Sev-Control	17.770	5.925	2.999	.003	.008
Mild-Control	16.815	6.279	2.678	.007	.022

Figure 69 Comparison of HUI Cognition attribute scores between TBI and control groups

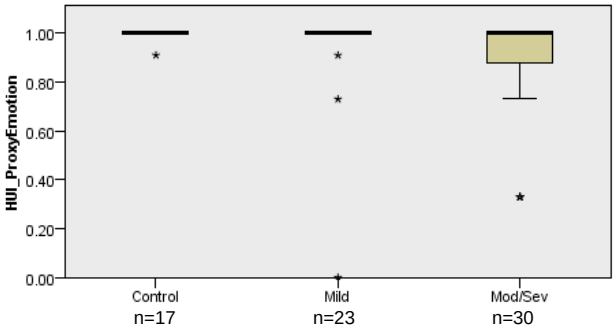
Cognition attribute scores were also lower with proxy-assessment (Kruskal-Wallis independent samples test, p=.004) but only between the mod/sev TBI and control group [Figure 70].



Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Mod/Sev-Mild	4.805	4.638	1.036	.300	.900
Mod/Sev-Control	17.797	5.402	3.295	.001	.003
Mild-Control	12.992	5.539	2.345	.019	.057

Figure 70 Comparison of HUI Cognition scores between TBI and non-TBI groups (proxy report)

Emotion attribute scores were not significantly different with self-assessment but were lower in the mod/sev TBI vs control group with proxy assessment (Kruskal-Wallis independent samples test, p=.016) [Figure 71].



Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Mod/Sev-Mild	8.780	3.835	2.289	.022	.066
Mod/Sev-Control	11.172	4.467	2.501	.012	.037
Mild-Control	2.392	4.580	.522	.602	1.000

Figure 71 Comparison of HUI Emotion scores between TBI and non-TBI groups (proxy report)

With two group comparison (TBI vs non-TBI), emotion attribute scores were significantly lower with both self [0.920 ± 0.217 vs 0.995 ± 0.022 (t-test for unequal variances, p=.017] and proxy assessment [0.885 ± 0.24 vs 0.992 ± 0.025 (t-test for unequal variances, p=.007].

3.5.3 Participation (Child and Adolescent Scale of Participation- [CASP])

Participation scores were lower in the mod/sev TBI group as seen in Table 15. The difference between the three groups was not statistically significant (Kruskal-Wallis independent samples test, p=.082).

	Mean	SD	95% Confidence Interval for Mean
Mild	95.5	6.5	91.9 - 99.1
Mod/Sev	89.6	12.7	83.2 - 95.9
Control	99.1	1.6	97.7 - 100

Table 15 CASP scores in mild, mod/sev TBI and control groups

Two group comparison (TBI vs non-TBI) showed that the mean CASP score in the TBI group was significantly lower, 92.3 ± 10.6 vs 99.1 ± 1.6 (t-test for unequal variances, p=.001) [Figure 72].

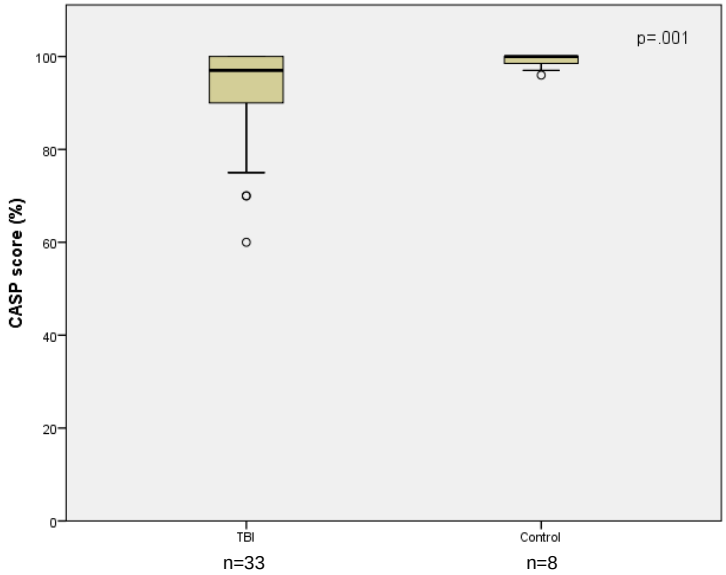


Figure 72 Comparison of CASP scores between TBI and non-TBI groups.

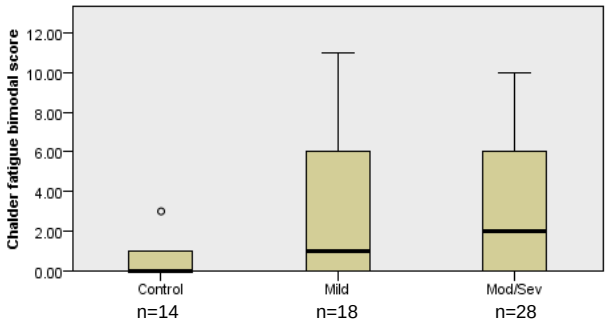
3.5.4 Fatigue

Fatigues scores as assessed with the Chalder fatigue scale are shown in Table 16. When comparing two groups (TBI vs non-TBI/control) the difference was statistically significant (t-test for unequal variances) using either bimodal or Likert scoring method. TBI participants had higher fatigue scores.

	Groups	Mean	SD	p
CFS Bimodal (0-11)	Control	.43	.852	<.001
	TBI	3.13	3.462	
CFS Likert (0-33)	Control	10.07	3.362	.035
	TBI	13.07	7.129	

Table 16 Fatigue scores in TBI and control groups.

Fatigue scores were significantly different between the 3 groups using bimodal scoring (scale 0-11) (Kruskal-Wallis independent samples test, p=.02) but not when using Likert scores (Kruskal-Wallis independent samples test, p=.339) [Figure 73].



Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Control-Mild	12.603	5.916	2.130	.033	.099
Control-Mod/Sev	14.857	5.434	2.734	.006	.019
Mild-Mod/Sev	-2.254	5.016	-.449	.653	1.000

Figure 73 Comparison of Fatigue scores between TBI and control groups

As the binomial fatigue score for the control group was .43 ± .85 (mean±SD) a bimodal score of ≥3 (same as the bimodal score initially recommended for identifying significant fatigue [239]) or ≥16 (Likert) was considered abnormal for our study sample and suggestive of fatigue levels above what would be expected in healthy controls.

20/46 of TBI participants had fatigue scores of 3 or above (mod/sev 13/28, mild 7/18) and 1/14 in the control group. The difference was statistically significant (Chi-Square, p=.013) [Figure 74].

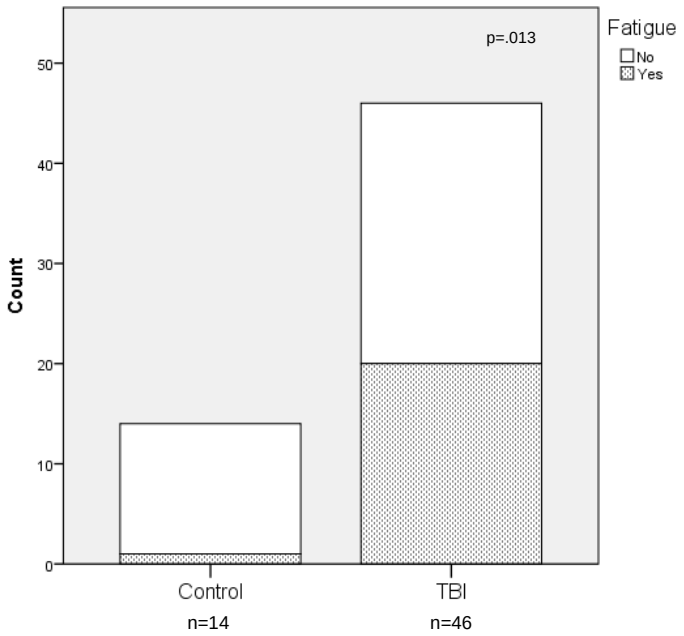


Figure 74 Frequency of significant fatigue among TBI patients.

Five participants (4 mod/sev and 1 mild TBI) had bimodal fatigue scores above 9, which is seen in Chronic Fatigue Syndrome (CFS) sufferers (mean 9.14 and SD 2.73 from large study involving 361 CFS sufferers and 1615 healthy individuals from the community [285])

There were more females with fatigue (8/20 vs 13/40 males) but the difference in fatigue between TBI and non-TBI females was not significant (Fisher's exact test, p=.656). The difference was significant in males as 13/29 in the TBI and 0/11 in the control group reported fatigue (Fisher's exact test, p=.006).

Fatigue scores correlated negatively with PedsQL scores (ρ= -.68, p<0.001). Participants with higher levels of fatigue tend to have poorer QoL (physical and psychosocial) (Mann-Whitney U, p<.001) [Figure 75].

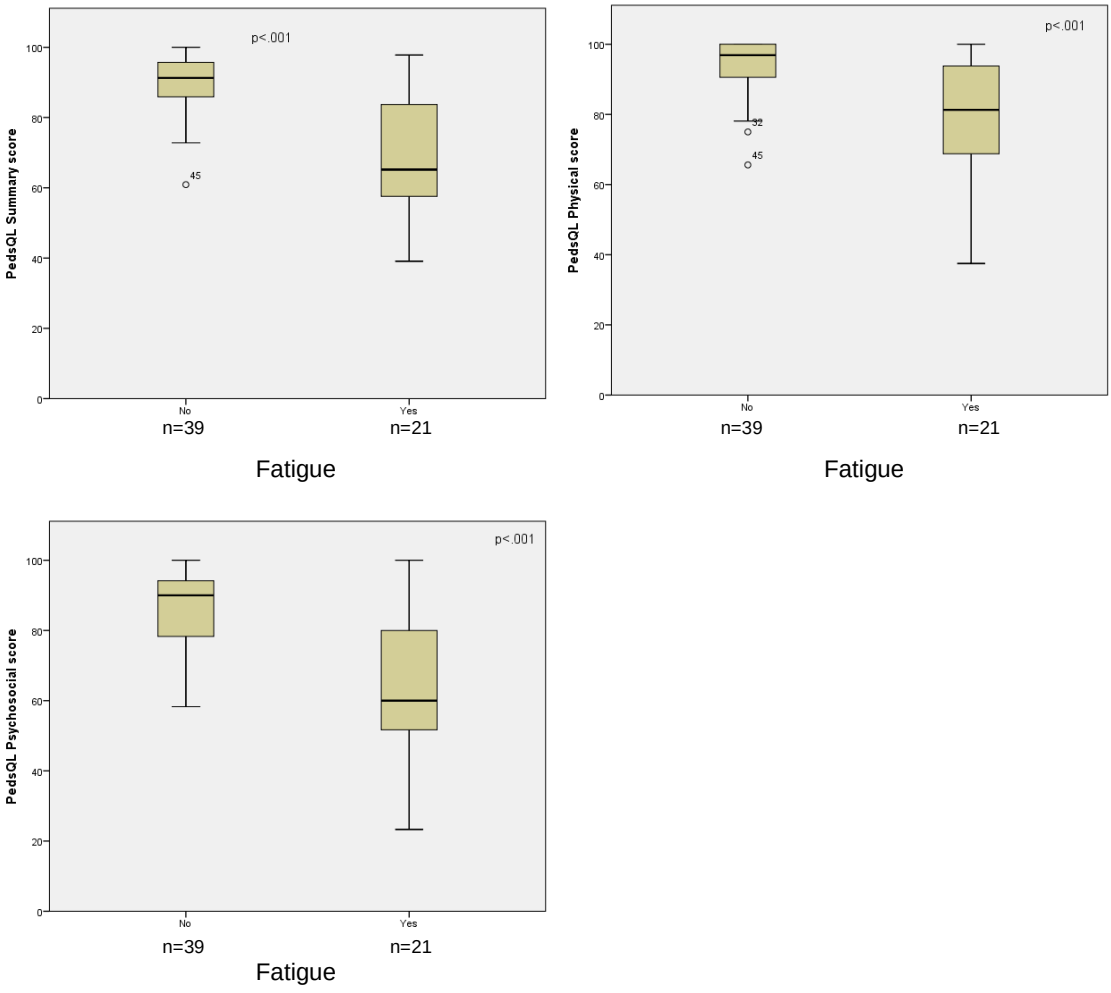


Figure 75 Association between Fatigue and PedsQL scores

Fatigue was also associated with higher Anxiety and Depression levels (Mann-Whitney U, p=.003 and <.001 respectively) [Figure 76].

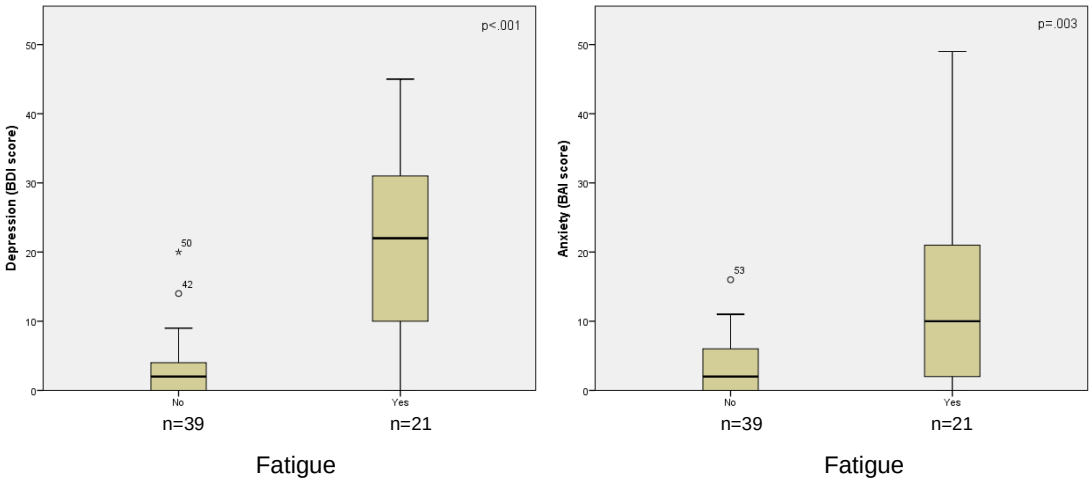


Figure 76 Association between anxiety scores (BAI), depression scores (BDI) and fatigue.

3.5.5 HRQL and fatigue results summary

Measures of HRQL associated with physical health (vision, speech, ambulation, dexterity) were not different between groups. Psychosocial measures of HRQL, although not statistically different when comparing the three groups (mid, mod/sev, controls) were lower when comparing all TBI participants (mild/mod/sev) to the non-TBI/control group. Cognition scores as assessed with the Health Utilities Index (HUI) were lower in the mod/sev TBI group. Cognition, emotion and participation (CASP) scores were different with two-group comparison (lower in TBI vs non-TBI). Self and proxy reports correlated strongly.

3.5.5.1 HRQL, fatigue and endocrine status summary

There was no significant difference in quality of life (PedsQL) or any of the various health attribute scores (HUI) between mod/sev TBI participants with normal and abnormal stimulated GH secretion.

Fatigue (using binomial or Likert score cut-offs) did not correlate with stimulated GH (Fisher exact Test, p=.208) [Figure 77] or cortisol status (Fisher exact Test, p=.739) as assessed with the ITT [Figure 77].

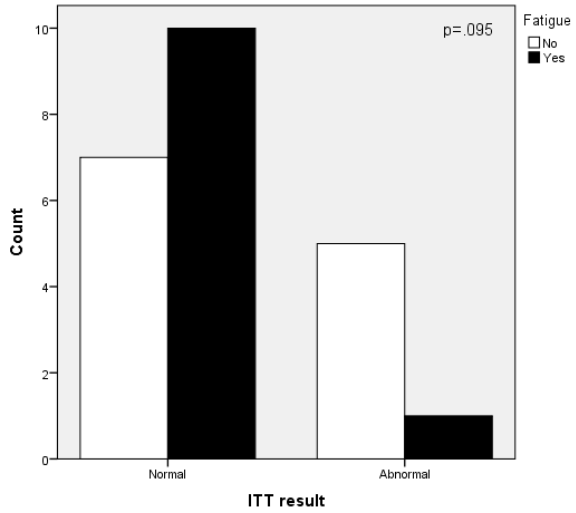


Figure 77 Fatigue (as assessed with Chandler fatigue scale) and GH status (as assessed with ITT) in mod/sev paediatric TBI survivors.

Measures of spontaneous GH secretion (mean secretion pulse height) were significantly different in participants with fatigue (t-test for unequal variances, p=.042) [Figure 78].

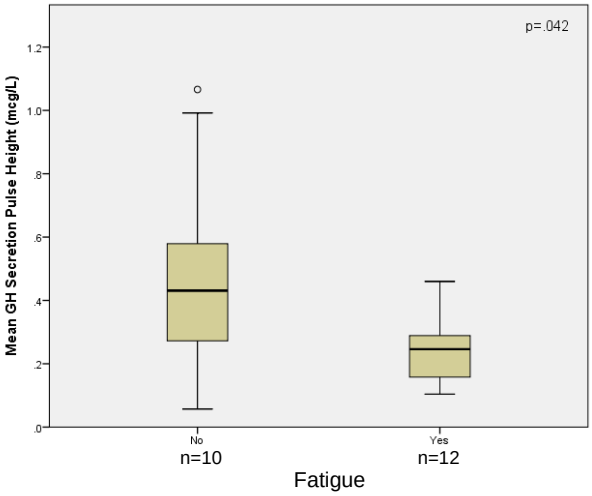


Figure 78 Fatigue and GH secretion pulse height in mod/sev TBI survivors

Measures of spontaneous cortisol secretion (overnight cortisol profile) were not different between mod/sev TBI participants with or without fatigue.

No difference was seen in CAR (Mann-Whitney U test, p=.414), or response to dexamethasone suppression (Mann-Whitney U test, p=.243) between the fatigue groups. The only significant difference was observed with bedtime salivary cortisone which was higher in participants with fatigue (Mann-Whitney U test, p=.007) [Figure 79]

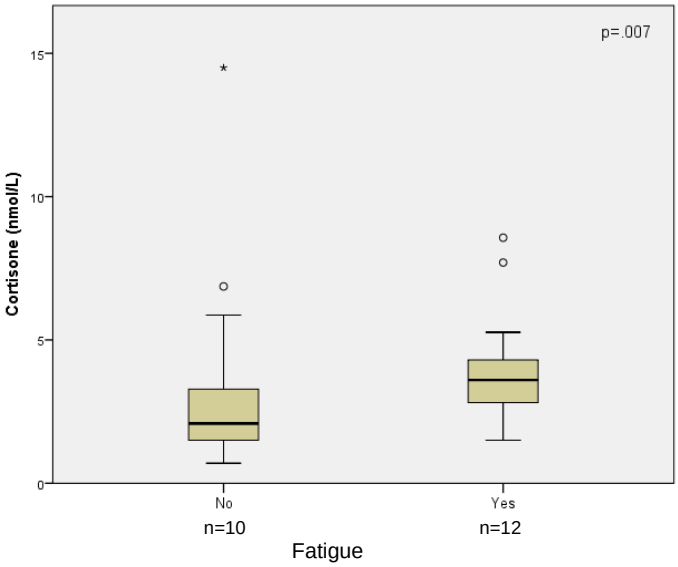


Figure 79 Bedtime salivary cortisone in mod/sev TBI survivors according to fatigue status

3.6 NEUROIMAGING RESULTS

Following screening, 56/72 participants (14 controls, 21 mild TBI, 21 mod/sev TBI) without any metallic implants or any other contraindication had a head MRI scan (10 from the mod/sev TBI, 3 from the mild TBI and 3 from the control group were excluded).

From the 42 TBI participants who had an MRI scan 19 were abnormal (4 in mild and 15 in mod/sev TBI). Reported abnormalities included contusions in 15, white matter sheering injury in 1, frontal lobe cavities in 1, reduced brain volume in 1 and acoustic neuroma in 1 (incidental finding). Pituitary morphology (both anterior and posterior) was normal in all participants.

Pituitary size as calculated with the ellipsoid formula was not different between groups when adjusting for pubertal stage (Kruskal-Wallis independent samples test, p=.309) [Figure 80].

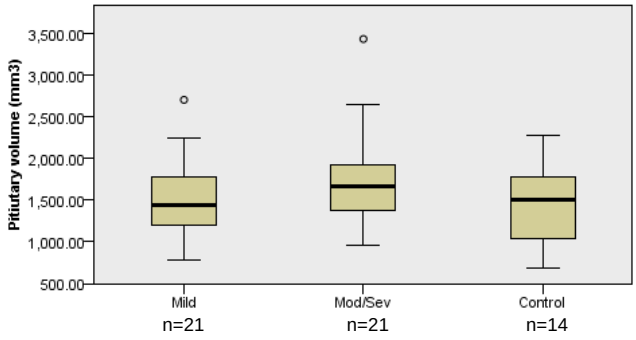


Figure 80 Pituitary volume in mild, mod/sev TBI study participants and healthy controls

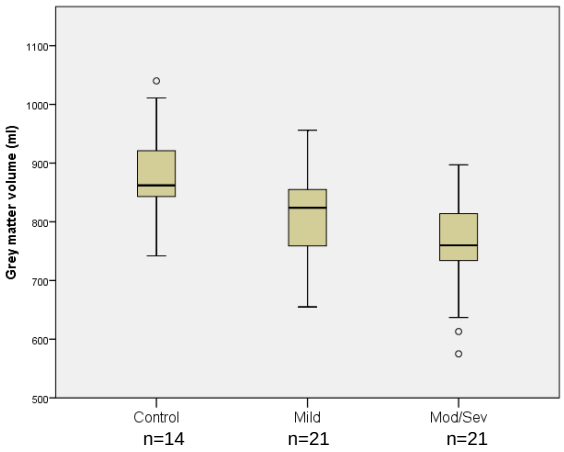
Pituitary volume did not correlate with measures of spontaneous or stimulated GH or cortisol secretion. Pituitary volume was greater in participants with fatigue (Mann-Whitney p=.039) but the difference was not significant when adjusting for pubertal stage (Mann-Whitney p=.285) as all participants with fatigue were at late stages or had completed puberty and as expected had larger pituitaries compared to younger participants.

3.6.1 Voxel Based Morphometry (VBM) results

VBM and ROI analysis for hippocampal areas demonstrated differences between the groups.

Grey matter volume (GM) was overall different between groups [F(2,53)=8.59, p=.001]. When a Bonferroni adjustment was applied for multiple comparisons, GM volume in

the mod/sev TBI group remained significantly lower than the control group (756±83 vs 874±84 ml) [Figure 81].

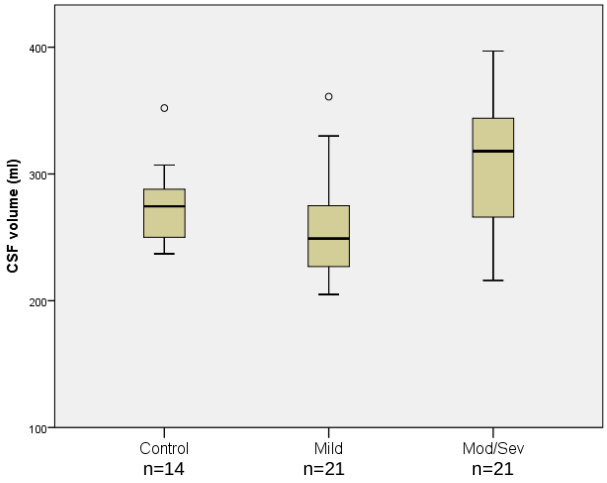


Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Mod/Sev-Mild	8.262	5.032	1.642	.101	.302
Mod/Sev-Control	-19.893	5.626	-3.536	.000	.001
Mild-Control	-11.631	5.626	-2.067	.039	.116

Figure 81 Comparison of grey matter volume between TBI and control groups

White matter volume (WMV) differences between groups did not reach statistical significance overall [F(2,53)=3.10, p=.053] so no further post hoc analyses were done. WMV in the mod/sev TBI group was 487±65ml vs 536±60ml in the control group.

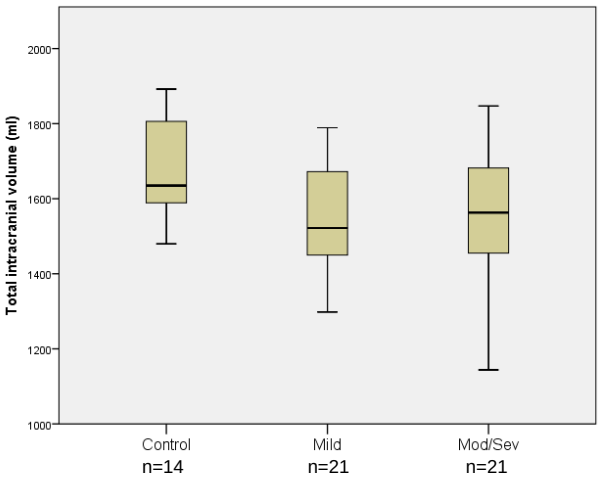
CSF volume was different between groups overall [F(2,53)=7.54, p=.001]. The only significant difference was between the mild and mod/sev TBI groups [Figure 82].



Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Mild-Control	-6.619	5.626	-1.176	.239	.718
Mild-Mod/Sev	-16.857	5.032	-3.350	.001	.002
Control-Mod/Sev	10.238	5.626	1.820	.069	.206

Figure 82 Comparison of CSF volume between TBI and control groups

Total intracranial volume (TIV) was lower in TBI participants (statistically significant difference between mild TBI and control group) [Figure 83].



Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Mild-Mod/Sev	-1.286	5.033	-.255	.798	1.000
Mild-Control	-14.071	5.627	-2.501	.012	.037
Mod/Sev-Control	-12.786	5.627	-2.272	.023	.069

Figure 83 Comparison of Total Intracranial Volume between TBI and control groups

Two-group comparisons (TBI vs non-TBI participants) demonstrated differences in GMV, WMV and TIV (t-test for unequal variances, p=.001, p=.015 and p=.008 respectively) [Figure 84].

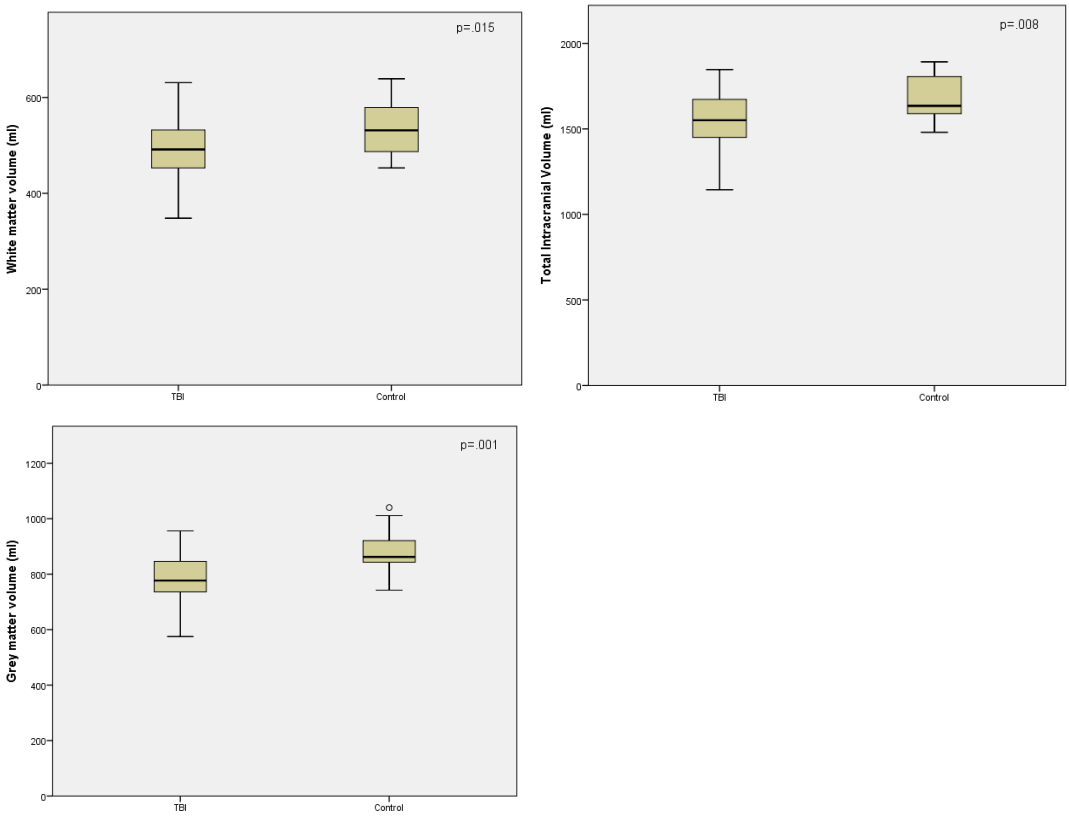


Figure 84 Comparison of Grey matter, White matter and Total Intracranial Volume between TBI (n=42) and non-TBI/control groups (n=14)

3.6.2 Region of Interest analysis

Only hippocampal areas were explored with ROI analysis but data are available for analyses of other ROI.

Left hippocampus volume was only just not statistically different between groups (Kruskal-Wallis independent samples test, p=.051) [Figure 85].

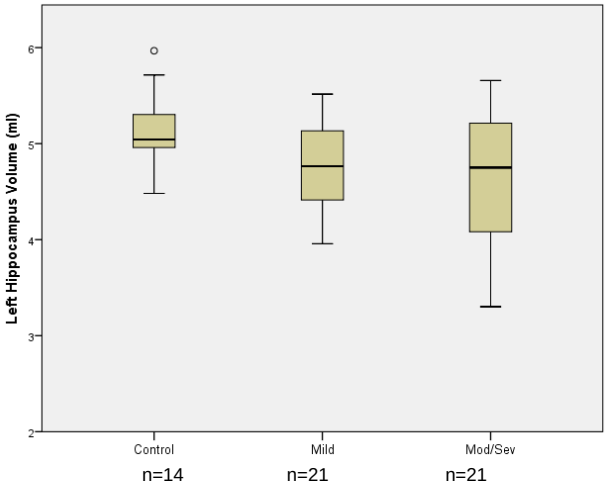
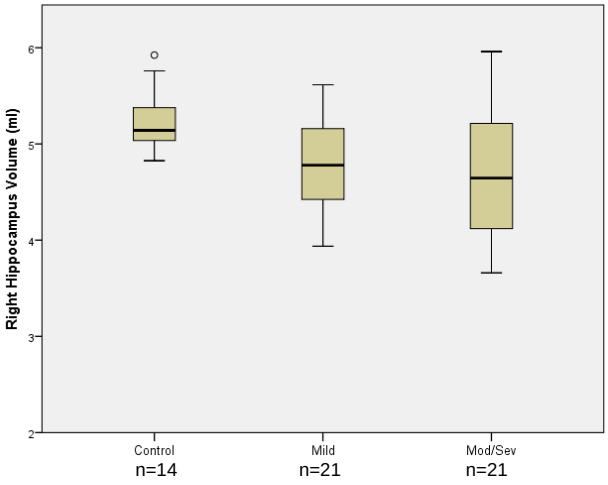


Figure 85 Comparison of Left Hippocampus volume between TBI and control groups

Right hippocampus volume was smaller in the mod/sev TBI group (Kruskal-Wallis independent samples test, p=.022). Pairwise comparisons showed the only significant difference being between the mod/sev TBI and control group [Figure 86].



Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Mod/Sev-Mild	2.524	5.033	.501	.616	1.000
Mod/Sev-Control	-14.976	5.627	-2.661	.008	.023
Mild-Control	-12.452	5.627	-2.213	.027	.081

Figure 86 Comparison of Right Hippocampus volume between TBI and control groups

When comparing all TBI participants (mild/mod/sev as one group) vs the control group, significant differences were seen in all VBM - except for CSF- and hippocampus volumes. WM, GM, TIV and both hippocampus volumes were smaller in the TBI group [Table 17, Mann-Whitney test)].

	Control group	TBI group	*p*
CSF volume (ml)	275±31	284±55	.718
Grey matter volume (ml)	874±84	781±85	.002
White matter volume (ml)	537±60	490±60	.032
Total Intracranial Volume (ml)	1686±140	1555±158	.008
Left hippocampus volume (ml)	5.16±.38	4.70±.55	.015
Right hippocampus volume (ml)	5.24±.32	4.77±.56	.006

Table 17 Comparison of brain (VBM analysis) and hippocampus volumes between TBI and control group. (mean±SD).

When only participants that had completed their growth were included in the analysis (9 from control group, 35 TBI group), GM and right hippocampus volumes were still significantly smaller in the TBI group [Table 18, t-test adjusted for unequal variances when indicated].

	Control group	TBI group	p^*
CSF volume (ml)	282±33	295±52	.357
Grey matter volume (ml)	839±65	769±82	.022
White matter volume (ml)	535±59	496±61	.092
Total Intracranial Volume (ml)	1657±131	1560±162	.106
Left hippocampus volume (ml)	5.08±.40	4.74±.58	.056
Right hippocampus volume (ml)	5.20±.33	4.81±.58	.014

Table 18 Brain (VBM analysis) and hippocampi volumes in TBI adolescents after completion of growth (mean±SD).

4 CHAPTER 4 – (DISCUSSION)

The KHINES study represents the first attempt to our knowledge to determine the long-term prevalence of PTHP following TBI during childhood with detailed endocrine assessments of GH and cortisol including both spontaneous secretion analysis and provocation testing. KHINES also included assessments of cognitive function, fatigue, depression and quantification of quality of life and health status. Finally, detailed neuroimaging was undertaken in all participants including matched controls.

KHINES succeeded in its aim to determine the long-term prevalence of PTHP following childhood TBI and support the limited number of previous paediatric TBI studies with sufficient patient numbers to recognise PTHP as a complication of paediatric TBI and estimate its prevalence [Table 19].

Although persistent endocrine dysfunction was identified only in a small number participants, KHINES raises questions regarding the use of dynamic testing for a condition where the *a priori* likelihood of pituitary/hypothalamic dysfunction is relatively small compared to high-risk populations where these tests have been traditionally used (i.e. oncology/neurosurgical patients with pituitary tumours, short children) and evaluates the use of spontaneous GH secretion and IGF1/BP3 as a diagnostic test for assessing pituitary function in TBI survivors.

4.1 INTRODUCTION

TBI remains a major cause of acquired neurological morbidity with an incidence of 100-350 per 100,000 in the general population [300]. Studies estimate that 3.8% of the population will experience at least one hospital admission due to TBI by 35 years of age which highlights the scale of the problem [9]. Injury severity can vary from mild to severe with few fatal cases of TBI especially when associated with multiple other injuries/trauma. Causes of TBI differ depending on the age group with falls being more common in preschool age children while road traffic accidents are more common in the older 10-15 age group [7]. A similar pattern was seen in KHINES as in 40% of participants TBI was secondary to falls (including one possible non-accidental injury in an infant) and road traffic accidents in the remaining 60% (pedestrian or cyclist hit by a vehicle). Males are more at risk for TBI irrespective of age group [11] and the same was seen in KHINES. Although mild TBI is more common than more severe types of TBI, the percentage of participants with mild TBI in KHINES was lower compared to moderate/severe TBI participants (33% vs 43%). The same distribution was seen in the non-participant group (mild 25% vs moderate/severe TBI 33%) and it is therefore

	Study design Period of data collection Interval since TBI	Patients eligible/included Age range	Eligibility criteria	Protocol	Pituitary deficiencies	Clinical outcome
Einaudi et al 2006 [50]	Retrospective and prospective 1994–2002 retrospective 2002–2003 prospective Interval 12 months	Retrospective n=98/22 (22%) Age 11.2–18y Prospective n=30 Age 0.25–15.5y	GCS at inclusion: severe, moderate or mild	-Basal & -Dynamic tests restricted to patients with height velocity <25 centile (n=2) GHRH+arginine	Complete hypopituitarism (n=1) LH/FSH (n=1) GHD (n=2) Hypocortisolism (n=1)	Retrospective group 1 precocious puberty 1 complete anterior hypopituitarism 1 hypogonadism 1 GHD Prospective group 18/20 normal auxological data, 1 GHD 1 asymptomatic hypocortisolism
Niederland et al 2007 [301]	Retrospective 2003–2004 Interval 30.6±8 months	n=38/26 (68%) Age 11.4±0.7	GCS at inclusion: severe to mild	-Dynamic test all L-DOPA and ITT GH sufficiency >7 ng/mL	42% abnormal GH 34% abnormal basal cortisol	No significant height reduction No clinical hypocortisolism
Poomthavor et al 2008 [49]	Retrospective 1995–2005 Interval 0.9–8.5 years	n=50/17 questionnaire; n=29 hormonal tests (24% of eligible patients) Age 3.5–20.1y	GCS at inclusion: severe	-Basal -Dynamic tests restricted to patients with low growth velocity (n=8) glucagon test GH sufficiency >10 ng/mL	Normal GH response Subnormal cortisol response (n=3)	1 precocious puberty 8 low growth velocity with normal GH tests 4 previously diagnosed with one or more pituitary deficiencies 1 central hypothyroidism
Khadr et al 2010 [48]	Cross-sectional 2001–2007 Interval 0.6-14.1	n=133/33 (24%) Age 5.4–21.7y	GCS inclusion: severe, moderate or mild short stature	-Basal -Dynamic tests in all ITT or glucagon GH sufficiency >5 µg/L	GHD (n=7) Low cortisol peak (n=9) Low basal prolactin (n=1)	32/33 mean height, weight and BMI SDS comparable with reference population 1 GH treatment
Norwood 2010 [302]	Cross-sectional 2007–2009 Interval 0.7–3.8 years	n=52/32 (62%) Age 18.2±2y	GCS inclusion: severe or moderate	-Basal -Dynamic tests in all Arginine/glucagon (GH sufficiency >7 ng/mL 18 years/>5 ng/mL <18 years) -Overnight profile (GH >5ng/ml)	GHD (n=10) Low basal cortisol (n=6) Low basal fT4 (n=1)	GHD Eexcessive weight gainafter TBI
Moon et al 2010 [303]	Cross-sectional Medical records 1999–2004 Interval 4.2–10.3 years	n=97/20 (20%) Age 9.2–23.2y	GCS inclusion: severe, moderate, mild	-Basal -Dynamic tests to patients with clinical hypopituitarism	No subject warranted further investigation	Mean height, weight and BMI SDS comparable with reference population * No precocious puberty *Measures of adiposity similar to controls *Quality-of-life assessment
Kaulfers et al 2010 [304]	Prospective 2011–2007 Interval 1.4–7.8 years	n=102/34 (33%) Age 5.4–21.7y	GCS inclusion: severe or moderate	-Basal -Overnight GH profile	Central hypothyroidism (n=2) GHD (n=1)	Mean height, weight and BMI SDS comparable with reference population Precocious puberty (n=3) No GH profile results
Heather et al 2012 [47]	Cross-sectional 2000–2010 Interval 6.5±3.2 years	n=345/198 (57%) Age 8.3±3.3y	GCS inclusion, moderate, severe	-Basal -Dynamic test all Arginine clonidine	GH peak <10 µg/dL (n=65) and	*Of those with GH peak <5 ng/dL Mean height SDS 1.0±1.2 and BMI 2.8

			or mild+structural TBI (skull fracture, intracranial haemorrhage or cerebral injury)	low-dose ACTH test	GH peak <5 µg/dL (n=16) IGF-1 within normal range Suboptimal cortisol response (n=17)	±0.8; follow-up with normal growth velocity *None were considered to have significant ACTH deficiency *One boy and one girl with precocious puberty
Casano-Sancho 2013 [305]	Prospective 2009–2011 Interval 3 and 12 months after TBI	n=51/37 (73%) Age 0.2–19.9y	GCS inclusion: severe, moderate or mild+structural TBI (skull fracture	-Basal -Dynamic test all two tests glucagon clonidine	GH peak <7.5 ng/mL after 1 year of TBI (n=8) IGF-1 within normal range Suboptimal cortisol response after 3 months of TBI (n=10), after 12 months (n=3) Abnormal response GnRH test (n=1)	*Growth velocity within normal limits in all patients except for one *None were considered to have significant ACTH deficiency *No precocious puberty
Salomón-Estébanez et al 2014 [42]	Cross-sectional 2004–2009 Interval 1.3–5.8 years after TBI	n=58/36 (61%) Age 2.7–15.1y	Did not include GCS score (abnormal TC and admission to PICU)	-Basal -Dynamic tests if clinical PTHP	1 Autoimmune hypothyroidism 2 Low morning cortisol (1 of them abnormal ITT) 4 IGF-1 levels below the 2.5th	*Normal height velocity *No precocious puberty
Auble et al 2014 [306]	Retrospective 2008-2011 Interval 1-9 years	n= 102/14 (13.7%) Age 2-9y	GCS inclusion: severe or moderate + subdural hematoma On CT or MRI *Abusive head trauma	-Basal -Overnight GH profile -Low-dose ACTH	GH peak low (n=2) TSH surge low (n=6) Hyperprolactinemia (n=9) Low morning cortisol (n=1) *GH profile mean GH>1.1mcg/l	Relatively smaller stature was noted in 29% of patients BMI z-sore median 0.5 (-2.3 to +1.9)
Personnier et al 2014 [43]	Prospective 2008–2011 Interval 9.5 ±3.4 months	n=103/87 (84%) Age 0.8–15.2y	GCS:severe (+acute subdural haematoma)	-Basal -Dynamic test glucagon	GH peak <7 ng/mL (n=27) Low fT4 (n=6) Suboptimal cortisol response (n=1)	*Decrease their height SDS >0.5 (n=6) *No precocious or delayed puberty

Table 19 Paediatric TBI studies published to date (adapted from Casano-Sancho P. Arch Dis Child 2017) [307]

unlikely that the higher representation of moderate/severe TBI participants in KHINES represents systematic error. It is possible this is simply a reflection of mild TBI patients being seen mostly in primary care rather in a hospital emergency department and therefore being less likely to be recruited to hospital based studies like KHINES.

4.2 TBI AND PTHP

In the acute phase of TBI PTHP is a recognised complication which usually resolves spontaneously and is considered to represent physiological adaptation to critical illness [308, 309]. However, the natural history of acute PTHP and the risk factors determining the development of permanent PTHP remain an area or controversy. Our knowledge of chronic PTHP has evolved from single case reports, to case series, to an increasing number of studies using different inclusion criteria and methodology making their results difficult to compare.

Spontaneous recovery from PTHP was initially described by Agha et al in a case report of a 25 year old male who was diagnosed with PTHP (GH and ACTH deficiency) 16 months post TBI and showed full recovery at retesting after 5 years [310]. Tanriverdi et al in a pilot, prospective study reported that 3 years post TBI, pituitary function recovers in about half of mild and moderate TBI survivors but persists in those with severe TBI [311]. PTHP may therefore not be as persistent as initially described. Conversely, development of PTHP with isolated or multiple hormone deficiencies years after the acute TBI phase in patients where the initial endocrine assessment was normal has been described, but longer-term prospective data are not available to determine if these remain persistent [312, 313]. There is a possibility that these evolving and recovering hormonal deficiencies are just a reflection of inaccuracies and poor reproducibility of diagnostic testing as previously discussed in Chapter 1.

The unclear natural history of TBI has impacted on the recommendations for assessing pituitary function following TBI which over the last decade have varied considerably. Some suggest that endocrine tests should be reserved only for survivors of moderate or severe TBI with risk factors including cerebral oedema with increased intracranial pressure, hypoxia/hypotension, diffuse axonal injury and basal skull fracture [20, 25, 312, 314] while others recommend universal testing [306, 315]. Although the former approach can underestimate the true incidence of PTHP the latter approach carries the risk of over diagnosing PTHP if strict diagnostic criteria are not used. Identifying patients for endocrine screening on the basis of clinical symptoms is also difficult as the symptomatology of PTHP is non-specific and may overlap with TBI sequelae. Fatigue for example is very common amongst TBI patients without PTHP [152] but also in patients with GHD who show a good response with improved energy levels when treated with GH [162].

The challenge therefore remains in deciding how, when and most importantly who to screen for PTHP considering that 3.8% of the population will experience at least one hospital admission due to TBI by 35 years of age.

4.3 METHODS DISCUSSION

Researchers have used various types of studies to explore the prevalence of PTHP following TBI in childhood. From the first case reports and case-control studies that led to the generation of the PTHP hypothesis there has been an exponential increase in the number of prospective studies in adults exploring risk factors and outcome measures of TBI. Similarly the first paediatric studies were retrospective with varied time intervals from TBI, inclusion criteria and methodology. Despite however the relatively flexible inclusion criteria most studies were not able to recruit high numbers of participants with some recruiting only 10-30% of eligible participants [Table 19]. Although KHINES followed the Kids' Head Injury Study (prospective study of outcome in children with severe, moderate and mild TBI [KHIS, 2002-2004]) by nearly10 years, almost half (79/172) of contactable participants agreed to take part in KHINES which is comparable to recent prospective TBI studies. This was a result of their previous positive experience with KHIS and ensuring that the research psychologist who had been a member of the original KHIS team made the initial contact. In order to assess the potential of bias between participants that agreed to participate and those that didn't, we compared these groups and we found no differences with regards to age, gender, time since TBI, severity of TBI, duration of post-traumatic amnesia, days in intensive case or type of structural abnormalities on acute neuroimaging (extradural/subdural haemorrhage, diffuse axonal injury or skull fracture). Although not statistically different, more participants with moderate/severe TBI, extradural haemorrhage and skull fracture agreed to take part in KHINES compared to non-participants.

TBI presents a new challenge to clinicians dealing with neuroendocrine conditions as the diagnosis of hypopituitarism is highly dependent on pre-analytical (confounders that are patient related such as age, BMI, gender, drugs, pre-existing medical conditions including mental health) and analytical factors. Previous studies have shown the prevalence of PTHP to vary greatly depending on inclusion criteria, testing methodology and analytical factors [38, 43, 47, 50, 316]. As outlined in Table 19 some researchers used only baseline endocrine tests while others employed dynamic tests in all or only in symptomatic participants. Only three paediatric TBI studies report assessing spontaneous GH secretion.

Even when applying the same inclusion criteria, sources or variation that affect the reported prevalence of PTHP cannot be ignored. Analytical factors such as changes in assays (mainly GH and cortisol which are the ones mostly measured with dynamic tests) can result in misclassification of a test result as abnormal. The use of new, automated, highly specific cortisol assays for example has resulted in a reduction of the cut-off used to diagnose adrenal insufficiency from 500 nmol/l to 420nmol/l [317]. For other hormones including GH and IGF1 consensus statements have been developed to standardise GH assays [318].

Assay changes however are only one of the sources of variation. Interpretation of dynamic tests relies on the use of specific cut-offs. For GH dynamic tests these cut-offs depend on the pharmacologic stimulus used but also on other confounders such as the age or BMI of the patient. Obesity blunts GH response to insulin [75], glucagon or GHRH+arginine [319] and therefore BMI specific cut-offs need to be applied. As these limitations/restrictions were not widely recognised previously, they may have resulted in over diagnosis of GHD in the past. In light of this, some researchers have reanalysed their data and subsequently reported a lower prevalence of PTHP [320]. All of the above apply for TBI studies that include assessment of spontaneous GH secretion with the addition of scarce normative data (most from studies using older non-standardised GH assays).

Another source of overestimating the prevalence of PTHP is not including confirmatory retesting considering the intra-individual variation of GH dynamic tests [321-323]. It is possible that the intra-individual variation of GH dynamic tests is associated with the specific dynamic test protocol and sampling intervals.

In the majority of paediatric TBI studies the somatotroph axis appears to be the one mostly affected, either in isolation or with other pituitary hormone deficiencies (thyroid, gonadotrophins) especially during the first year post TBI. It has been suggested that this is a result of the location of somatotroph and gonadotroph cells in the periphery of the anterior pituitary and fragility of the infundibular-hypothalamic-pituitary structure due to its unique anatomical and vascular structure [324]. In our study, only one female participant presented with hypogonadism, which was not isolated but combined with GHD. Thyroid function was normal in all participants.

In view of the vulnerability of the somatotroph axis, the emphasis of the endocrine arm of KHINES was to assess the long term impact of TBI on GH status. Although various stimuli have been used for assessing GH status by stimulating pituitary GH release, KHINES chose the ITT as a) it is regarded to be the “gold standard” for assessing GH

status, b) it can assess both the somatotroph and HPA axes and c) the test is safe when done by experienced staff in a centre that already has extensive experience in performing the test.

In contrast to GH stimulation tests where cut-offs for diagnosing GHD have been mostly agreed (in adults having an ITT, a GH cut-off of 3mcg/L has been accepted [76], while in children 7-10 mcg/L is being used in most centres), diagnostic criteria for defining “normal” spontaneous GH secretion are lacking. Some studies have used the presence of at least one GH concentration peak above the pharmacological test cut-off used for that age group as a criterion [84] while others have used mean overnight GH secretion instead, as this was found to have 100% specificity depending on the applied diagnostic cut-off (<1 mcg/L) [87, 88, 249]. Nevertheless, with KHINES we chose spontaneous GH secretion analysis as the second endocrine test as this was the only way to explore associations between neurosecretory dysfunction (i.e. abnormal spontaneous secretion in the presence of normal stimulated secretion) and PTHP symptoms. Although studies comparing normally growing vs poorly growing children but also lean vs overweight children have shown that differences in spontaneous GH secretion are due to lower amplitude of GH peaks rather than reduced number of GH secretory episodes, it is not known if TBI is associated with a similar GH secretory pattern or impaired GH pulsatility.

4.4 RESULTS DISCUSSION

4.4.1 Endocrine (growth hormone)

Stimulated GH secretion as assessed with the ITT was abnormal in 7/25 moderate/severe TBI participants. Although the rate of abnormal tests was in line with that reported in some of the earlier TBI studies that did not use confirmatory retesting, all KHINES participants with abnormal ITT were growing normally (lowest height SDS was -0.33) and were not overweight (highest BMI +1.3 SDS). IGF1 which is mainly regulated by GH and is a surrogate marker of GH status was within the reference range in all but one participant (IGF1 -2.39 SDS). IGF1 levels did not correlate with peak GH response to ITT even when controlling for their (normal) weight. In the study by Heather et al [47] that included young children with structural TBI, 8% (16/198) demonstrated abnormal GH responses to two stimulation tests (clonidine and arginine) but all 16 children had normal IGF1 levels and demonstrated normal growth at follow-up making the clinical significance of even two, abnormal GH stimulation tests questionable.

Using the overnight GH profile as the second test to assess GH status, 4/22 (using mean overnight GH as the diagnostic criterion) and 1/22 (using peak spontaneous GH secretion as the diagnostic criterion) participants would be diagnosed with GHD. IGF1 was less than -2 SDS in two out of the four abnormal overnight profiles using mean overnight GH secretion as the diagnostic criterion and was also low (-2.39 SDS) in the single abnormal profile using peak spontaneous GH secretion as the diagnostic criterion. There was no correlation between peak stimulated and peak spontaneous GH secretion. Peak GH during sleep was generally higher compared to stimulated (ITT) for the same participant but the difference was not statistically significant.

Although some studies report less than 15% discordance between stimulated and spontaneous GH secretion results, this does not appear to be the case with TBI patients as there was no concordance between the ITT and overnight GH profile result using either mean or peak overnight GH spontaneous secretion criteria to define a profile as being normal or abnormal. It would be reasonable to expect good concordance between these two methods of assessing GH status in clinical scenarios where GH secretion is severely impaired (patients with hypothalamo-pituitary pathology and multiple hormone deficiencies) but in TBI patients who have been growing normally and are asymptomatic, an abnormal result on a GH stimulation test is of questionable significance especially when both IGF1 and spontaneous GH secretion are normal.

A possible explanation for the deceptively high rate of false positive ITT's in TBI patients can be found in the design of the ITT itself. Although most ITT protocols use sampling at 0, 20, 30, 60, 90 and 120 min as originally described by Roth [71] these may not be the optimal sampling time points especially when diagnostic decisions are going to be based on the result of a single test. The results from deconvolution analysis of KHINES overnight GH profiles, but also from GH kinetic studies where GH and somatostatin were given in combination (somatostatin given in order to supress endogenous GH secretion), show that the half-life of GH is between 9-15 minutes with a mono-exponential disappearance curve [325]. This would suggest that in order to detect GH concentration peaks, GH samples should be taken at much shorter intervals. As peak GH levels with the ITT are achieved on average 45 min after the administration of insulin [326] it would be reasonable to intensify sampling around that time point to increase the probability of capturing the "true" peak GH response.

KHINES TBI participants had only one stimulation test (ITT) and the high prevalence of GHD based on the ITT result alone without associated clinical findings, highlights the limitations of the ITT (more so a single ITT) to diagnose GHD in this context. As there

is no physiological reason for GH kinetics to change with other pharmacological stimuli used in stimulation tests, it is likely that the same limitations will apply if sampling intervals are longer than the estimated half-life of GH.

Analysis of spontaneous GH secretion is therefore more likely to provide accurate information regarding not only overall GH secretion but also the pulsatile pattern of GH secretion which acts as a signal for tissue specific responses and can be disrupted, sometimes without changes in overall GH secretion. It has been shown that IGF1 production differs in muscle and liver depending on the pattern of GH secretion [327]. Although basic information regarding pulsatility can be obtained by plotting GH concentration over time, interpretation and definition of what is a peak or pulse can be very subjective and does not take into account the kinetics of GH (secretion, distribution, elimination) which can affect the form of the GH concentration curve. It is therefore preferable to use pulse detection or deconvolution algorithms as described in detail in Section 1. Although not perfect, these almost fully automated methods enable direct comparisons and consistency between analyses. Analysis of KHINES participant GH profiles using AutoDecon demonstrated intact GH pulsatility exposing secretory and in contrast to the ITT, measures of spontaneous GH secretion derived from deconvolution correlated strongly with IGF1 levels. ApEn for GH was higher in female TBI participants indicating greater irregularity. This pattern reflects the physiological gender separation in GH secretion. ApEn was not different between participants with fatigue.

For the paediatric TBI population, growth is a very sensitive indicator of somatotroph axis integrity and measurement of height velocity in particular has been shown to aid identification of TBI survivors with PTHP, providing that growth velocity remains *consistently* under the 25th centile during a follow up period or at least one year. Using this screening method, a third of paediatric patients will demonstrate suboptimal GH response to a single stimulation test with GHRH & Arginine [328].

The prevalence of GHD in KHINES provides information about the natural history of PTHP after TBI in childhood. In another study with similar design (Wamstad et al. [329]) that enrolled slightly younger children and adolescents 3-4 years post TBI, the prevalence of GHD was 17% based on the overnight GH profile and 33% based on dynamic testing. The results of the stimulation test are almost the same (33% vs 28% in KHINES) using a different stimulation test (arginine/glucagon). Overnight GH secretion was abnormal in a smaller percentage in KHINES (5% vs 17%) using however more strict criteria (peak overnight GH cut-off same as the one used for the ITT). In the study by Wamstad the same peak overnight GH level of < 5mcg/L was

used to diagnose GHD even in participants that were over 18 years of age. If the same cut-off (5 mcg/L) was applied in KHINES, the prevalence of GHD would be 13% (3/22) based on the profile only and 4.5% (1/22) based on both tests (14% in the study by Wamstad).

In summary and by assessing both stimulated and spontaneous GH secretion in moderate/severe TBI survivors the long-term prevalence of GHD is between 5-10%.

4.4.2 Endocrine (cortisol)

HPA axis abnormalities in KHINES were mild. In two participants stimulated cortisol secretion (392 and 483 nmol/l) was suboptimal using the local laboratory diagnostic cut-off value of 500 nmol/l. One of the participants had also suboptimal GH response to the ITT. In all moderate/severe TBI participants cortisol was secreted predominantly in the second half of the night with gradually increasing pulsatility. Salivary cortisol profiles did not differ between TBI participants and controls, or between participants with depression or fatigue. The circadian rhythm was preserved and all participants responded with low morning cortisol levels following suppression with oral dexamethasone. The two participants with suboptimal stimulated cortisol response had high fatigue scores. One had a bimodal score of 10 (patients with Chronic Fatigue Syndrome have a score of 9 or above). Although chronic stress is associated with chronic elevation of cortisol levels, preceded by changes of trough cortisol levels and loss of circadian rhythm [330, 331] this was not seen in KHINES participants. A similar response has been described in adult patients with depressive disorders [332]. Conversely, chronic pain syndromes, anxiety disorders and posttraumatic stress are associated with hypocortisolism [333].

Although changes in CAR have been described in patients with PTSD [334], chronic fatigue [335], hippocampal lesions [336] (blunted response) and chronically stressed, recovered depressed patients (enhanced response) [337], neither was seen in KHINES. In two participants (one healthy control and one mod/sev TBI) the CAR was negative over three consecutive days. The most likely explanation for these results would be delayed collection of the first sample after awakening or mislabelling of the samples.

4.4.3 Cognitive function

The findings of this study confirm the presence of significant cognitive impairment in paediatric TBI survivors 10 years following TBI as previously demonstrated in adult patients [338]. Anthropometric and physical function scores in our study group of TBI survivors suggest a good outcome from the physical disability point of view but

significant residual neurocognitive disability. In addition to cognitive impairment our study participants (mostly moderate/severe TBI) demonstrated reduced QoL, high rates of fatigue and depression scores. Cognitive deficits involve mainly working memory which are comparable to previous studies with shorter follow up post TBI [179]. Although significant cognitive recovery takes place during the first year after TBI, the rate of improvement slows down in severe TBI survivors particularly in the domains of performance IQ, adaptive problem solving, memory and motor skills [339]. The persistent deficits and lack of catch-up over time suggest the possibility of reduction in the rate of acquisition of new skills after severe TBI. Although TBI KHINES participants had total IQ scores (Full Scale Intelligence Quotient) that were not significantly different to that of the control group, only the moderate/severe TBI group included participants with IQ scores under 80. Performance IQ which is one of the last domains to recover was not different between groups but again only the moderate/severe TBI group included participants with performance IQ in the lower centiles. Verbal IQ was significantly lower in the moderate/severe TBI group and being linked to academic achievement highlights the persistent consequences and deleterious effects of TBI on academic performance.

A link between the somatotropic system and cognitive function has been hypothesised once it was shown that GH is permeable to the brain and receptors for both GH and IGF1 exist in areas of the brain associated with cognitive function and behaviour [141]. GH treatment in adults with non-TBI related GHD has been shown to improve memory and attention [340] but these findings have not been reproduced in other studies [341]. Children with congenital hypopituitarism have an intellectual ability that is in the low-average range compared to the population norm but not significantly different when compared to their siblings except for performance IQ (reflection of reduced ability to perform tasks requiring perceptual organisational skills) [165]. These children are susceptible to hypoglycaemia early in life and are also likely to have other associated brain malformations [166]. GH therefore appears to have a less important role in brain development compared to thyroid hormone. Furthermore, intelligence in children with GH insensitivity due to abnormal GH receptor is not different compared to controls indicating that GH and GH-induced IGF-1 production is not necessary for normal brain growth and development [167]. It is however possible that the beneficial effects of GH treatment in regards to cognitive function and memory are a result of neuroprotective properties of GH and IGF1 as demonstrated in the hippocampus [342, 343]. There is however agreement in that memory, attention and executive function decline in adults with PTHP [344].

4.4.4 Behaviour

Behavioural and psychosocial problems following TBI in childhood have not received the same attention as the physical and cognitive consequences but many children with TBI demonstrate low self-esteem. This is closely linked with anxiety and depression and can impair academic performance leading to further psychosocial problems [345]. Recent studies have reported a surprisingly high incidence (31%) of novel psychiatric disorders in children with mild TBI in the second year post injury [346] but a systematic review of mild and moderate paediatric TBI studies from 2008-2013 did not show a clear effect [347]. Frontal white matter is particularly susceptible to injury and persistent changes in the area were demonstrated in KHINES participants. Executive functioning (task planning, inhibition, attention control, sustained effort, and mental flexibility) relies on frontal–striatal networks that are particularly susceptible to diffuse axonal injury [178]. Approximately 20 to 40% of young children (5-15 years of age) with TBI show significant executive dysfunction within the first year of injury [179]. In KHINES the prevalence of behavioural problems in the moderate/severe TBI group was significantly higher. This included changes in externalizing behaviour consisting of three other behaviour syndromes (aggressive, rule breaking and intrusive) that mainly involve conflicts with other people and with social mores but also problems with attention.

Scores for internalizing behaviour consisting of three syndromes (anxious/depressed, withdrawal, somatic complains) were also higher in all TBI participants including those with mild TBI. Depression scores were significantly higher in the moderate/severe TBI group.

Considering the potential neuroprotective effects of GH, studies looking at psychiatric outcomes in TBI patients showed a high incidence of depression, anxiety and psychosis, which correlated negatively with peak, stimulated GH responses. In the study by Wamsad et al [329], abnormal spontaneous (but not stimulated) GH secretion was associated with impaired visual memory and lower HRQL but this was not demonstrated in KHINES as there were no differences in regards to anxiety, depression and cognitive function between participants with normal and abnormal stimulated or spontaneous GH status.

Depression has also been reported to be associated with reduced spontaneous (mainly nocturnal [348]) and stimulated GH secretion [349]. A similar effect was seen in KHINES but did not reach statistical significance, possibly due to sample size. In KHINES participants with depression

, GH pulse mass, pulse height and average GH secretion but not number of detected pulses was lower. It has been hypothesised that increased activity of central beta receptors [350] and CRH hypersecretion (which is found in patients with depression) exert an inhibitory effect on GH release.

4.4.5 HRQL

Although it is encouraging that only a small proportion of TBI survivors are left with significant physical disability, it is the subtle but significant issues in childhood such as decline in school performance (but still within the “normal” range, hence not causing major concerns) and behavioural changes that are often missed. To complicate things further, TBI survivors (much like cancer survivors) often perceive their quality of life as satisfactory after having to live for many years with the complications and sequelae of their condition. All these symptoms are difficult to capture as even detailed neuropsychological assessments have their limitations and can be affected by deficits in self-awareness (common sequelae of severe TBI) that render adolescents less capable of accurately rating their HRQL. Furthermore popular QoL measurement systems fail to consider whether the measured outcomes are relevant to the adolescent and capture the subtlety of the adolescent social experience [351].

None of KHINES TBI participants had significant physical disability as assessed with the PedsQL sub-score for physical functioning. Although summary and PedsQL sub-scores (physical and psychosocial) were similar between the three KHINES groups (control vs mild vs moderate/severe TBI), differences emerged once comparisons were made between TBI and non-TBI participants. Both summary and psychosocial PedsQL scores (but not Physical sub-scores) were significantly lower in the TBI group. Overall, sixteen participants (7 mild, 9 moderate/severe TBI) had a summary PedsQL score of less than 69.7% (equivalent to one SD below the population mean) which has been shown to represent poorer HRQL. Similar results were obtained using the Health Utilities Index (HUI), a system of measuring health status once comparisons were made between TBI and non-TBI participants. Similarly to the PedsQL results, the HUI did not show any differences for ambulation, dexterity and pain between groups but attribute scores for cognition and emotion were lower in the TBI group.

4.4.6 Fatigue

Fatigue was one of the most prominent symptoms in KHINES TBI participants. Almost half (13/28) of moderate/severe TBI participants had high fatigue levels (some of them in the range seen only in CFS sufferers [285]) as assessed with Chalder’s fatigue scale. This is similar to what has been reported previously within the first two [352] or

five years [153] post TBI. Although not always clear unless using a standardised tool to measure fatigue, it is important to distinguish between *physical* and *central* fatigue. The former refers to impaired physical performance or exercise tolerance. This can be seen in TBI survivors with musculoskeletal injury and prolonged immobilisation [353]. One of the proposed mechanisms linking PTHP and physical fatigue is impaired muscle mitochondrial function secondary to GHD [354]. The latter (central fatigue) refers to the lack of motivation to initiate or sustain mental or physical tasks [355]. Although functional gains appear to continue for long periods after TBI – even after rehabilitation intervention has stopped – fatigue levels do not seem to follow the same pattern of improvement over time. This could be a result of TBI survivors realising and having to deal with the consequences of TBI when returning to their previous activities. Maintaining the same achievement level and having to "catch-up" adds an additional burden on TBI survivors, which over time results in increased levels of fatigue. Considering the high incidence of cognitive impairment in domains as executive functioning, memory and attention following TBI this is not unexpected [338] and is reflected in the employment and studying status of TBI survivors, which declines over time. In the study by Olver et al [153] only half of TBI survivors who were employed at the time of injury were employed at 2 years and even fewer after 5 years (40%).

Although fatigue levels between GHD and non-GHD TBI survivors (irrespective of TBI severity) have been reported to be comparable, TBI survivors with PTHP show a similar benefit from GH replacement in terms of improved HRQL when compared to a matched group of GHD patients due to non-functioning pituitary adenoma. It is not known if non-GHD survivors with fatigue would respond to GH treatment in the same way. In a randomised, placebo controlled study evaluating the efficacy of GH therapy in patients with chronic fatigue and low spontaneous GH levels, Moorkens et al. showed there was no significant improvement in HRQL after 12 months of treatment.

KHINES did not demonstrate any correlation between stimulated GH secretion and fatigue levels (even in those participants where stimulated GH response was clearly abnormal) and in conjunction with available evidence we cannot advocate the use of GH for treating fatigue symptoms in TBI survivors without solid evidence of PTHP using strict diagnostic criteria.

4.5 STRENGTHS AND LIMITATIONS OF THE STUDY

KHINES has significant strengths compared to other paediatric TBI studies. Recruitment was high considering that almost half of contactable KHIS participants agreed to take part in KHINES. KHIS, being a prospective study of outcome in children

with severe, moderate and mild TBI, consecutively recruited participants over a short period of time. This resulted in little variability in time from injury for KHINES participants (all between 7-11 years) and no selection bias. Clinical characteristics between participants and non-participants were not different (age, time from TBI, gender, TBI severity, socioeconomic status).

From the endocrine perspective, all study participants had the same stimulation test (ITT) and overnight sampling for GH and cortisol was done in the same hospital. Samples were analysed at the same lab using the same assay and method for blood and salivary samples. All tests were done by the same researcher.

One of the limitations of the study was not having a control group for the endocrine investigation arm of KHINES involving blood tests. Ethics approval was obtained for studying the mod/sev TBI group but not for mild TBI or controls. These groups did however have an assessment of their cortisol status and integrity of the HPA axis feedback using salivary cortisol sampling.

Although height SDS was used for all analyses, parental adjusted height SDS would have been another useful outcome measure. As accurate parental heights were not available for most KHINES participants this was not possible.

Spontaneous GH secretion can also show marked night to night variation (-50% to +100% change in night GH secretion) in the same individual [356]. Considering the effect of sleep on GH secretion this is probably not unexpected. Although overnight testing conditions were kept as much as possible the same, it is not possible to exclude without EEG monitoring differences in sleep quality and their effect in individual GH secretion.

The final limitation to consider for this study is the number of participants that could not have an MRI for research purposes due to strict safety criteria (mainly metal plates from neurosurgery). Most of these participants had had brain surgery and were more likely to have suffered more severe brain damage. It is therefore possible that the chronic neuroimaging brain changes of TBI could be underestimated as some of the most severe TBI were excluded.

4.6 FUTURE PERSPECTIVES AND POTENTIAL STUDIES

KHINES has succeeded in its aim to determine the long term prevalence of PTHP after childhood TBI and has raised a number of further questions. These could be the theme of further studies and research papers that may include the following themes.

4.6.1 Methods for diagnosing PTHP

By using strict diagnostic criteria KHINES has shown that PTHP is less frequent than has been previously reported. The ITT at its current implementation may not be suitable for diagnosing hypopituitarism in conditions where the *a priori* likelihood of pituitary disease is low as with PTHP. While the hypothesis that other GH stimulation tests may have similar limitations remains to be tested, we would recommend that in the absence of multiple hormone deficiencies and in order to facilitate prospective studies the diagnosis of PTHP is supported by at least two abnormal dynamic tests (or modified dynamic test with frequent sampling) and IGF1 levels lower than -2 SD and patients Link to prospective clinical scores.

4.6.2 Identification of predictors of neuroendocrine test eligibility

Considering the controversy of using dynamic GH endocrine tests in diagnosing PTHP and the practical difficulties of evaluating spontaneous GH secretion in a clinical setting, novel neuroimaging modalities can provide additional support in identifying TBI survivors that are at risk of developing PTHP. DWI has been used to indirectly assess the hormone secreting capacity of the pituitary in TBI patients [357] and very recently DTI has been successfully used to visualise the hypothalamo-hypophyseal tract preoperatively, reducing the risk of tract injury [358]. The same modality could potentially be used in TBI patients to assess the integrity of the same tract with prospective follow-up of clinical outcomes and to identify those most at risk of PTHP.

4.6.3 Efficacy of GH treatment in TBI survivors with PTHP

TBI survivors with PTHP show a similar benefit from GH replacement in terms of improved HRQL when compared to GHD patients due to pituitary adenomas. However, a randomised placebo controlled study evaluating the efficacy of GH therapy in patients with chronic fatigue and low spontaneous GH levels, showed no significant improvement in HRQL. It is not known if TBI survivors with fatigue and abnormal GH parameters using would respond to GH treatment in the same way.

4.6.4 Role of GH treatment on brain repair after TBI

There is mounting evidence about the neuroprotective effects of GH but more studies are necessary in order to understand the role of GH in these processes. A prospective randomised study comparing clinical outcomes after GH treatment in both PTHP and non-PTHP TBI patients would help to evaluate the contribution of GH treatment with regards to speed of recovery and rehabilitation.

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