The endocrine and metabolic factors influencing neuroinflammation and recovery following traumatic brain injury

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Abstract

Background: Traumatic brain injury (TBI) is a major cause of psychological and cognitive disability in young adults in the developed world. The prognosis for these patients is uncertain. Neuroinflammation may be an important underlying mechanism. Growth hormone deficiency (GHD) is a recognised consequence of TBI and may influence recovery. The metabolic syndrome, a state of insulin resistance, may also influence outcome from TBI.

Objectives: i) To study the prevalence and consequences of pituitary dysfunction in soldiers who have suffered a blast TBI (bTBI) ii) To investigate the effect of GH deficiency and serum IGF-I levels on recovery from TBI iii)To evaluate a new positron emission tomography (PET) radioligand [^{18}F]GE-180 purported to measure TSPO neuroinflammation in the healthy human brain iv) To quantify neuroinflammation using [^{18}F]GE-180 in patient following TBI and correlate with metabolic factors.

Methods: i) Cross-sectional comparative study of 19 soldiers with bTBI vs. 39 civilians with non-blast TBI. Full endocrine testing, neuropsychological testing, diffusion tensor imaging (DTI) ii) Longitudinal study of 39 patients following TBI; IGF-I and GHD testing at baseline, DTI and neuropsychological testing at two study visits designed to be one year apart. Intervention study of 10 patients with GHD pre- and post- 1 year of GH replacement therapy iii) Cross-sectional PET study using [^{18}F]GE-180 in 10 healthy volunteers iv) Longitudinal [^{18}F]GE-180 PET study in 12 patients following TBI: MRI, PET, neuropsychological and metabolic testing at two visits 6 months apart.

Results: i) Higher prevalence of pituitary dysfunction in bTBI (31.6%) compared to nbTBI (2.6%), P =0.004 ii) Greater improvement in fractional anisotropy (FA) in patients with a higher IGF-I at baseline, in the splenium of the corpus callosum (SPCC) and in logical memory scores; no effect of GH replacement *per se* seen on WM recovery or memory, but significant improvement in QoL and depression scores iii) Low brain uptake of [^{18}F]GE-180 in healthy volunteers, two compartmental 4K-fix (2TC) model provided best fit of the data, no effect of TSPO polymorphism seen iv)

no significant effect of genotype seen in TBI patients or in outcome measures between patients and controls.

Conclusions

i) Novel finding of greater prevalence of pituitary dysfunction in patients with bTBI ii) IGF-I, irrespective of the presence of GHD, improves WM recovery in the SPCC and memory iii) GH replacement does not influence cognition or brain structure in this study but did improve quality of life iv) The TSPO ligand [^{18}F]GE-180 appears to be limited by poor brain uptake v) the 2TC-fix model provides the best model fit iv) distribution volumes are low and there appears to be no effect of the TSPO polymorphism on PET outcome measures in patients with TBI and controls.

Statement of Publications

The results from Chapter 3 have been published in:

Pituitary Dysfunction after Blast Traumatic Brain Injury: UK BIOSAP Study. D Baxter, D Sharp, **C Feeney**, D Papadopoulou, Timothy E Ham, S Jilka, P Hellyer M Patel, A Bennett, A Mistlin, E McGilloway, M Midwinter, A Goldstone. *Annals of Neurology* June 2013. PMID 23794460

The results from Chapter 6 have been published in the European Journal of Nuclear Medicine and Molecular Imaging:

Kinetic analysis of the translocator protein positron emission tomography ligand [^{18}F]GE-180 in the human brain. Claire Feeney*, Gregory Scott*, Joel Raffel, S Roberts, Christopher Coello, Amy Jolly, Graham Searle, AP Goldstone, David J Brooks, Richard S Nicholas, William Trigg, Roger N Gunn, David J Sharp. (*=joint first author). Eur J Nucl Med Mol Imaging. June 2016. PMID: 27349244**.**

The results from Chapter 4 are under review at Annals of Neurology from July 2016:

Association of serum IGF-I with white matter, neuropsychological and cognitive recovery following traumatic brain injury. Feeney C, Sharp DJ, Hellyer PJ, J H Cole, Scott G, Baxter D, Jilka S, Ham T, Leech R , Midwinter M, Goldstone AP

Other Publications during PhD:

Seeds of Neuroendocrine Doubt.
C Feeney, G Scott, J Cole, M Sastre, AP Goldstone, R Leech. *Nature* July 2016. doi:10.1038/nature18602

Prevalence and correlates of Vitamin D deficiency in Adults after Traumatic Brain Injury. Omer A Jamall, **Claire Feeney,** AP Goldstone *et al*. Clinical Endocrinology Feb 2016. PMID: 26921561

Using Nonechoplanar Diffusion-weighted MRI to Assess Treatment Response in Active Graves Orbitopathy: A Novel Approach with 2 Case Reports. Ritchie AE, Lee V**, Feeney C**, Lingham RK. *Ophthal Plast Reconstr Surg*. Aug 2014. PMID: 25141074

Abstracts (during PhD)

Kinetic analysis of the translocator protein positron emission tomography ligand [18F]GE-180 in the healthy human brain. C Feeney, G Scott *et al* Society for Neuroscience Annual Meeting 2015, Chicago

Tract-based spatial statistics: a voxel-wise approach to longitudinal analysis of diffusion tensor imaging (DTI) in traumatic brain injury. C Feeney *et al.* Presented at the Society for Neuroscience Annual Meeting, San Diego Nov 2013**.**

High prevalence of pituitary dysfunction in soldiers after blast traumatic brain injury: Blast Injury Outcome Study in Armed forces Personnel (BIOSAP). C Feeney, D Baxter, DJ Sharp, AP Goldstone *et al*. 15th Clinicopathological Pituitary Conference (CPC), Royal College of Physicians Feb 2013
Awarded 1st Poster Prize

Statement of Originality

Patient recruitment was primarily from the multidisciplinary TBI clinic at St Mary's Hospital, the Defence Medical Research Council (DMRC) and Richard Greenwood's clinic at Homerton Hospital. Patient recruitment for content in chapters 3 and 4 was carried out by Dr Tim Ham, Dr David Baxter, Prof Sharp, Dr Goldstone and myself towards the later stage of recruitment. Patient recruitment for content in Chapters 6 and 7 was led my myself with help where necessary from members of the research team.

Radiographers at the Robert Steiner Unit and the Clinical Imaging Facility (CIF) carried out the MRI and PET scanning. Physician supervision and PET tracer administration was carried out my myself. Antonio de Marvo and Tim Dawes inserted the arterial lines in return for a professional fee. Amy Jolly contributed to the collection of cognitive data and helped me run the study. Scanning paradigms and cognitive tests were developed with the help of Dr Adam Hampshire.

The analysis of all the data was carried out by myself with assistance from P Hellyer, G Scott, R Leech and supervisors where necessary. Pipeline software developed in Imanova was used for the PET analyses. Roger Gunn, Graham Searle and Christopher Coelho developed this software and helped with our analyses and interpretation.

All other work in this thesis is my own and conforms to the rules and guidelines set out for PhD theses by Imperial College London. The work of others is appropriately referenced.

Copyright Declaration

List of Abbreviations

AIS	Abbreviated Injury Score
ADLs	Activities of daily living
ACTH	Adrenocorticotropic hormone
FLIRT	FMRIB's Linear Image Registration Tool
AIC	Akaike information criterion
AD	Alzheimer's disease
ARCM	American Congress of Rehabilitation Medicine
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
APAs	Antipituitary antibodies
APOE	Apolipoprotein
ASL	Arterial Spin Labelling
BDI-II	Beck Depression Inventory II
BP_{ND}	Non displaceable binding potential
bTBI	Blast traumatic brain injury
BBB	Blood brain barrier
BET	Brain extraction
CRP	C-reactive protein
CRT	Choice reaction time
CM	Compartmental modelling
CT	Computed tomography
CGM	Cortical grey matter
CDC	Cortisol day curve
DI	Diabetes insipidus
DAI	Diffuse axonal injury
DTI	Diffusion tensor imaging
DVR	Distributed volume ratio
DEXA	Dual-energy X-ray absorptiometry
ECG	Electrocardiogram
FBP	Filtered back projection

FDG	Fludeoxyglucose
FSL	FMRIB Software Library
FSH	Follicle-stimulating hormone
FA	Fractional anisotropy
FAI	Free androgen index
FFA	Free fatty acids
GLM	General linear model
GCS	Glasgow coma scale
GST	Glucagon stimulation test
GLP-1	Glucagon-like peptide-1
GCP	Good Clinical Practice
GH	Growth hormone
GHD	Growth hormone deficiency
HABs	High affinity binders
HDF	High fat diet
HPLC	High performance liquid chromatography
HOMA	Homeostatic Model Assessment Score
IED	Improvised explosive device
ISS	Injury severity score
IR	insulin resistance
ITT	Insulin tolerance test
IGF-I	Insulin-like growth factor 1
IDF	International Diabetes Federation
IM	Intramuscular
LABs	Low affinity binders
LDL	Low-density lipoprotein cholesterol
LH	Lutenising hormone
MRI	Magnetic resonance imaging
MRS	Magnetic Resonance Spectroscopy
MD	Mean diffusivity
MetS	Metabolic Syndrome
MST	Metyrapone stimulation test

MABs	Mixed affinity binders
MIAKAT	Molecular Imaging And Kinetic Analysis Toolbox
NICE	National Institute for Health and Care Excellence
nbTBI	Non-blast TBI
NHP	Nottingham Health Profile
NMR	Nuclear Magnetic Resonance
1TC	One tissue compartmental model (2 rate constants)
POB	Plasma over blood ratio
PTFE	Polytetrafluoroethylene
PET	Positron emission tomography
PTA	Post traumatic amnesia
PTSD	Post-traumatic stress disorder
PLIC	Posterior limb of the internal capsule
QoL	Quality of Life
RF	Radiofrequency
ROI	Region of interest
RTA	Road Traffic Accident
SHBG	Sex hormone binding globulin
SF-36	Short Form 36 survey
SRTM	Simplified reference tissue model
SPCC	Splenium of corpus callosum
SOP	Standard operating procedure
SUVR	Standardised uptake value ratio
SUV	Standardised uptake value
SPSS	Statistical Package for the Social Sciences
SPM	Statistical parametric mapping
SCID	Structured clinical interview
SAH	Subarachnoid haemorrhage
SWI	Susceptibility weighted imaging
AGHDA-QoL	The Quality of Life Assessment of Growth Hormone Deficiency in Adults
TSH	Thyroid-stimulating hormone

TAC	Time activity curve
TBSS	Tract based spatial statistics
TSPO	Translocator protein
TBI	Traumatic brain injury
2TC	Two tissue compartmental model (4 rate constants)
VT	Volume of distribution
WM	White matter
WHO	World Health Organisation

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1 Introduction

1.1 Background

1.1.1 Definition of traumatic brain injury (TBI)

The most recent definition of TBI was formulated by the Woking Group on Demographics and Clinical Assessment of the International Interagency Initiative toward Common Data Elements for Research in TBI and Psychological Health in 2010 (Menon, Schwab, Wright, & Maas, 2010). Their definition is as follows:

"An alteration in brain function or other evidence of brain pathology caused by an external force"

Other definitions of TBI have been proposed from the American Congress of Rehabilitation Medicine (ARCM) (Medicine, 1993) and the WHO Task Force (Carroll, Cassidy, Holm, Kraus, & Coronado, 2004), however these definitions have been criticised for focussing on mild TBI and for not acknowledging blast injuries as an important cause of TBI (Roozenbeek, Maas, & Menon, 2013).

Hence the list of external forces defined in the Common Data Elements definition now include: 'the head being struck; the head striking an object; the brain undergoing an acceleration-deceleration movement without direct external trauma to the head; a foreign body penetrating the brain; forces generated from events such as a blast or explosion; and other force yet to be defined."

An "*alteration in brain function*" is defined as 1 of the following clinical signs:

i) Any period of loss of or a decreased consciousness level.
ii) Any loss of memory for events immediately before (retrograde amnesia) or after the injury (PTA).

iii) Neurologic deficits (weakness, loss of balance, change in vision, dyspraxia, paresis/plegia, sensory loss, aphasia)

iv) Any alteration in mental state at the time of the injury (confusion, disorientation, slowed thinking).

The definition recognises that factors other than TBI may be responsible for alterations in mental state at the time of the injury (e.g. alcohol/recreational drug use, medication, pain and post-traumatic shock) but this should not preclude a diagnosis of TBI (Menon et al., 2010).

1.1.2 Epidemiology

TBI is global public health problem and is a major cause of death and lifelong disability in those who survive. There are approximately 5.3 million people in the USA who live with a disability related to TBI (Langlois & Sattin, 2005) and approximately 7 million individuals in the European Union (Brazinova et al., 2015). This accounts for approximately 15% of the burden of death and disability worldwide. The World Health Organisation (WHO) projects this to rise to 20% in 2020 (Finfer & Cohen, 2001).

The incidence of TBI (all severity) hospitalisations in England in 2002 was 229 per 100,000 (Tennant, 2005) which is similar to the incidence of stroke in European populations (Carolei et al., 1997). The incidence was found to vary considerably with age and geography. 31% were aged between 0-15 yrs.; 56% were aged 16-74 and 13% were aged 75 yrs. and over. The highest incidence was found in Liverpool (419 per 100,000) and the lowest in Brent and Harrow (91 per 100,000) (Tennant, 2005).

Regarding the incidence of TBI by severity, a multicentre study in Scotland found that 90% of their TBI admissions were classified as ‘mild’ (GCS 13-15), 5% ‘moderate’ (GCS 9-12) and 3% ‘severe’ (GCS 3-8). However, initial severity was not related to disability at 1 year follow-up, and occurred in nearly half of each severity group (mild 47%, moderate 45%, severe 48%) (Thornhill et al., 2000).

1.1.3 Aetiology

Data from the International Mission on Prognosis and Analysis of Clinical Trials in TBI (IMPACT) database gives important aetiological information. Road traffic accidents (RTA) are the leading cause of all TBI accounting for 64.1% of cases, followed by falls (19.3%), 'other' (6.9%), assault (5.1%), work-related (2.6%) and sports/recreation (2.0%). RTA included all accidents on the street, including those in motor vehicles, on bicycles and as pedestrians and the group 'falls' included those that had taken place in a domestic setting as well as those under the influence of alcohol (Butcher et al., 2007). Falls were associated with a poorer outcome but this was explained by the older age of the patients.

Age and gender are other important aetiological factors. TBI is most common in very young children (aged 0-4 yrs.) and in adolescence and young adulthood (age 15-24 yrs.) with a subsequent peak in incidence in older adults i.e. over 65 yrs. of age (Thornhill et al., 2000; Thurman, Branche, & Sniezek, 1998). Older people have the highest rates of TBI-related hospitalisations and deaths . Male to female ratio in all subgroups of TBI is approximately 3:1 and mortality rates and complications tend to be greater in men compared to women (Berry et al., 2009).

Alcohol consumption is strongly associated with TBI occurrence. Several studies across Europe have reported alcohol intoxication to be a factor in 25-50% of TBI cases and the association is stronger in males versus females (Tagliaferri, Compagnone, Korsic, Servadei, & Kraus, 2006).

Sports-related injuries account for a significant proportion of TBI with approximately 300,000 of these types of injuries in the United States each year (Thurman et al., 1998). Blast TBI (bTBI) from improvised explosive devices (IEDs) also contribute significantly to the global burden of TBI. Estimates of bTBI from the Iraq and Afghanistan conflicts have been as high as 320,000 US military personnel (Tanielian T, 2012).

1.1.4 Clinical consequences and economic impact

TBI is a heterogeneous condition that can lead to a wide variety of clinical consequences; ranging from death or coma to disabling neurological, cognitive and psychiatric symptoms. Pituitary dysfunction is also an important clinical consequence that will be discussed later.

In survivors of TBI, disability is reported in 50% at one year with similar proportions at 5-7 years after TBI and 12-14 years (same cohort) (McMillan, Teasdale, & Stewart, 2012; Thornhill et al., 2000; Whitnall, McMillan, Murray, & Teasdale, 2006). The presence of reported disability at one year is a particularly bad prognostic factor with 80% either deceased or disabled at 12-14 years. These three observational studies also provide evidence for change in disability over time with between one quarter and a third reporting an improvement in disability over time.

Neurological consequences (particularly pertaining to moderate and severe TBI) include post-traumatic epilepsy, parkinsonism, Dementia of the Alzheimer's type, Dementia pugilistica (in boxing only), Benign Paroxysmal Positional Vertigo (BPPV), anosmia and ocular/visual motor deterioration (Bazarian, Cernak, Noble-Haeusslein, Potolicchio, & Temkin, 2009).

In patients who may otherwise have a full neurological they may still suffer from disabling cognitive symptoms. Executive functions such reasoning, mental flexibility, concept formation and self-monitoring are particularly vulnerable in TBI due to the high prevalence of frontal lobe injury and can have a devastating effect on job performance activities of daily living (ADLs) (McDonald, Flashman, & Saykin, 2002). Impairments in short-term memory and speed of information processing are also commonly reported following TBI (Draper & Ponsford, 2008; Kinnunen et al., 2011; Scheid, Walther, Guthke, Preul, & von Cramon, 2006).

Patients can also experience troubling neuropsychiatric symptoms following a TBI. Symptoms of anxiety and depression are common along with personality changes that include disinhibition, aggression and apathy (Arnould, Rochat, Azouvi, & Van der Linden, 2015; Fleminger, 2008). Post-traumatic stress disorder (PTSD), substance abuse and even psychosis can all be attributed to a TBI (Bryant et al., 2010). A multicentre study (n=592) found that 6 years after a severe injury 28% of patients met criteria for at least 1 psychiatric disorder, the most common being a major depressive episode (11%) followed by substance use disorder (9%), PTSD (6%) and generalised anxiety disorder (6%) (O'Donnell et al., 2016).

Hypopituitarism is also a recognised phenomenon following TBI. Initial studies reported prevalence rates of up to 90% but more recent studies using robust testing approaches and stricter inclusion criteria report prevalence rates of 5-10% (Klose et al., 2014; Kokshoorn et al., 2011). Growth hormone deficiency is the most commonly reported deficiency, followed by ACTH and gonadotrophin deficiencies. TSH deficiency is very unusual and permanent diabetes insipidus is also rare (Capatina, Paluzzi, Mitchell, & Karavitaki, 2015; Kokshoorn et al., 2011). Untreated post-traumatic hypopituitarism is associated with impaired lipid profile, unfavourable body composition and decreased quality of life one year after injury (Klose, Watt, Brennum, & Feldt-Rasmussen, 2007). Growth hormone deficiency in particular is associated with impaired cognition that is partially reversible with GH replacement (High et al., 2010).

The economic impact of TBI is considerable. The total direct and indirect costs of TBI in 2010 in the UK was approximately 5.5 billion Euros (Fineberg et al., 2013) and in Europe 64 billion Euros (Gustavsson et al., 2011). Data from the Trauma Audit Research Network estimate that the mean cost of hospital stay per patient with TBI was £15,462 and this figure varied by severity of injury, associated injuries, patients aged 45-64 years, length of stay in critical care and overall length of stay(Morris, Ridley, Lecky, Munro, & Christensen, 2008).

1.1.5 Pharmacotherapies in post-acute TBI

Since this thesis deals with the post-acute (i.e. >6 weeks from injury) sequelae from TBI, I shall briefly review here the medical therapies used to treat the most common symptoms in the post-acute phase of a TBI namely; memory and attention impairment, sleep disturbance, headache and depression. (Bhatnagar, Iaccarino, & Zafonte, 2016). The evidence base for cognitive rehabilitation and other non-medical interventions will not be reviewed here. Pituitary dysfunction and its treatment will be reviewed separately on page 39.

1.1.6 Sleep disturbance

The sleep-wake cycle is commonly disturbed following a TBI. A meta-analysis reported that 25-29% of patients have a diagnosed sleep disorder (insomnia, hypersomnia, apnoea) and 50% report some form of sleep disturbance (Mathias & Alvaro, 2012). Whilst the diagnosis and treatment of apnoea is a separate entity, the management of sleep disturbance in general is challenging. Sleep hygiene counselling can help as can cognitive behavioural therapy.

Melatonin is a hormone produced by the pineal gland that is involved in the circadian regulation of sleep-wakefulness. Patients with TBI have significantly lower levels of evening melatonin (Shekleton et al., 2010) and in a single cross-over study, patients reported improved day-time alertness on melatonin, and increased sleep duration on amitriptyline (Kemp, Biswas, Neumann, & Coughlan, 2004). Modafinil has been used in the treatment of daytime somnolence and has shown some efficacy at higher doses (250mg) (Menn, Yang, & Lankford, 2014). The mechanism of action is not clear.

Other drugs such as benzodiazepines, hypnotics, trazadone and prazosin have theoretical applications to sleep disturbance following TBI, although their use has not been well-studied in TBI populations and may be limited by side-effects such as impaired cognition and drowsiness.

1.1.7 Headache

Headaches are very common following a TBI and can take various forms i.e. cervicogenic, migraine-like, tension-type and neuralgic (related to the greater or lesser occipital nerves) (A. W. Brown et al., 2015). Several underlying mechanisms for the development of headache following TBI have been proposed including changes in cerebral blood flow, decrease in glucose metabolism and disturbances in release of inhibitory neurotransmitters (Solomon, 2009).

Treatment of headache following TBI is similar to treatment of headache more generally. Symptomatic treatment includes NSAIDs, paracetamol, triptan medication and antiemetic medications if necessary. To avoid headache induction by medication over-use, these treatments should be restricted to 2-3 days of the week or 10 days in month (Bhatnagar et al., 2016). Headache prophylaxis in the form of beta-blockers, antiepileptic and antidepressant agents may also be warranted. Post-traumatic headache may also be treated with occipital nerve blocks and onabotulinum toxin A has shown some efficacy in mild bTBI (Yerry, Kuehn, & Finkel, 2015).

1.1.8 Impaired information processing

Deficits in information processing and attention are common consequences following a TBI (Draper & Ponsford, 2008). Dopamine, noradrenaline and acetylcholine have all been implicated in attentional neural pathways and have all been targets for therapeutic intervention. Methylphenidate is a noradrenaline and dopamine reuptake inhibitor and is the most commonly studied therapy in this domain. Several studies have reported benefit in processing speed following TBI but mixed results for other cognitive domains (McAllister et al., 2016; Sivan, Neumann, Kent, Stroud, & Bhakta, 2010; Whyte et al., 2004; Willmott & Ponsford, 2009). Studies to evaluate the effect of bromocriptine, atomoxetine, amantadine on information processing speed have shown no effect (Ripley et al., 2014; Sivan et al., 2010; Whyte et al., 2008), while donepezil and lisdexamfetamine dimesylate (a neurostimulant) have demonstrated a positive effect, although more studies are

needed (Tramontana, Cowan, Zald, Prokop, & Guillamondegui, 2014; Whyte et al., 2004).

1.1.9 Memory

Targeted pharmacotherapy to improve memory is not commonplace in the TBI clinic. Animal studies have reported mixed effects of donepezil on memory following a TBI (Shaw et al., 2013; Yu, Kim, & Kernie, 2015). In humans, a small trial and case series of donepezil following TBI improved measures of memory (Trovato, Slomine, Pidcock, & Christensen, 2006; Zhang, Plotkin, Wang, Sandel, & Lee, 2004) and a multicentre placebo controlled study to evaluate memory remediation following TBI is actively recruiting at present. Rivastigmine, an agent used in AD, has shown little promise in memory impairment following TBI (Silver et al., 2009; Tenovuo, Alin, & Helenius, 2009).

1.1.10 Depression

There is a 30% risk of developing depression for up to 5 years following TBI and severity of injury does not seem to be a particular risk factor (Guillamondegui et al., 2011; Osborn, Mathias, & Fairweather-Schmidt, 2014). Suicide is 3-4 times more common than in the general population (Gordon et al., 2006). Selective serotonin reuptake inhibitors e.g. citalopram and sertralline are considered first line treatment for depression following TBI based on limited but supportive evidence in the literature (Ashman et al., 2009; Rapoport et al., 2008; Rapoport et al., 2010). In the absence of TBI-specific studies, the management of depression beyond first-line treatment is akin to the general management of depression outside of TBI.

1.2 Pathophysiology of TBI

There are a variety of different types of cellular injury following TBI that can broadly be classified into focal injuries (contusions, intraparenchymal haematomas, subdural and epidural haematomas). Traumatic subarachnoid haemorrhage (SAH) can also be the result of focal damage. Diffuse axonal injury (DAI) results predominantly from rapid acceleration-deceleration of the head and is described in more detail below.

1.2.1 Focal injuries

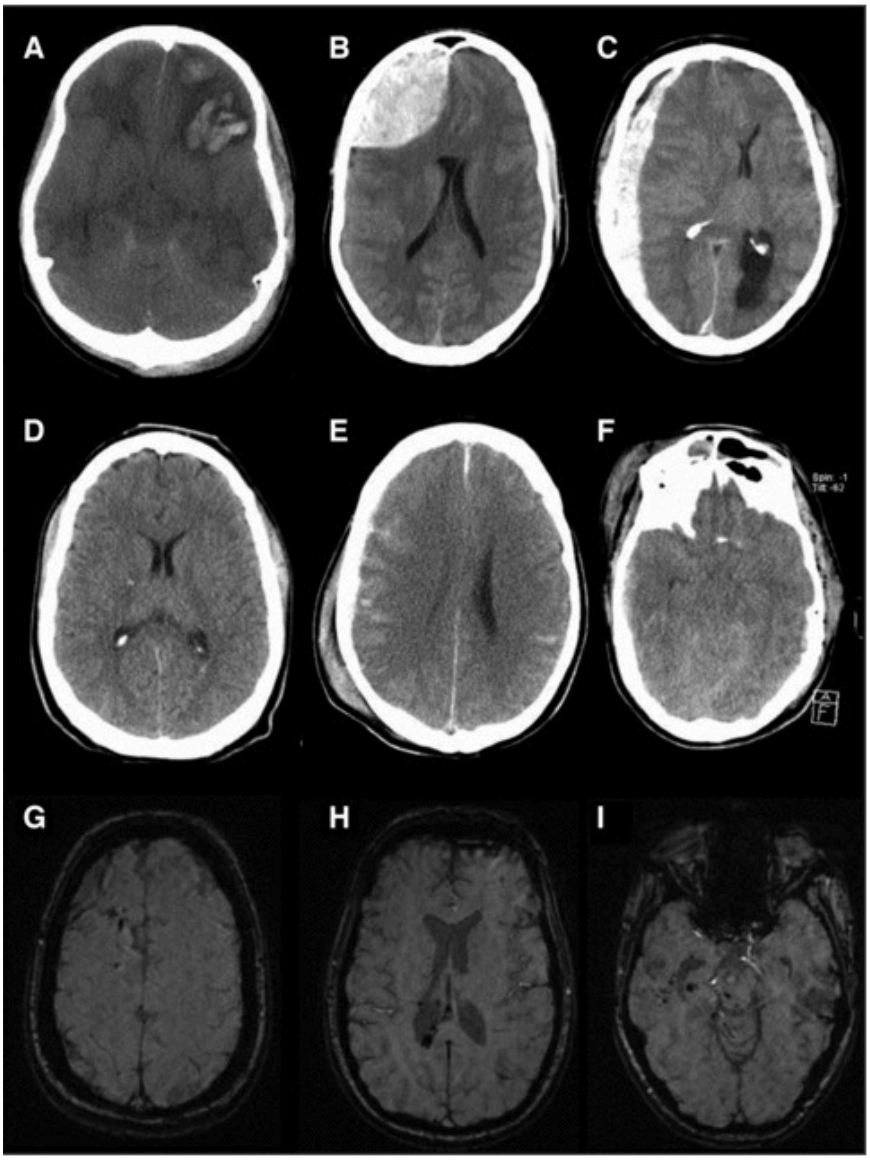
Focal brain damage results from collision forces acting on the skull, causing compression of the tissue under the cranium. Damage can occur at the site of impact (coup) or opposite to site of impact (contre-coup) (Pudenz & Shelden, 1946). The most common type of focal brain injury is a contusion. These are localised areas of damage that result from the primary traumatic insult followed by the activation of several cellular pathways (dysregulation of cerebral blood flow, reduction in nitric oxide, accumulation of lactic acid, oedema and glutamate release), ultimately leading to neuronal apoptosis (Werner & Engelhard, 2007).

1.2.2 Diffuse injuries

Diffuse brain injury consists of widespread damage to axons, diffuse vascular injury (causing microbleeds), oedema formation and hypoxic-ischaemic injury. It is thought to result from rapid acceleration and deceleration forces, typically resulting from high speed RTAs (Gennarelli, 1983). When subjected to such forces, brain segments move at variable rates causing additional shear and compressive forces within the brain parenchyma (Gentry, Godersky, & Thompson, 1988). Axonal injury was first reported by Strich in 1956 and described as the diffuse degeneration of the white matter of the cerebral hemispheres (Strich, 1956). Later the term ‘diffuse axonal injury’ was defined as its own entity with three grades of severity relating to lobar injury only (grade 1), additional injuries in the corpus callosum (grade 2) and further

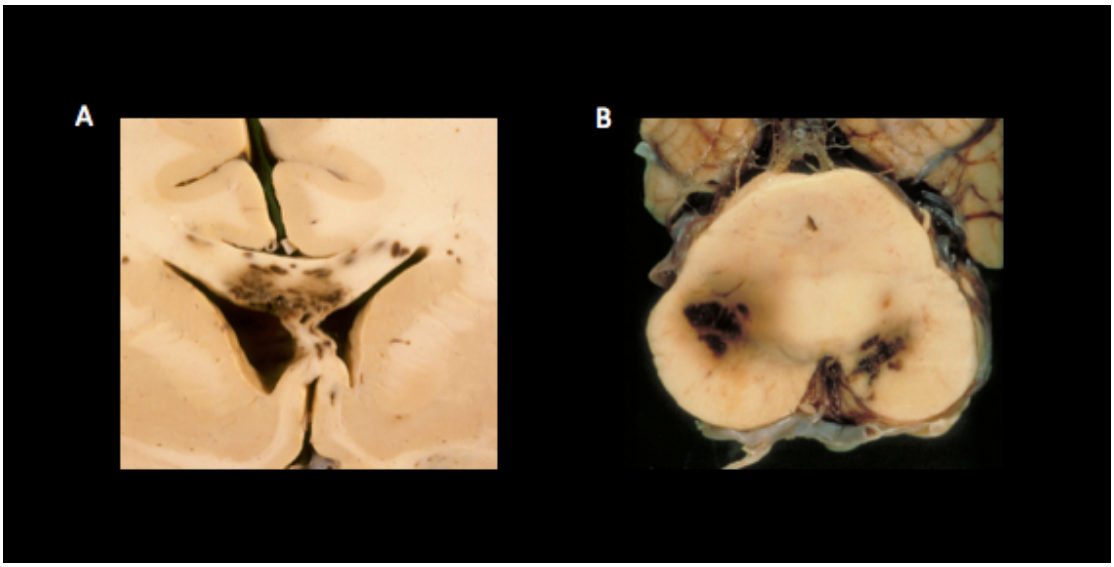
additional injuries in the brainstem (grade 3) (Adams et al., 1989). Examples of focal and diffuse injuries are depicted in **Figure 1-1** and the neuropathological features of DAI in **Figure 1-2** .

Figure 1-1 Focal and diffuse TBI. Examples of focal and diffuse TBI on CT (rows 1 and 2) and MRI (row 3).



Focal injury: (A) Left frontal contusion with midline shift to the right and compression of the lateral ventricles. (B) Right frontal epidural haematoma with midline shift to the left and compression of the anterior part of the lateral ventricle. (C) Right frontotemporoparietal subdural haematoma with a midline shift to the left. Diffuse injury: (D) Punctate haemorrhage within the right posterior limb of the internal capsule, a sign of DAI; (E) Diffuse SAH; (F) Diffuse swelling with bilateral compression of the basal cisterns. DAI on MRI: Susceptibility weighted images of one patient revealing punctate haemorrhages (hypo-intense foci) within (G) the right frontal hemisphere, (H) Splenium of the corpus callosum, and (I) mesencephalon, corresponding to grade 3 DAI. Reproduced with permission from (Andriessen, Jacobs, & Vos, 2010)

Figure 1-2 Typical neuropathological appearance of diffuse axonal injury.



A) Grade 2 DAI: lesions in corpus callosum and B) Grade 3 DAI: lesions also in brain stem (right image). Reproduced with permission from (Adams et al., 1989)

1.3 Structural Imaging Techniques in TBI

This section of the Introduction will describe the principles of the structural imaging techniques used in this thesis.

1.3.1 Principles of Magnetic Resonance Imaging (MRI)

MRI provides detailed images of tissues using the principle of nuclear magnetic resonance (NMR). NMR occurs when atomic nuclei are absorbed and re-emit electromagnetic radio waves when placed in a magnetic field. The specific resonance frequency depends on the strength of the magnetic field and the properties of the atomic isotope. Only isotopes that contain an odd number of protons have the ability to produce radio waves under magnetic conditions.

The isotope of choice in MRI is hydrogen as this is abundant through the body but varies in content in different tissues. MRI allows differentiation of tissues based on hydrogen content (e.g. grey and white matter in the brain).

1.3.2 Summary of how MRI images are generated.

Hydrogen nuclei with lone protons carry angular momentum or 'spin' as it is commonly referred to. When a strong magnetic field (B_0) is applied to these nuclei, they reorder themselves with respect to the $B_{0,}$ either parallel or anti-parallel to it ('low energy' or 'high energy' atoms respectively). The stronger the magnetic field then the greater the number of parallel atoms. Once aligned, these protons precess around the B_0 axis.

When excitatory radiofrequency pulses are applied to the aligned nuclei, they will 'flip' from low-energy to high-energy states. The 'flip angle' is the degree of displacement of protons from their original position and the net magnetisation vector created by the RF pulse. Increasing the strength or duration of the excitatory RF pulses will increase the flip angle. In MRI, the source of RF pulses is an electromagnetic coil around the magnetic field.

When the RF pulse ceases, then the hydrogen atoms return to their low-energy state and signal is emitted which is dependent on the flip angle, B_0, and atomic density. A receiver coil around the tissue of interest is used to detect this electromagnetic signal. Two principal components of this signal are the T1 and the T2 relaxation times. The T1 relaxation time is the time taken for the magnetisation vector to return to the z-axis and the T2 (or transverse) relaxation time is the rate that the xy component of the magnetisation vector decays. Manipulation of the magnetic field can be applied to manipulate these relaxation times. T2* accelerates the transverse decay and this can be useful for some MRI images.

1.3.3 T1 weighted-images

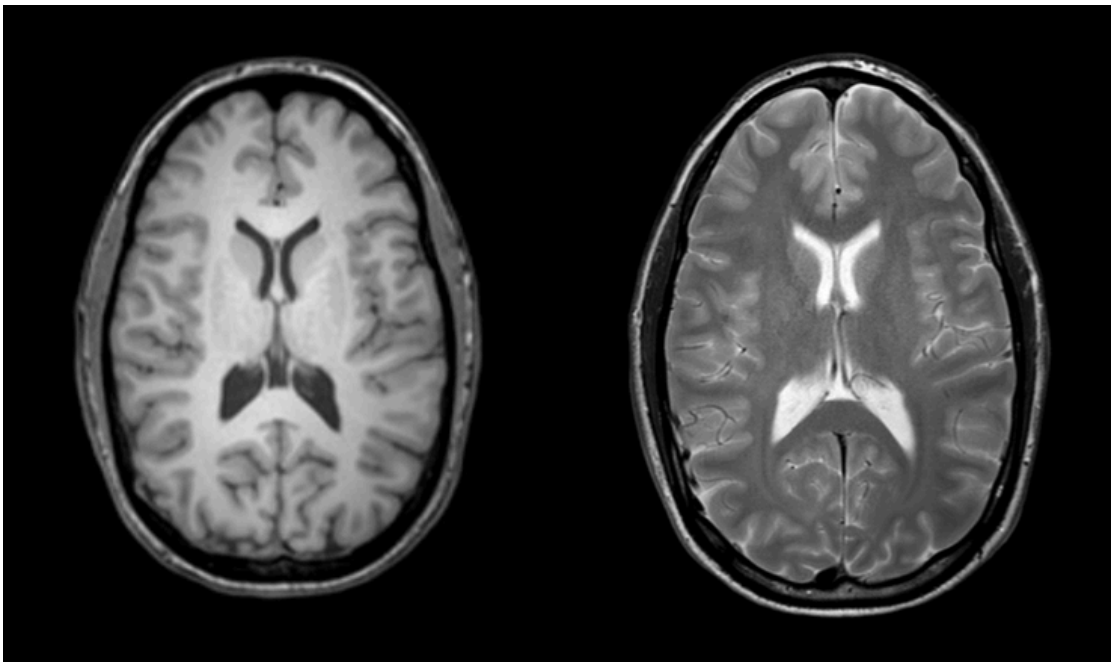
These image reconstructions use the T1 relaxation time to make contrasts between tissues. Grey matter has a significantly longer T1 time than white matter and

therefore T1-weighted images provide good anatomical information and contrast between grey and white matter.

1.3.4 T2 weighted-images

These images are produced using the T2 or transverse relaxation times. Grey and white matter is less differentiated this way because the T2 relaxation times are not so different. However fat and water do have differences in the T2 time and therefore is useful to assess pathological states such as oedema. **Figure 1-3** shows axial slices of a healthy brain to demonstrate contrast differences between T1 and T2 weighted images.

Figure 1-3 MR axial images of a healthy brain. T1 weighted (left), T2 weighted (right)

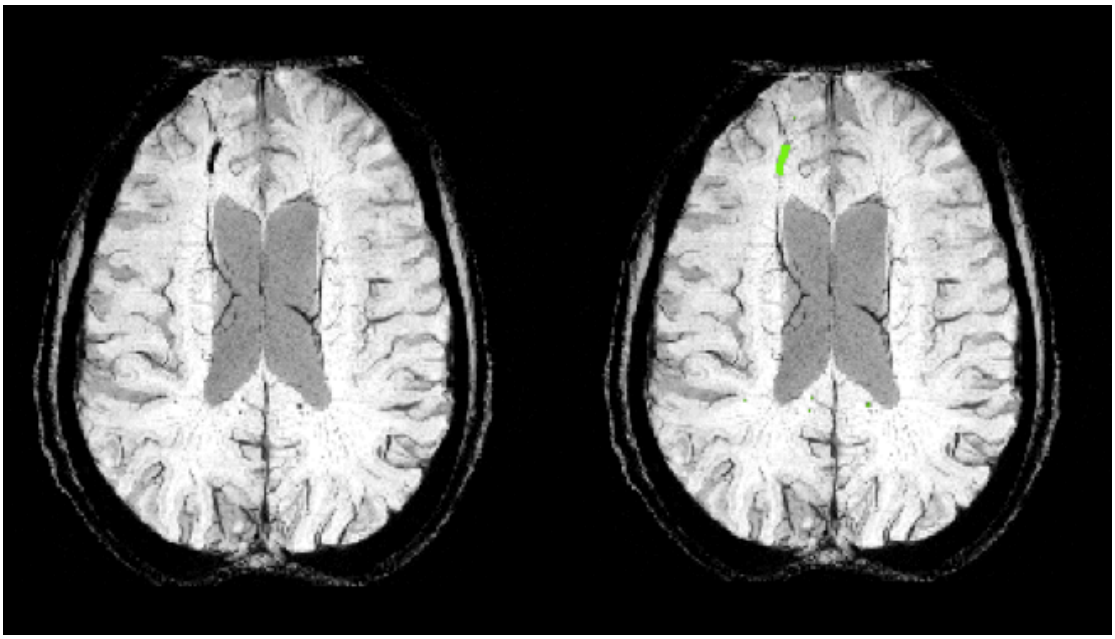


1.3.5 Susceptibility-weighted images (SWI)

These images are constructed differently to T1/T2 and T2*. SWI uses a special gradient-recalled echo pulse sequence that takes advantage of the magnetic

susceptibility differences between tissues. It is sensitive to venous blood, iron and haemorrhage in the brain (Haacke, Xu, Cheng, & Reichenbach, 2004) and is an effective technique to identify microhaemorrhages from TBI (Barnes & Haacke, 2009). An example of a microbleed visible on a SWI image is depicted in **Figure 1-4**.

Figure 1-4 Single microbleed (overlaid in green) visible on SWI imaging in a patient following TBI



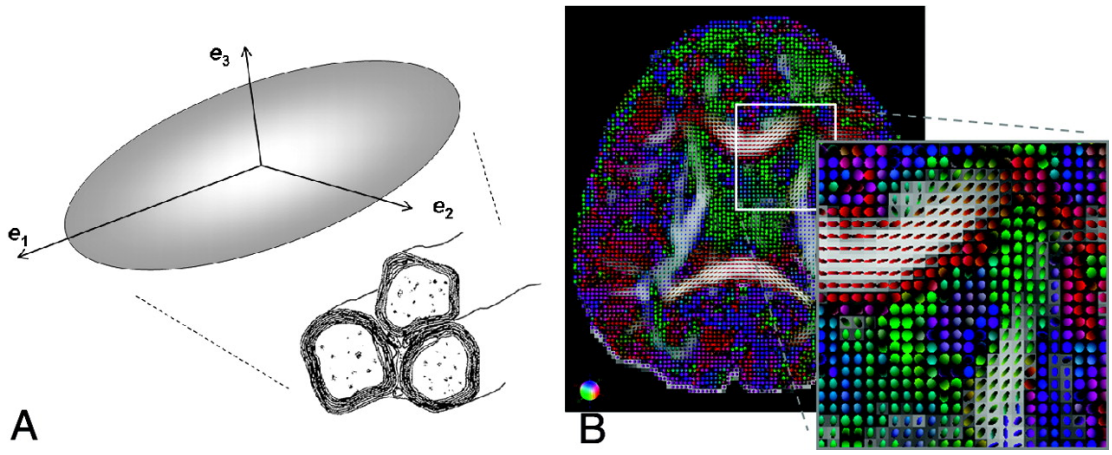
1.3.6 Diffusion tensor imaging (DTI)

DTI is a unique MR technique that measures water diffusion in the brain (Basser & Jones, 2002) and is usually performed in research settings. In tissues where there is no restriction or limited restriction to water diffusion e.g. cerebrospinal fluid (CSF), then water molecules diffuse in a random fashion. This type of diffusion is called isotropic. However, in a white matter fibre, water will preferentially diffuse along the white matter tract (where there is less restriction to movement) rather than across it. This type of diffusion is called anisotropic.

DTI can tell us about the type of water diffusion in brain tissue and this information can be useful. In brief, the application of multiple gradient fields while acquiring MR

data, followed by application of a tensor model results in the generation of an ellipsoid tensor at each voxel **Figure 1-5.**

Figure 1-5 Schematic of the diffusion tensor ellipsoid

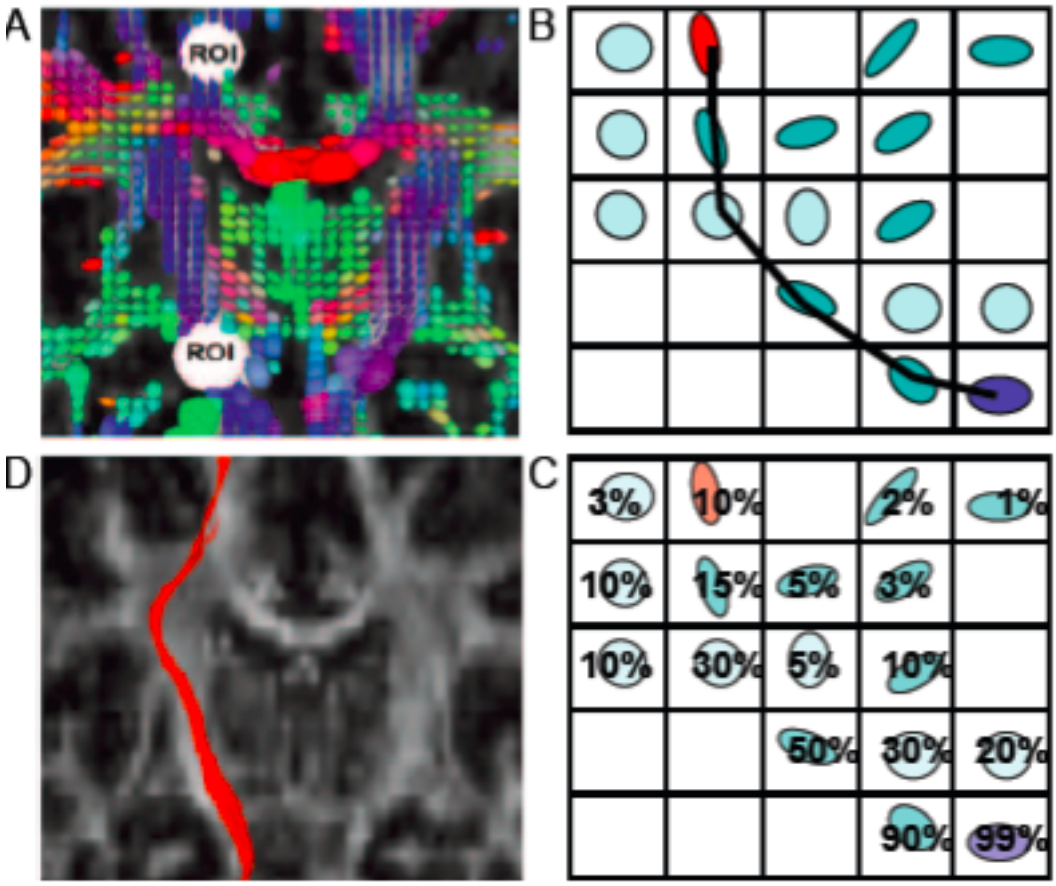


A, Schematic drawing showing the relationship between the diffusion tensor ellipsoid the underlying white matter fibre bundle. The direction of the longest axis of the ellipsoid, mathematically called the principal eigenvector of the diffusion tensor (e1), corresponds to the major fibre direction where water molecules exhibit the fastest diffusion due to lack of a barrier. B, The diffusion tensor ellipsoid can be obtained on a voxel-by-voxel basis. Reproduced with permission from (Chung, Chou, & Chen, 2011)

The ellipsoid eigenvalues vary across different cell types in the brain and this can give important information regarding the underlying structures. For example, in CSF, the eigenvalues are large as there is predominantly isotropic diffusion with little to constrain the movement of water molecules. There is more constraint of water molecules in the grey matter, but eigenvalues are relatively equal, suggesting that there is less anisotropic diffusion but no net direction of water diffusion within voxels. White matter which is made up of bundles of axons does have high constraint of water molecules along them and therefore DTI is a useful modality to study white matter in particular.

There are two principal ways in which DTI can be useful. Tractography is a technique which uses the eigenvectors to map out pathways of white matter fibres in the brain **(Figure 1-6)**

Figure 1-6 Principles of tractography



Diffusion ellipsoid map with two regions of interest. B) Schematic representation of diffusion tensors in a 5x5 grid, with seed ROI in dark blue and termination ROI in red. The black line represents the propagation of the deterministic tractography streamline in the direction of principal eigenvector. C) Probabilistic tractography produces a likelihood map of the diffusion path between two ROIs. Rather than delineating a single best path, the likelihood map shows the probability that a particle diffusing between ROIs traverses each voxel. D) Resulting tract connecting the two ROIs. Adapted with permission from (Nucifora, Verma, Lee, & Melhem, 2007)

Eigenvalues and eigenvectors can also be used to generate diffusivity measures that can describe the direction and quantity of diffusion within an individual voxel (Basser & Pierpaoli, 1996). Fractional Anisotropy (FA) describes the overall constraint of water diffusion within a voxel and is represented by the equation here:

$$FA = \sqrt{1/2}\,\frac{\sqrt{((\lambda_1, - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2)}}{\sqrt{\lambda_1{}^2 + \lambda_2{}^2 + \lambda_3{}^2}}$$

FA values close to 1 suggest that diffusion is highly anisotrophic (constrained) and values close to 0 imply that diffusion is isotropic. Mean diffusivity (MD) describes the overall amount of water diffusion in a molecule. When MD is low there is little water movement and when it is high there is high diffusivity of water. MD does not however tell us about the directionality of water diffusion. Axial (AD) and radial diffusivity (RD) are the other diffusivity measures that represent the degree of water restriction in the axial and radial planes of the ellipsoid:

$$\text{Radial diffusivity} = \tfrac{1}{2}\,(\lambda_1 + \lambda_3)$$

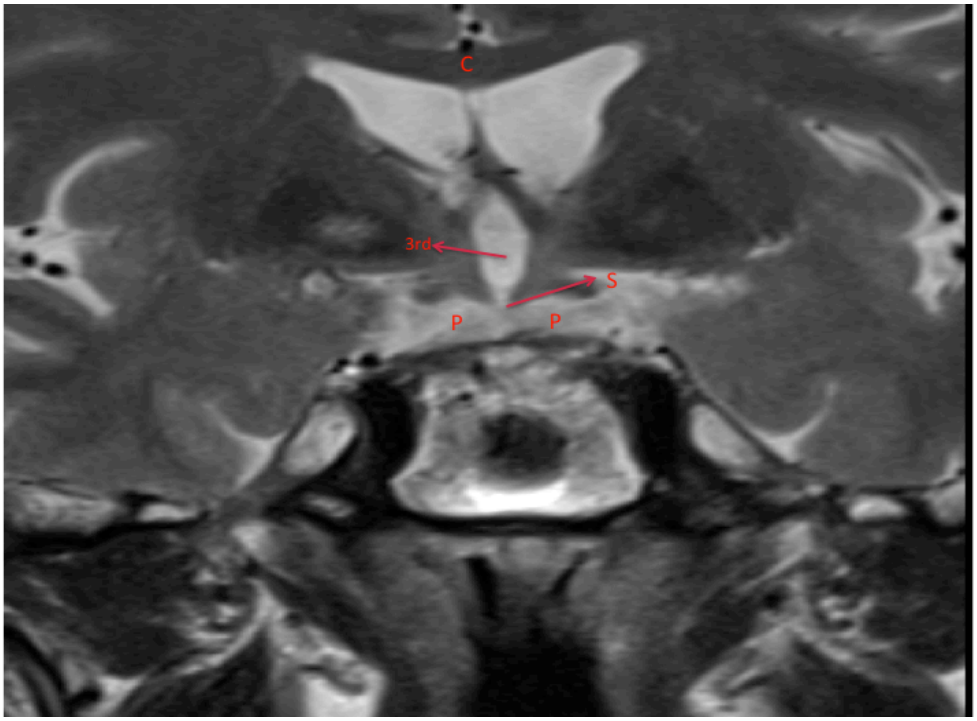
$$\text{Axial Diffusivity} = \lambda_1$$

In white matter, FA is generally thought to reflect loss of white matter integrity either reflecting damage to myelin or axon membrane, reduced axonal packing density or reduced axonal coherence. There may be limitations to the biological interpretation of DTI, however, it is still considered a useful imaging modality for investigation of white matter pathology in stroke, multiple sclerosis (MS), Alzheimer's (AD) and psychiatric disorders as well as TBI (Assaf & Pasternak, 2008; Kinnunen et al., 2011; Kou et al., 2010).

1.4 Pituitary dysfunction following TBI

The pituitary gland is a small endocrine gland, weighing approximately 0.5 g that sits in the sella turcica at the base of the brain as is connected to the hypothalamus via the pituitary stalk (**Figure 1-7**) The gland itself is composed of two embryologically and functionally distinct structures; the adenohypophysis (anterior pituitary) and the neurohypophysis (posterior pituitary). The anterior pituitary gland releases 6 principal hormones and the posterior pituitary secretes 2 (**Table 1-1**).

Figure 1-7 T2 MRI coronal pituitary view with gadolinium enhancement



(P=pituitary, s=pituitary stalk, c=corpus callosum, 3rd=third ventricle)

Table 1-1 Anterior and posterior pituitary hormones and their downstream effectors

Anterior pituitary gland	Cell type	Principal target	Principal downstream mediator	Effect
Growth hormone (GH)	Somatotroph	Liver	Insulin-like growth factor (IGF-I)	Growth/metabolism
Prolactin (PRL)	Lactotroph	Mammary glands	n/a	Lactation
Adrenocorticotropic hormone (ACTH)	Corticotroph	Adrenal cortex	Cortisol	Secretion of stress hormones
Thyroid stimulating hormone (TSH)	Thyrotroph	Thyroid gland	Thyroxine (T4)	Energy metabolism
Follicle stimulating hormone (FSH)	Gonadotroph	Ovaries/testes	Oestrogen /testosterone	Regulation of reproduction
Lutenising hormone (LH)	Gonadotroph	Ovaries/testes	Progesterone/ testosterone	Production of sex hormones
Posterior pituitary gland				
Antidiuretic hormone (ADH)	n/a	Kidneys/arterioles	n/a	Water retention and blood pressure
Oxytocin	n/a	Uterus/mammary glands	n/a	Uterine contractions and lactation

Pituitary dysfunction secondary to TBI was considered rare prior to 2000. It was first described in 1918, but only became more widely appreciated until two prevalence studies were published in 2000 (Benvenga, Campenni, Ruggeri, & Trimarchi, 2000; E, 1918; Lieberman, Oberoi, Gilkison, Masel, & Urban, 2001). Pituitary dysfunction in the context of TBI refers to hypofunction of the pituitary gland (usually anterior) i.e. hypopituitarism or panhypopituitarism, or abnormal prolactin measurements. Excess anterior pituitary hormone production is not described following TBI.

Here I describe the prevalence, consequences and treatment of pituitary dysfunction following TBI. Please note that additional introductory material on this subject is provided at the beginning of Chapters 3, 4, and 5.

1.4.1 Prevalence of chronic post-traumatic anterior pituitary dysfunction following TBI

The first systematic review on the prevalence of pituitary dysfunction following TBI suggested that this condition was common (27.5%) and even more so following SAH (47%) (Schneider, Kreitschmann-Andermahr, Ghigo, Stalla, & Agha, 2007). However more recent studies have reported much lower prevalence rates (Kokshoorn et al., 2011). There are several reasons that could explain this variability including the choice of dynamic endocrine tests and cut-offs particularly for growth hormone deficiency (GHD), use of confirmatory testing (Klose et al., 2007; Kokshoorn et al., 2010), time since TBI to endocrine testing and severity of injury. A recent systematic review attempts to control for these factors (Tanriverdi et al., 2015). 1203 patients were analysed across 14 studies. 28% were found to have at least one hormone deficiency and 6% were found to have multiple deficiencies. The frequencies of hypopituitarism following TBI are displayed in **Table 1-2**.

Table 1-2 Frequencies of anterior hypopituitarism in the chronic phase following TBI.

First Author, Year	N	GH	LH/FSH	ACTH	TSH	Hypopituitarism	Multiple Deficiencies
Bondanelli, 2004	50	4	7	0	5	14	6
Aimaretti, 2005	70	14	8	4	5	16	7
Agha, 2004	102	18	12	23	1	29	6
Popovic, 2004	67	10	6	5	3	23	7
Agha, 2005	48	5	6	9	1	ND	ND
Schnieder, 2006	70	7	14	6	2	25	3
Tanriverdi, 2006	52	17	4	10	3	26	5
Klose, 2007	104	18	2	5	2	18	6
Bushnik, 2007	64	23	7	39	12	58	ND
Bavisetty, 2008	70	11	7	0	0	15	5
Kleindienst, 2009	23	9	0	11	0	ND	ND
Srinivasan, 2009	18	4	0	9	4	10	5
Van der Eerden, 2010	107	1	7	6	1	15	0
Kokshoorn, 2011	112	3	1	2	ND	6	0
Leal-Cerro, 2005	170	6	29	11	10	42	15
Hermann, 2006	76	6	13	2	2	18	5
Total TBI, n	1203	156	123	142	51	315	70
Total TBI, %	100%	13.0%	10.2%	11.8%	4.2%	27.8%	6.2%
Total TBI all GCS, n	957	144	81	129	39	255	50
Total TBI all GCS, %	100%	15.0%	8.5%	13.5%	4.1%	28.8%	5.6%
Total TBI GCS ≤8, n	246	12	42	13	12	60	20
Total TBI GCS ≤8, %	100%	4.9%	17.1%	5.3%	4.9%	24.4%	8.1%

Abbreviations: ND=not defined. Adapted from (Tanriverdi et al., 2015)

However, when using an additional confirmatory test at least three months after a TBI, 15% were found to have any hormone deficiency and 4% had multiple hormone deficiencies (n=313). Consistent with other studies, GHD was the most common deficit (9%), followed by ACTH (6%), LH/FSH (5%) and TSH (1%). Frequencies of hypopituitarism in the chronic phase (at least 3 months after TBI) and studies only with confirmatory tests are shown in **Table 1-3**.

Table 1-3 Frequencies of hypopituitarism in the chronic phase (at least 3 months after TBI) and studies only with confirmatory tests

First Author, Year (Ref)	N	GH	LH/FSH	ACTH	TSH	Hypopituitarism	Multiple Deficiencies
Pathological screening tests							
Agha, 2004	102	18	12	23	1	29	6
Klose, 2007	104	18	2	5	2	18	6
Van der Eerden, 2010	107	1	7	6	1	15	0
Total N	313	37	21	34	4	62	12
Total %	100%	11.8%	6.7%	10.9%	1.3%	19.8%	3.8%
Pathological confirmatory tests							
Agha, 2004	102	11	12	13	1	29	6
Klose, 2007	104	16	2	5	2	16	6
Van der Eerden, 2010	107	0	0	1	0	1	0
Total N	313	27	14	19	3	46	12
Total %	100%	8.6%	4.5%	6.1%	1.0%	14.7%	3.8%

Adapted from (Tanriverdi et al., 2015)

1.4.2 Prevalence of pituitary dysfunction in the multidisciplinary TBI clinic at Imperial Healthcare NHS Trust

A weekly multidisciplinary TBI clinic was set up in 2009 to provide neurological, endocrine, cognitive and psychological support to patients in the chronic phase following a TBI. From April 2012 to March 2016 I have assisted Dr Tony Goldstone in providing the neuroendocrine care for these patients. I have seen approximately 470 new and follow-up patients in this clinic. Our preference has been to perform two dynamic tests of pituitary function before confirming the diagnosis of either GH or ACTH deficiency.

In our cohort of TBI patients seen at Imperial College Healthcare Trust between 2009 and 2012, 93 out of 248 (37.5%) had a sub-optimal GH response to glucagon testing (peak GH <5 mcg/L). However the final prevalence of GHD was only 7.4% (13/176 patients) defined through inadequate GH response during 2 dynamic tests: glucagon

test followed by GHRH-Arginine test. We found the glucagon test alone to have a relatively poor specificity and sensitivity in the diagnosis of GHD after TBI when compared to the GHRH-Arginine test (using age- and BMI- adjusted cut-offs). At <5 mcg/L GH cut-off in the glucagon test sensitivity was 86.7% and specificity 14.3%, while at <3 mcg/L sensitivity was 66.7% and specificity 60.0% (n=49) (Zaw-Linn J, Feeney C, Goldstone AP. BSc Project 2013).

1.4.3 Proposed pathophysiological mechanisms for developing TBI-induced chronic hypopituitarism

It is not completely clear how post-traumatic hypopituitarism develops following TBI; although several hypotheses have been proposed including direct damage to the pituitary, hypothalamus or stalk; vascular damage to the long hypophyseal vessels; autoimmunity and genetic factors.

Supporting a vascular hypothesis, in one study, 80% of patients with traumatic hypopituitarism had at least one radiological abnormality of the sella (including loss of volume or empty sella, signal inhomogeneities, perfusion deficit, and lack of neurohypophyseal signal) compared to 29% of those without hypopituitarism (P=0.032) (Schneider, Samann, et al., 2007). In the acute phase following a moderate or severe TBI, another study found that 30% of patients had pituitary gland enlargement or evidence of haemorrhage/infarction or signal abnormalities on MRI (Maiya et al., 2008). The commonly observed pattern of GH, followed by gonadotrophin deficiencies following TBI may also be explained by the somatotrophs and gonadotrophs being located in the area of the pituitary perfused by the long hypophyseal vessels, although this is purely speculative.

Autoimmunity has also been proposed as a potential mechanism. Pituitary antibodies may develop after blood-brain barrier (BBB) disruption allows leakage of pituitary proteins into the circulation which in turn induces an immune response (Papa et al., 2012; Tanriverdi et al., 2010). One study has reported a prevalence of 45% of antipituitary antibodies (APAs) following TBI (compared to 0% in controls)

and this was associated with the development of hypopituitarism (Tanriverdi, De Bellis, et al., 2008). This finding has also been replicated in boxers who have been exposed to repetitive head trauma (Tanriverdi et al., 2010).

Genetic factors have also been implicated as contributory mechanisms. Apolipoprotein (APOE) is a key protein which has a role in neuronal repair and is heavily expressed in the hypothalamo-pituitary area (Nishida, Yoshioka, & St-Amand, 2005). The E4 allele is thought to be a strong risk factor for the development of Alzheimer's disease (AD) (Welsh-Bohmer, Gearing, Saunders, Roses, & Mirra, 1997), but the E3 allele can inhibit the neuroinflammatory response (Laskowitz et al., 2001). A preliminary study has found pituitary dysfunction was significantly lower in those with APOE E3/E3 genotype (17.7% in those with the E3 allele vs. 42% without P=0.01) (Tanriverdi, Taheri, et al., 2008).

1.4.4 Clinical consequences of TBI-induced chronic hypopituitarism

The clinical consequences of post-traumatic hypopituitarism can be divided into metabolic (body fat composition, body mass index (BMI), hypertension, glucose metabolism, lipid profile, bone metabolism) and cognitive/psychological. These shall be addressed in turn.

Metabolic consequences

Outside of TBI, untreated hypopituitarism can lead to a number of metabolic abnormalities. Growth hormone deficiency in particular induces truncal obesity, reduces lean body mass and bone mass that reverse on treatment (Mukherjee, Murray, & Shalet, 2004). GHD also contributes to an adverse cardiovascular risk profile, including dyslipidaemia, hypertension and increased mortality from cardiovascular causes (Verhelst & Abs, 2009). Untreated hypogonadism i.e. testosterone deficiency can other induce a averse metabolic profile as well as thyroid and ACTH deficiencies (although the latter two deficiencies are usually immediately treated, leaving less scope for metabolic abnormalities to develop).

TBI patients who developed hypopituitarism following injury were more insulin resistant, had worse lipid profile and had altered glucose metabolism, that was not explained by an increase in BMI (Prodam et al., 2013). Another study also confirmed higher waist circumference, total fat mass and low-density lipoprotein-cholesterol (LDL) in patients with hypopituitarism at 12 month follow-up after TBI (Klose et al., 2007).

Cognitive/psychological consequences

There are several targets for GH and its downstream mediator IGF-I throughout the brain (Lobie et al., 1993). GH and IGF-I are thought to have neuroprotective effects and influence cognitive domains relating to learning and memory (Nyberg & Hallberg, 2013). The influence of GHD and GH replacement will be discussed in more detail in the introductory sections of Chapter 4 and 5 respectively. In gonadotrophin deficiency, testosterone replacement has been shown to have positive effect on spatial and verbal memory; an effect also seen in healthy older males and those with AD who are not hypogonadal (Cherrier, 2009; Cherrier et al., 2001; Cherrier, Craft, & Matsumoto, 2003). The effect of ACTH/cortisol/thyroxine/prolactin (Cherrier, 2009; Cherrier et al., 2001; Cherrier et al., 2003) on cognition is not clearly delineated.

1.4.5 Diagnostic tests and treatment of TBI-induced chronic hypopituitarism

Diagnosis

The diagnosis of thyroid, prolactin, LH, FSH abnormalities is generally straightforward and can be diagnosed on single or multiple serum samples. However the diagnosis of ACTH and GH deficiency is more complicated due to the pulsatile release and diurnal profile of these hormones. For this reason, dynamic function tests are required whereby the pituitary is stimulated by some means (e.g. hypoglycaemic stress, glucagon) and measurements of cortisol and GH taken thereafter. These measurements are then analysed against cut-off thresholds to determine whether ACTH or GH deficiency is present. Examples of these dynamic function tests include the insulin tolerance test (ITT) and the glucagon stimulation test (GST). Diagnostic testing for hypopituitarism following TBI will be explained in more detail in Chapter 3.

Treatments

The most commonly diagnosed pituitary hormone abnormality following TBI is GHD and criteria for starting recombinant GH replacement is generally governed by guidelines produced by the National Institute for Health and Care Excellence (NICE). There are six manufacturers that produce somatrophin and is usually administered once a day, subcutaneously, via a pen device. The current NICE guideline recommends that treatment should only be considered in those with severe GHD (i.e. peak GH <9 mU/L on an ITT or another reliable test) and impaired quality of life (<11/25 on the Assessment of GH Deficiency in Adults questionnaire (AGHDA) (QoL-AGHDA)). NICE also suggests that QoL should be reassessed at 9 months and if it has not improved by 7 points then consideration should be given to stopping treatment (guideline, 2003). In the case of multiple hormone deficiencies it is generally accepted practice to replace other hormones prior to GH and reassess QoL thereafter. In the case of GHD following TBI it is not recommended to start GH less than a year after injury, in case of resolution of the condition. The effect of GH replacement on cognition and recovery from TBI will be discussed in Chapter 5.

TSH deficiency following TBI is extremely rare (Tanriverdi et al., 2015). Indeed in our cohort of patients at Imperial Healthcare NHS Trust, we have not diagnosed a single case since 2009. However if present, thyroxine replacement should be implemented in the standard manner. ACTH deficiency, once confirmed, necessitates prompt replacement with replacement doses of glucocorticoid (usually hydrocortisone 20mg in divided doses per day), including education on doubling dosage in intercurrent illness and seeking prompt medical attention in case of vomiting. Usually patients carry a steroid card or a medialert bracelet. Testosterone replacement in males will depend on a variety of factors including reported sexual symptoms and reduced bone mineral density. When instigated, replacement can take various forms. Our preference is to initiate 3 monthly depot injections. There are limited data available to draw conclusions about the effect of ACTH/FSH/LH and TSH deficiencies following TBI.

1.5 Neuroinflammation following TBI

The second half of this thesis (Chapters 6 and 7) addresses a different theme; neuroinflammation following TBI. I shall describe here why neuroinflammation in TBI is an important area of research and how metabolic factors may map onto this.

1.5.1 Definition

Inflammation in general terms is the body's response to injury. Outside of the CNS it is easier to describe where blood borne cytokine and chemokines facilitate recruitment of monocytes, neutrophils and macrophages to the site of injury with the goal of repairing injurious damage to the body. Within the CNS, this traditional role has been extended to include the activation of microglia and astroglia. These brain glial cells are both sources and targets of inflammatory mediators and their activation is a central response to a variety of CNS injury; including trauma, infection, drugs, and various disease processes (O'Callaghan, Sriram, & Miller, 2008). Given that microglial are the dominant immune cell and can be studied with positron-emission tomography, this thesis will focus on microglial activation following TBI.

1.5.2 The role of microglia in mediating the neuroinflammatory response

Microglia are the brain's resident macrophages (representing up to 10% of the total cell population of the brain) and are key orchestrators in the brain's inflammatory processes. Microglia are thought to be mobile and have a highly branched morphology allowing constant surveillance of their environment (Morganti-Kossmann, Satgunaseelan, Bye, & Kossmann, 2007). In their quiescent state, they may exert a role in synaptic pruning. When microglia become activated in response to injury, they rapidly change their morphology and upregulate a number of cell-surface and intracellular antigens (Finnie, 2013).

In their activated state, microglia release a variety of pro-inflammatory cytokines including IL-1B and TNF-alpha which can cause damage by the production of superoxide free radicals (Koshinaga et al., 2000). However they can also serve neuroprotective functions e.g. clearing toxic debris by phagocytosis, release of trophic factors and recruiting new stem cells to the site of injury (Colton, 2009)

The ability of microglia to exert both beneficial and harmful effects is due to their ability to adopt different phenotypes under different conditions. The M1, or 'classically activated' phenotype is pro-inflammatory and destructive, marked by production of high levels of TNF-alpha, interferon gamma, IL-1B, IL-12 and low levels of IL-10. Conversely, the M2 or 'alternatively activated' phenotype releases anti-inflammatory cytokines such as IL-4, TGF-beta and IL-10 and promotes tissue remodelling and angiogenesis (Nimmerjahn, Kirchhoff, & Helmchen, 2005). It is well recognised however that the M1/M2 dichotomy of microglial differentiation is likely to be an oversimplification and instead represent 2 extremes of phenotypic variation in vivo.

How exactly microglia can alter their phenotype following CNS injury is dependent on a variety of different factors within the neural microenvironment, including variation of microglial populations within the cortex, variation in neurotransmitter signalling and differences in complement production and leucocyte infiltration (Nimmerjahn et al., 2005)

Increasing evidence suggest that microglia can also be influenced by signals outside the CNS. Systemic inflammation, for example, can invoke microglial activation through signalling pathways that are not completely understood (Perry, Cunningham, & Holmes, 2007).

1.5.3 Neuropathological evidence for chronic microglial activation following TBI

Pathological markers for microglial activation (CD68, CR3/43) appear to be present peri-lesionally following an acute TBI but there is also evidence for a long-term microglial response that may be harmful (Gentleman et al., 2004; Johnson et al., 2013; Shitaka et al., 2011). In one study of head-injured patients who survived at least a year following their head injury, CD68 immunoreactivity was greater in both parasagittal and hippocampal white matter up to 4 years following injury and CR3/43 mmunoreactivity was greater in both parasagittal and hippocampal white matter up to 16 years following the injury. This appeared not to be influenced by age (Gentleman et al., 2004).

Another study also replicated these findings by reporting on-going pathological evidence of neuroinflammation, especially in the corpus callosum, up to 18 years after a single moderate-severe TBI in humans (Johnson et al., 2013). However they also described an association of chronic microglial activation with on-going axonal degeneration consistent with other animal models that demonstrate white matter degeneration following TBI (X. H. Chen et al., 2004; Gale, Johnson, Bigler, & Blatter, 1995).

1.5.4 Microglia in neurodegeneration

In addition to their phagocytic role (neuroprotective role), activated microglia synthesize and secrete potential neurotoxins that may cause or aggravate CNS pathology. These toxins include free radicals, nitric oxide, IL-1, IL-6, TNF α, among others (Chao, Hu, Molitor, Shaskan, & Peterson, 1992; Chao, Hu, & Peterson, 1995; Chao, Hu, Sheng, & Peterson, 1995; Giulian, Baker, Shih, & Lachman, 1986; Righi et al., 1989). For example, in AD, a classical neurodegenerative disease, microglial activation has been shown in regions associated with amyloid β deposition (Edison et al., 2008).

TBI is now considered to have a neurodegenerative component and is a risk factor for the development of dementia (D. H. Smith, Johnson, & Stewart, 2013). Neurodegeneration may be an underlying mechanism that could explain the variable recovery from TBI over the long-term (McMillan et al., 2012). Recently, amyloid pathology has been shown to correlate with axonal injury following TBI (Scott et al., 2016).

1.5.5 Therapies targeted at reducing microglial activation

Minocycline, a tetracycline antibiotic, inhibits microglial activation and has other neuroprotective effects at an experimental level. It is the most widely studied agent specifically targeting neurological conditions that have a neuroinflammatory component (Plane, Shen, Pleasure, & Deng, 2010). Human clinical studies of minocycline have had positive and negative effects on disease outcomes in stroke (Lampl et al., 2007), multiple sclerosis (Metz et al., 2009), Parkinson's disease ("A pilot clinical trial of creatine and minocycline in early Parkinson disease: 18-month results," 2008), Huntington's disease (Thomas, Ashizawa, & Jankovic, 2004). Results from a clinical trial in TBI are awaited (Scott G, Sharp DJ *et al.).*

1.5.6 The Translocator Protein (TSPO)

The 18 kDA TSPO protein (formerly known as the benzodiazepine receptor) is present in the outer mitochondrial membrane of cells and is thought to play a crucial role in cholesterol transport across cells; the first step in steroidogenesis (R. C. Brown & Papadopoulos, 2001). However this traditional view of TSPO function has recently been challenged by a number of experimental studies (for review see (Selvaraj, Stocco, & Tu, 2015). TSPO has a number of other roles related to mitochondrial function including immune modulation, apoptosis, cell proliferation, protein import, porphyrin transport, haem biosynthesis, ion transport, cellular respiration and oxidative processes (Girard et al., 2012).

There are low levels of TSPO in the healthy nervous system but are then rapidly up-regulated in microglia and astrocytes in the context of disease or injury (M. K. Chen & Guilarte, 2008; Cosenza-Nashat et al., 2009). TSPO has therefore become a target for measuring microglial activation via positron-emission tomography (PET).

1.5.7 TSPO PET Imaging

PET is a functional imaging technique where radiotracers labelled with a positron-emitter are injected into the body to measure a biological process. A positron is the antiparticle of the electron and has a positive charge. Unstable radioisotopes (i.e. chemical elements with varying number of neutrons) are able to emit positrons when they undergo decay. The radiotracer will concentrate around the receptor or metabolic process of interest.

Once a positron is emitted it will travel a short distance (1-3mm) before it collides with an electron. This collision is known as annihilation and once it occurs two gamma rays are produced at 180 degree angles to one another. These gamma rays are detected by a gamma counter which allows 3D images to be reconstructed.

A number of radioligands have been developed to measure the TSPO receptor. The first ligand developed for this purpose was [^{11}C]PK-11195 and has been used widely to measure microglial activation in AD, mild cognitive impairment, Huntington's disease, Parkinson's disease, multiple sclerosis, schizophrenia, fronto-temporal dementia, multiple system atrophy, ischaemic stroke and TBI (Ramlackhansingh et al., 2011; Schweitzer, Fallon, Mann, & Kumar, 2010).

Although [^{11}C]PK-11195 has some utility in measuring neuroinflammation in a wide variety of neurological and psychiatric disease, there are certain limitations to its use. The kinetics of the molecule are highly variable in part due to high plasma protein binding, high non-specific binding due to the lipophilicity of the molecule and low brain uptake (Ching et al., 2012). As a result of these factors, there is no standardised approach to analysis and interpretation. Practically-speaking, the half

life of carbon-11 (20.38 mins) necessitates the availability of an onsite cyclotron to manufacture the molecule and this can restrict its use increase its cost.

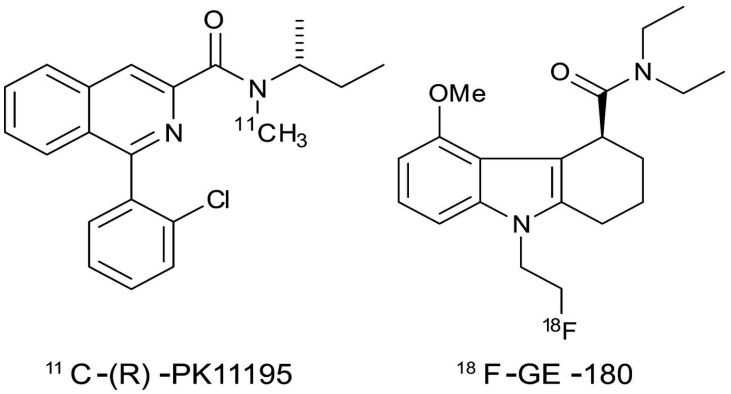
Over the last decade, approximately 20 new ligands for TSPO have been developed and evaluated in imaging studies with the hope of addressing some of the limitations outlined with [^{11}C]PK-11195 (Chauveau, Boutin, Van Camp, Dolle, & Tavitian, 2008). Several second-generation TSPO ligands including [^{11}C]PBR-28, [^{11}C]DAA1106, [^{11}C]DPA713, [^{18}F]PBR06, [^{18}F]FEPPA and [^{18}F]PBR111 with improved signal-noise ratio have been investigated in humans.

A major observation with these ligands was that there was considerable interindividual variability in their affinity for TSPO (Owen et al., 2011; Owen et al., 2010). It seemed that these ligands bound with a trimodal distribution: either high, low or mixed affinity depending upon the subject. Subsequently, it was found that a Ala147Thr polymorphism for the TSPO receptor is responsible for this trimodal distribution (Owen et al., 2012).

1.5.8 [18F]GE-180

The TSPO ligand of choice for this thesis (chapters 6 and 7) is a novel fluorinated tracer, [^{18}F]GE-180 (S-N,N-diethyl- 9-2-18F-fluoroethyl]-5-methoxy 2,3,4,9-tetrahydro-1H-carba- zole-4-carboxamide) (**Figure 1-8).** This ligand was identified as the lead compound from series of 85 tricyclic indoles all assessed for binding affinity, brain uptake and specific binding in an established neuroinflammation model (Wadsworth et al., 2012). A fluorinated molecule with a half life of 109.7 minutes has advantages over carbon-11 in terms of obviating requirement for on-site cyclotron and cost.

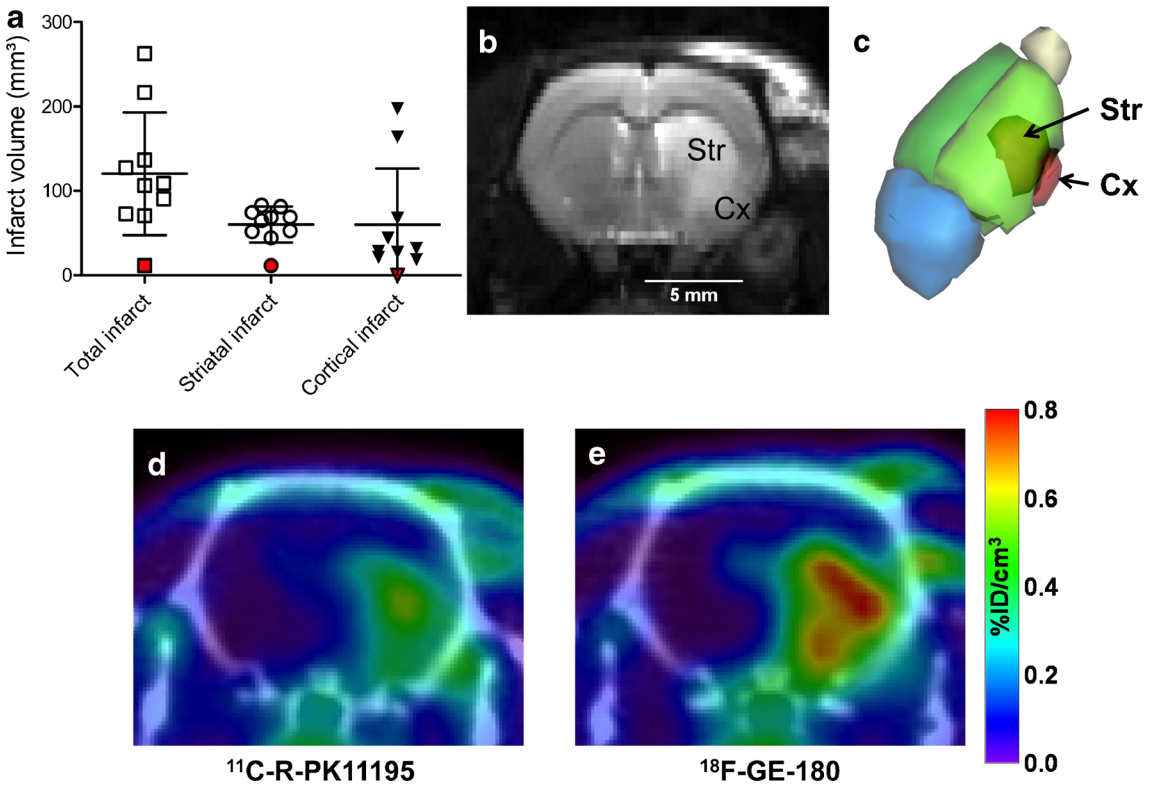
Figure 1-8 Chemical structures of [^{11}C]PK-11195 and [^{18}F]GE-180



^{11}C-(R)-PK11195 ^{18}F-GE-180

[^{18}F]GE-180 was able to identify sites of microglial activation by in vivo PET imaging that was confirmed with immunohistochemistry in an acute model of neuroinflammation and appeared to have superior binding to [^{11}C]PK-11195 (Dickens et al., 2014). This pattern was maintained in a preclinical model of stroke, where [^{18}F]GE-180 demonstrated better signal to noise ratio compared to [^{11}C]PK-11195 **Figure 1-9** (Boutin et al., 2015). Not only did [^{18}F]GE-180 have greater signal at infarct site, the level of non-specific binding in healthy tissue was lower when compared to [^{11}C]PK-11195.

Figure 1-9 [^{11}C]PK-11195 vs. [^{18}F]GE-180 in an animal mode of stroke



a) Infarct volumes (red points-excluded animals due to very small infarcts) b) Representative T2 MR image in a rat showing visible infarct (Str striatum, Cx cortex) c) 3D rendering of infarct areas d)e) coregistered quantitative PET/CT images (40-60min summed image after injection). Reproduced with permission from (Boutin et al., 2015)

One of the first clinical studies using [^{18}F]GE-180 is described in Chapter 6.

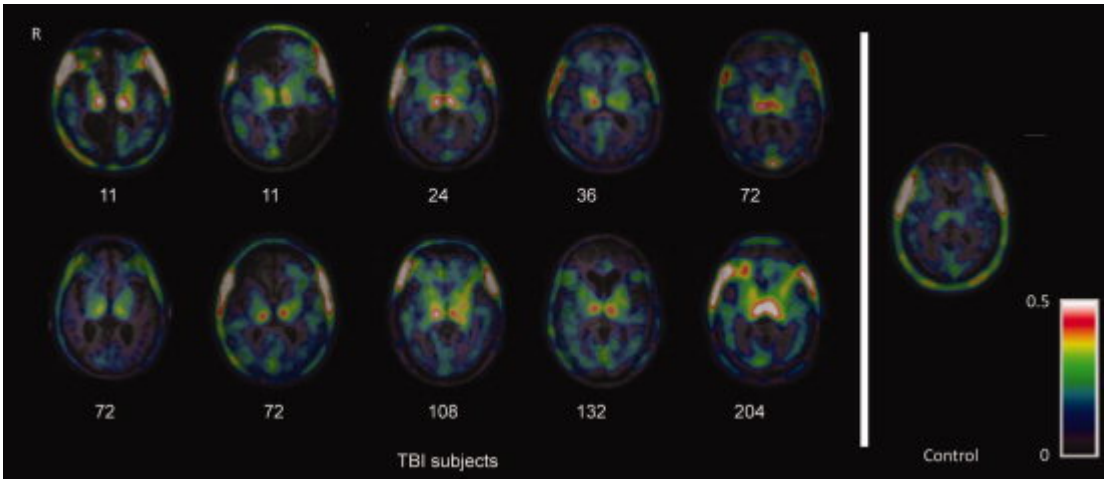
1.5.9 TSPO imaging in TBI

The role of neuroinflammation in the pathophysiology of TBI has been purported as a potential mechanism to explain the variable recovery from TBI as well as the development of de novo diseases including post-traumatic epilepsy and dementia . (Annegers, Hauser, Coan, & Rocca, 1998; Z. Guo et al., 2000; McMillan, Teasdale, Weir, & Stewart, 2011). Animal models have demonstrated that the initial inflammatory process can continue for a year following TBI, especially in the thalamus (Nagamoto-Combs, McNeal, Morecraft, & Combs, 2007). Human post-mortem studies have also shown microglial activation in long-term survivors of TBI

(Gentleman et al., 2004) and sites of activation coincide with Wallerian degeneration (Wilson et al., 2004).

In a TSPO PET study using [^{11}C]PK-11195 in ten patients at least 11 months after moderate-severe TBI, all patients had increased thalamic binding compared to controls. This effect seemed to be present even in patients who had a very long time since injury (**Figure 1-10**).

Figure 1-10 [^{11}C]PK-11195 images of TBI patients overlaid on T1 transverse images at the level of the thalamus.



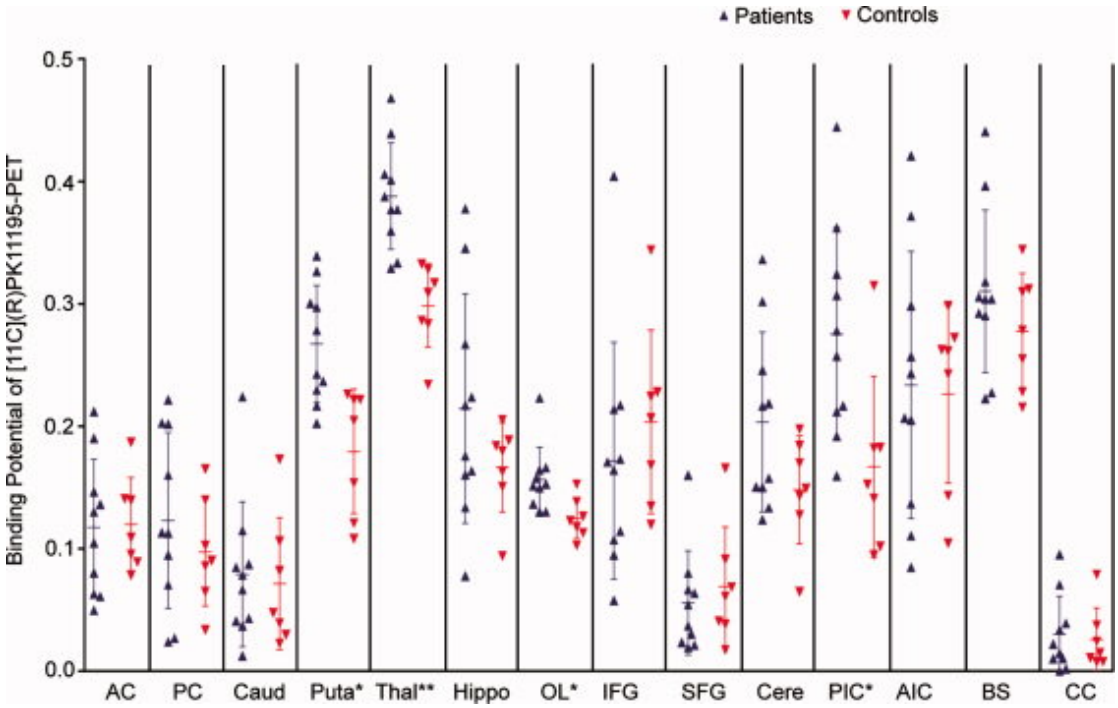
Numbers indicate time from TBI to time of PET scan. Reproduced with permission from (Ramlackhansingh et al., 2011)

[^{11}C]PK-11195 binding varied between regions with highest binding in the thalamus, putamen and posterior limb of the internal capsule (PLIC) (**Figure 1-11**). [^{11}C]PK-11195 in the thalamus was associated with greater cognitive impairment. Increased binding was not found at the sites of focal damage, but more distant subcortical areas which may reflect the dense connectivity of these structures.

In Chapters 6&7 I will be expanding on our group's TSPO research by using the tracer [^{18}F]GE-180 in patients following a TBI. I will be investigating whether the Metabolic

Syndrome (MetS) correlates with TSPO neuroinflammation and recovery following a TBI.

Figure 1-11. [11C](R)PK11195 binding in the regions of interests investigated.



*Individual subjects' binding potentials are plotted with patients in blue and control subjects in red. PET = positron emission tomography; AC = anterior cingulate; PC = posterior cingulate; Caud = caudate; Puta = putamen; Thal = thalami; Hippo = hippocampus; OL = occipital lobe; IFG = inferior frontal gyrus; SFG = superior frontal gyrus; Cere = cerebellum; PIC = posterior limb of internal capsule; AIC = anterior limb of internal capsule; BS = brainstem; CC = corpus callosum. *p < 0.05 significance; **survives Bonferroni multiple comparison correction. Reproduced with permission from (Ramlackhansingh et al., 2011)*

1.6 The Metabolic Syndrome (MetS)

1.6.1 Definition

MetS is the name given to a group of disorders , which, when occurring together, significantly increases the risk of developing cardiovascular disease and diabetes. This condition exists in approximately 20-25% of adults in the developed world (Cameron, Shaw, & Zimmet, 2004; Ford, Giles, & Dietz, 2002).

There are several definitions of MetS based on consensus statements from worldwide organisations such as the International Diabetes Federation (IDF) (Cameron et al., 2004), the World Health Organisation (WHO) (Alberti & Zimmet, 1998) and the US National Cholesterol Education Program Adult Treatment Panel III (NCEP) ("Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III)," 2001). All incorporate the core elements of abdominal obesity, hypertension, impaired glucose metabolism and dyslipidaemia, but differ in the exact cut-offs or scoring criteria.

1.6.2 MetS and the brain

The link between MetS and cardiovascular decline is well established, but there may also be an important association with neurodegenerative processes. Type 2 diabetes is a strong risk factor for the development of AD (Ballard et al., 2011) and can delay recovery from stroke (Kissela & Air, 2006) and peripheral nerve injuries (Kennedy & Zochodne, 2000).

Several longitudinal studies have linked MetS with cognitive impairment and interestingly more so in those who had raised markers of systemic inflammation e.g. CRP, IL-6 and antichymotrypsin (Dik et al., 2007; Yaffe et al., 2007; Yaffe et al., 2004). The Framingham study demonstrated that cognition was inversely associated with blood pressure and type 2 diabetes (Elias, Wolf, D'Agostino, Cobb, & White, 1993)

and another study suggested that the presence of MetS confers a 74% risk of cognitive impairment (Whitmer, Gunderson, Barrett-Connor, Quesenberry, & Yaffe, 2005).

Metformin is a biguanide drug which counteracts the effects of MetS and increases insulin sensitivity. In animals, metformin has been shown to improve learning and memory impairment in High Fat Diet (HFD)-fed rats (Pintana, Apaijai, Pratchayasakul, Chattipakorn, & Chattipakorn, 2012).

1.6.3 MetS and TBI

In the case of TBI, type 2 diabetes increases mortality acutely (Ley et al., 2011), but the long term effects of metabolic abnormalities on brain repair are less clear. Some animal studies however suggest that MetS and a neurodegenerative process following TBI may be linked. Insulin resistant diabetic rats or rats fed a HFD had worse cognition function, greater neurodegeneration and abnormal neural plasticity after a cortical contusion injury (Hoane, Swan, & Heck, 2011). Another study demonstrated that hippocampal brain-derived neurotrophic factor (BDNF) was reduced and cognition was worse in rats fed a high-fat sucrose diet following a TBI (A. Wu, Molteni, Ying, & Gomez-Pinilla, 2003).

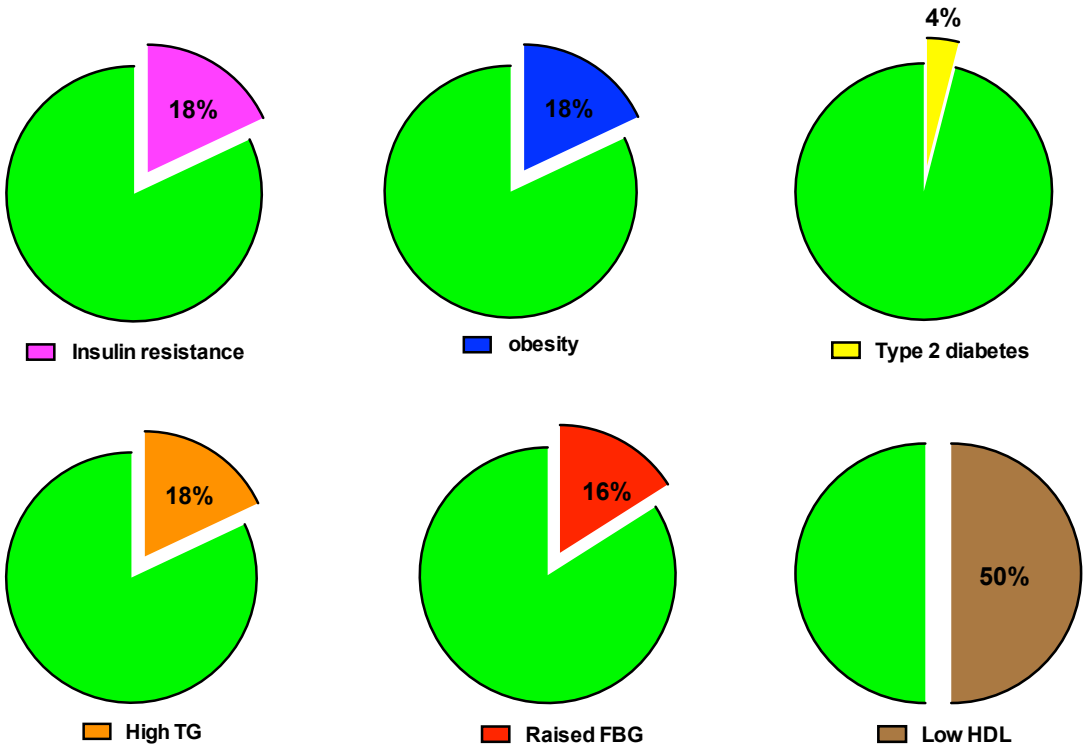
MetS is a potentially reversible condition either by lifestyle interventions (diet, exercise) or by medications, or both. Simple exercise has been found to improve brain plasticity and neurocognitive performance after a TBI and improves features of the MetS (Archer, 2012). In vitro and an in vivo work has demonstrated that metformin has been shown to enhance neurogenesis especially in the hippocampus and olfactory bulb and enhances spatial memory following a TBI(Wang et al., 2012). Finally, statin therapy has been shown to reduce axonal injury, enhance neurite outgrowth and promote neurological functional recovery after experimental TBI (H. Wu, Mahmood, Qu, Xiong, & Chopp, 2012). Statins may exert their effects by reducing neuroinflammation. Simvastatin has been shown to inhibit microglial and astrocytic activation and reduce IL-1β following a TBI (Li et al., 2009).

Glucagon-like peptide-1 (GLP-1) agonists are another class of drugs which counteract the effect of MetS. They are licensed treatments for type 2 diabetes and act to induce glucose-dependent insulin secretion. As GLP-1 receptors are distributed widely in the brain, these drugs are likely to have a central mode of action as well (Alvarez et al., 2005; Blazquez et al., 1998). Exendin-4 (a GLP-1 agonist) treatment produced neuroprotective effects in vitro and improved cognitive function in an animal model of TBI (Eakin et al., 2013). Pioglitazone, a thiazolidinedione, acting through the PPAR-Ɣ receptor has shown some promise in reducing histological damage and inflammation in the acute phase following TBI (Thal et al., 2011).

1.6.4 Prevalence of MetS in TBI patients at Imperial Healthcare NHS Trust

I investigated the prevalence of metabolic abnormalities in 241 patients attending the TBI clinic. A high proportion of these patients had features of MetS (**Figure 1-12):** 18% had insulin resistance using a Homeostatic Model Assessment Score (HOMA) of >3, 18% obesity (BMI>30 kg/m^2), 16% elevated fasting glucose (>5.6 mmol/L), 3.4 % had diagnosed type 2 diabetes, 18% hypertriglyceridemia, and 50% low HDL-cholesterol.

Figure 1-12 Prevalence MetS variables in patients attending the multidisciplinary TBI clinic (n=241).



TG=Triglyceride, HDL=High Density Lipoprotein, FBG = Fasting Blood Glucose

1.6.5 MetS and systemic inflammation

There is evidence to suggest that MetS increases systemic inflammation. MetS principally results from a combination of poor diet, lack of physical exercise and genetic factors. Under normal physiology, excess glucose and free fatty acids (FFA) are taken up from the circulation by adipose tissue, liver and skeletal muscle by insulin. They are then converted to glycogen or triglycerides where they are stored for future use.

In a state of chronic over-nutrition, these storage tissues exceed their capacity to store excess energy. Adipocytes hypertrophy and free fatty acids (FFA) in the circulation multiply. These two processes ultimately activate M1 macrophages in the adipose tissue which then release pro-inflammatory cytokines such as IL-β, TNF-α and IL-6 (Hotamisligil et al., 1996). This exacerbates adipocyte insulin resistance and

further recruits further macrophages (Xu et al., 2003). In addition, excess FFA and adiopcytokines pass directly to the liver impairing insulin action and promoting visceral adiposity. Visceral adiposity in itself is associated with a pro-inflammatory state with high plasminogen activator inhibitor-1 (PAI-1) and low adiopnectin (Amato et al., 2014).

This smouldering inflammatory response in the periphery is now thought to drive the cardiovascular disease seen in MetS and the efficacy of treatments such as aspirin and statins are thought to be, at least in part, mediated by their anti-inflammatory effects (Rocha & Libby, 2009)

1.6.6 Link between systemic inflammation and neuroinflammation

It has long been observed that systemic infections can induce a flare of multiple sclerosis (MS) for example. Microglial activation appears to be a mechanism through which this occurs. Markers of inflammation in the systemic circulation can signal to microglia in a number of ways (Perry et al., 2007).

Firstly, damage from TBI could disrupt the blood brain barrier (BBB) to the extent that cytokines and inflammatory mediators in the blood are able to communicate directly with macrophages. The signal is then communicated to the microglia and spreads though the CNS.

Alternatively, systemic inflammatory mediators may interact directly with the brain endothelium, communicating across the BBB via perivascular macrophages, possibly through induction of lipid mediators e.g. prostaglandin E2. Finally, direct signalling can also occur from the abdominal cavity to the brain via the vagus nerve.

In AD, factor such as systemic inflammation and obesity appear to promote disease progression (Heneka et al., 2015). MetS, diabetes and obesity all invoke a state of low grade peripheral inflammation and it may be this pathway that could promote a harmful neuroinflammatory process.

Given that TBI can induce neuroinflammation and neurodegeneration, it may be that peripheral factors such as MetS, diabetes and obesity could modulate the long term outcome from TBI in much the same way as AD and other neurodegenerative diseases.

1.7 Thesis overview

1.7.1 Chapter 1. Introduction

In this chapter I define TBI, review the epidemiology, aetiology and its clinical consequences, economic impact and treatments. I then describe the typical pathophysiological features of TBI. I then introduce the reader to a clinical consequence that is a major theme in this thesis; namely pituitary dysfunction following TBI and its metabolic and psychological consequences. Neuroinflammation is then introduced as another major theme in this thesis. I discuss its significance in TBI, how it is quantified and how it may be influenced by the metabolic syndrome.

1.7.2 Chapter 2. Methods

This chapter describes the study designs, patient recruitment and data collection for each chapter. Neuroimaging techniques used in this study are introduced including DTI and tract based spatial statistics. PET methodology is presented including an overview of kinetic compartmental modelling, generation of an arterial input function and the analysis processing steps. Methods for diagnosing endocrine dysfunction and metabolic impairments are also presented here.

1.7.3 Chapter 3. Pituitary Dysfunction following Blast Traumatic Brain Injury

Here I describe the high prevalence of pituitary dysfunction in 19 soldiers with blast TBI (bTBI) compared to 39 patients with non-blast TBI (nbTBI). I present the endocrine analyses I performed, quality of life (QoL) scores, cognitive outcome and

diffusion tensor imaging (DTI) results in soldiers. This work has already been published in Annals of Neurology (Baxter et al., 2013).

1.7.4 Chapter 4: Association of serum IGF-I with white matter, neuropsychological and cognitive recovery following traumatic brain injury

Here I present a longitudinal DTI and cognitive study in 39 patients following TBI. I use baseline serum IGF-I measurements and compare outcome measures for patients with an IGF-I above the median for the whole group compared to those with an IGF-I below the median value. I present whole brain and ROI FA results and cognitive/QoL analyses.

1.7.5 Chapter 5: The effect of GH replacement therapy on the recovery from TBI

In this chapter I present the whole brain and ROI FA results for 10 patients who received growth hormone replacement (GHR) for a median duration of 14 months following TBI compared to a group of TBI patients who did not receive this treatment. I present the patient characteristics, whole brain and ROI FA results, cognitive QoL analyses and show the effect of GHR on metabolic impairment.

1.7.6 Chapter 6: Neuroinflammation in TBI: A kinetic analysis of the translocator protein (TSPO) positron-emission tomography ligand [18F]GE-180 in the human brain

In this chapter I present a PET study using the novel TSPO ligand [18F]GE-180 in healthy volunteers. I present the blood and imaging data and kinetic modelling. I also investigate whether there is an effect of the TSPO polymorphism on outcome measures as has been seen with other TSPO tracers. This work has been accepted for publication in the European Journal of nuclear medicine and molecular imaging.

1.7.7 Chapter 7: Analysis of neuroinflammation in TBI using the novel PET radioligand [^{18}F]GE-180 and its relationship with the metabolic syndrome

In this chapter I present the [18F]GE-180 PET data in TBI patients at baseline and follow-up six months later. I examine for an effect of the TSPO polymorphism (high affinity and mixed affinity binders). I also investigate for a influence of metabolic syndrome on [18F]GE-180 outcome measures.

1.7.8 Chapter 8: Discussion

In this final chapter I summarise the results from all the chapters, discuss their significance and limitations.

2 Methods

This chapter will provide a broad overview of the participants, materials, methods and analyses used in this thesis. Please note that each chapter has its own methods section and will refer to sections in this chapter where necessary.

2.1 Participants

2.1.1 Recruitment for Chapter 3

19 military bTBI patients were recruited using the Academic Department of Military Emergency Medicine (Birmingham, UK) trauma database to identify soldiers injured between December 2009 and March 2012. This part of the recruitment process was carried out by D Baxter. Comparison was made with an age- and gender-matched control group of 39 patients who had suffered a non-blast TBI and who were seen in the multidisciplinary TBI clinic at Charing Cross Hospital between August 2009 and March 2012, met the inclusion and exclusion criteria and were within age range of the bTBI group. Soldiers were scanned on a 3 Tesla (Phillips Achieva scanner, Phillips Medical Systems, Netherlands) in the Robert Steiner Unit at Hammersmith Hospital London.

2.1.2 Recruitment for Chapter 4

39 patients with a history of TBI were recruited from the multidisciplinary TBI clinic at Charing Cross and St Mary's Hospitals or via Richard Greenwood's clinic at Homerton Hospital. Endocrine testing was performed as part of routine clinical care at either Charing Cross or St Mary's Hospital.

Inclusion criteria were as follows:

i) On-going cognitive or psychological symptoms following TBI

ii) Aged 18-80

iii) Capacity to give informed consent

Exclusion criteria were as follows:

i) History of neurosurgery or craniotomy
ii) History of psychiatric or neurological illness prior to TBI
iii) History of significant previous TBI
iv) Current or previous drug or alcohol abuse
v) Completion of endocrine testing
vi) Contraindication to MRI scanning

Research ethics approval was obtained from the Ealing and West London Research Ethics Committee REC 09/H0707/82. A control group of 35 age and gender matched healthy volunteers was recruited from various sources and had been collected by the group over the last few years. All controls had no history of neurological or psychiatric illness and were not taking any medication. Patients were scanned on a 3 Tesla (Phillips Achieva scanner, Phillips Medical Systems, Netherlands) in the Robert Steiner Unit at Hammersmith Hospital London.

2.1.3 Recruitment for Chapter 5

14 patients with confirmed growth hormone deficiency (GHD) were recruited from the multidisciplinary TBI clinic at Charing Cross and St Mary's Hospitals under the same ethics as for Chapter 4. 10 of these patients were scanned prior to starting GH and 10 one year afterwards. The 4 other patients with GHD were either not started on GH (n=3, 1 due to bladder carcinoma, 1 asymptomatic, 1 requiring investigation for nasal polyps) or lost to follow-up (n=2). This group was compared to a control group of 23 patients with TBI (but no GHD) and who were age and gender matched.

2.1.4 Recruitment for Chapter 6

10 healthy controls were recruited via various routes including word of mouth, existing volunteer databases and advertisements on noticeboards. Inclusion criteria were as follows:

i) age between 20 and 65 years

ii) in good general health
iii) no significant current or previous neurological or psychiatric illness
iv) capable of giving written informed consent
v) not currently on any medication that would interfere with the study or compromise participant safety.

Exclusion criteria were as follows:

i) unwillingness or inability to follow the procedures required
ii) significant prior neurological or psychiatric illness
iii) history of a drug or other allergy that, in the opinion of the investigators, contraindicates their participation in the study
iv) use of any medication or substance that, in the opinion of the investigators, would interfere with the study or compromise participant safety
v) contraindication to MRI scanning, assessed by a standard pre-MRI questionnaire (e.g. presence of ferromagnetic implants, claustrophobia, pregnancy)
vi) contraindication to PET-CT scanning (e.g. pregnancy)
vii) contraindication to arterial line insertion (e.g. positive Allen's test indicating ulnar arterial insufficiency, prolonged PT)
viii) contraindication to receiving contrast e.g. previous allergy or chronic renal impairment
ix) Exposure to ionising radiation (above normal background levels) in the last two years.
x) Low affinity binders (LABs) were excluded (see section 2.6.1 TSPO Genotyping page 80).

The study was approved by the Westminster Research Ethics committee (Project ref no: 13/LO/1596 version 2.0 date 3.12.13) and by the Administration of Radioactive Substances Advisory Committee (ARSAC).

2.1.5 Recruitment for Chapter 7

28 patients were recruited from the multidisciplinary TBI clinic at St Mary's Hospital. They then had either a face to face or telephone consultation to screen for the inclusion and exclusion criteria and to ensure they had read the patient information leaflet. They were then invited to come for an initial visit to have a metabolic assessment and a genetic test to screen for the TSPO polymorphism. From this point 12 patients were suitable for inclusion into the scanning part of the study. Of these 10 completed a follow-up scanning session 6 months later. A proportion of these patients were selected because they had confirmed Metabolic syndrome (MetS)

Inclusion criteria were as follows:

i) age between 20 and 65 years
ii) a diagnosis of traumatic brain injury (TBI)
iii) no significant neurological or psychiatric illness prior to TBI
iv) capable of giving written informed consent
v) not currently on any medication that would interfere with the study or compromise participant safety.

Additional inclusion criteria for TBI patients with MetS

i) Raised waist circumference i.e. Male ≥ 94 cm, Female ≥ 80cm (European measurements, other ethnic specific measurement apply). If body mass index (BMI) >30 kg/m2 then waist circumference criteria can be assumed.

Plus TWO or more of:

ii) Fasting hypertriglyceridemia (≥1.7 mmol/L or on specific treatment for this lipid abnormality)
iii) Hypertension (systolic ≥130mmHg, diastolic ≥85 or on anti-hypertensive drugs)
iv) Raised fasting glucose (≥5.6 mmol/L or previously diagnosed Type 2 Diabetes, or on anti-diabetic medication).

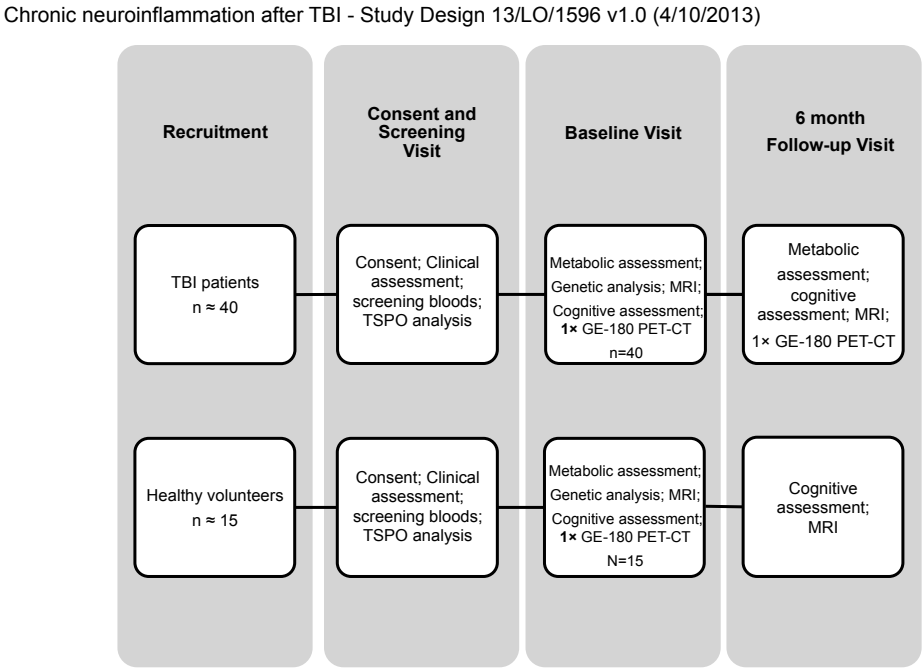
v) Low HDL-cholesterol (males <1.03 mmol/L, females <1.29 mmol/L, or on specific treatment for this lipid abnormality).

Exclusion criteria were as follows:

i) unwillingness or inability to follow the procedures required
ii) significant prior neurological or psychiatric illness
iii) history of a drug or other allergy that, in the opinion of the investigators, contraindicates their participation in the study
iv) use of any medication or substance that, in the opinion of the investigators, would interfere with the study or compromise participant safety
v) contraindication to MRI scanning, assessed by a standard pre-MRI questionnaire (e.g. presence of ferromagnetic implants, claustrophobia, pregnancy)
vi) contraindication to PET-CT scanning (e.g. pregnancy)
vii) contraindication to arterial line insertion (e.g. positive Allen's test indicating ulnar arterial insufficiency, prolonged PT)
viii) Contraindication to receiving contrast e.g. previous allergy or chronic renal impairment

The study was approved by the Westminster Research Ethics committee (Project ref no: 13/LO/1596 version 2.0 date 3.12.13) and by the Administration of Radioactive Substances Advisory Committee (ARSAC). A high-level overview of the study design is depicted in **Figure 2-1**

Figure 2-1 High level overview of study design (PET studies).



My studies accounted for n=12 TBI patients and n=10 healthy volunteers. TSPO=translocator protein.

2.2 TBI severity classification

For all chapters, TBI severity was assessed according to the Mayo Classification system (Malec et al., 2007). These scoring criteria integrate post-traumatic amnesia (PTA); lowest Glasgow Coma Scale (GCS) score in the first 24 hours, duration of loss of consciousness and neuroimaging findings. Criteria are shown below in **Table 2-1.**

Table 2-1 TBI Mayo classification severity score adapted from (Malec et al., 2007)

Injury Severity	**Diagnostic criteria**
Moderate to severe TBI	If one of more of the following criteria apply: i) Death Secondary to TBI ii) LOC 30 mins or greater iii) PTA 24 hours or greater iv) GCS<13/15 in the first 24 hours after injury (attributable to TBI, not other causes) v) One of the following imaging findings: -Intracerebral, subdural or epidural haematoma -cerebral contusion -penetration of the dura -subarachnoid haemorrhage -brainstem injury
Probable (Mild) TBI	If none of the above apply but patients have one or more of the following: i) LOC <30 mins ii) PTA <24 hours iii) Depressed basilar or linear skull fracture with intact dura.
Possible (Symptomatic) TBI	If none of the above apply, but patients report one or more of the following symptoms: i) Blurred vision ii) Headache iii) Focal neurological symptoms iv) Dazed v) Dizziness vi) Nausea vii) Confusion

2.3 Study visit at Robert Steiner MRI Unit (Chapter 3, 4 and 5)

All participants were sent a patient information leaflet and screened for inclusion or exclusion criteria either in clinic or over the telephone prior to arrival on the study day. Consent forms were signed at the study visit. A full clinical assessment was carried out including recording of symptoms and medications. A cognitive assessment was carried out. Quality of life questionnaires were filled out. Following this, brain MRI scans were acquired.

At the follow-up visit, the same study protocol was applied. A clinical assessment was taken to assess on-going symptoms and medication changes. In the case of those patients on GH, enquiry was made as to current dosing, dose changes during the year and any periods off treatment.

2.3.1 Quality of Life (QoL) assessments

Questionnaires were given to the participants to fill out under supervision. These questionnaires were chosen to reflect some of the common symptoms reported by patients following a TBI, including depression and sleep disturbance and included:

i) Quality of Life. Assessment of GH deficiency in Adults (AGHDA) (QoL-AGHDA)
ii) Beck Depression Inventory-II (BDI-II) (Beck, Steer, Ball, & Ranieri, 1996)
iii) Short Form-36 Survey (SF-36) (Ware & Sherbourne, 1992)
iv) Nottingham Health Profile (NHP) (Hunt, McEwen, & McKenna, 1985)
v) Pittsburgh Sleep Quality Index (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989)
vi) Epworth sleepiness scale (Johns, 1991)

2.3.2 Neuropsychological assessment

All patients underwent a standardised neuropsychological battery of tests which our group has used previously in TBI (Bonnelle et al., 2012; Kinnunen et al., 2011).

i) The Wechsler Test of Adult Reading (WTAR) (Wechsler, 1997b) was used to assess pre-morbid intellectual functioning.

ii) The Wechsler Abbreviated Scale of Intelligence (WASI) Similarities and Matrix Reasoning subtests were used to assess current non-verbal and verbal reasoning ability (Wechsler, 1999)

iii) Wechsler Memory Scale-Third Edition (WMS-III) subtest Digit Span, was used to assess working memory (Wechsler, 1997b)

iv) Wechsler Memory Scale-Third Edition (WMS-III) subtest Logical Memory I and II, was used to measure immediate and delayed memory recall of structured verbal material (Wechsler, 1997a)

v) The Peoples' test from the Doors and People Test to assess associative learning and recall (immediate and delayed) (Baddeley, 1994).

vi) The Delis-Kaplan Executive Function System (D-KEFS) subtests Verbal Fluency (F-A-S) and Colour-Word Interference (Stroop) to assess word generation fluency, cognitive flexibility and set-shifting and information processing speed (Delis, 2001).

vii) The Trail Making Test (Forms A and B) was used to further assess executive functions (R, 1958)

2.3.3 MRI brain acquisition

Each participant had standard high-resolution T1 and gradient-echo (T2*) ($1.75x1.75x2mm^3$) imaging to assess focal brain injury and evidence of microbleeds, superficial siderosis, presence and location of contusions and gross pituitary injury. All structural MR scans were reviewed by a single experienced consultant neuroradiologist, Dr Manesh Patel (Hammersmith Hospital, London).

MRI was performed on 3T Achieva scanner (Philips Medical Systems, Netherlands) using an 8 channel head coil. The T1 and T2*-weighted images were obtained prior to DTI. For DTI, diffusion-weighted volumes with gradients applied in 16 non-collinear directions were collected in each of the four DTI runs, resulting in a total of 64 directions. The following parameters were used: 73 contiguous slices, slice thickness 2mm, field of view 224mm, matrix 128 x 128 (voxel size 1.75x1.75x2 mm^3), b value 1000 and four images with no diffusion weighting (b=0s/mm^2).

2.3.4 Generation of FA maps

The images were registered to the b0 image by affine transformations to minimize distortion due to motion and eddy currents and then brain-extracted using Brain Extraction Tool (S. M. Smith, 2002) from the FMRIB Software Library image processing toolbox (S. M. Smith et al., 2004; Woolrich et al., 2009). FA maps were generated using the Diffusion Toolbox (Behrens et al., 2003).

2.3.5 Tract based spatial statistics (TBSS)

TBSS is a voxel-wise approach to analysing FA maps (and other DTI metrics). Given that there is significant inter-individual variability in brain structure and volume, DTI data is particularly vulnerable to partial volume effects. TBSS is a method of tackling this problem by reducing white matter (WM) structure to smaller regions of brain tracts' where the WM alone is consistent across groups (S. M. Smith et al., 2006)

TBSS was carried out using the FMRIB Software Library (S. M. Smith et al., 2006; S. M. Smith et al., 2004) and involved a number of steps:

(i) Non-linear alignment of all subjects' FA images into common FMRIB58 FA template space;
(ii) Affine-transformation of the aligned images into standard MNI152 1mm space;
(iii) Averaging of the aligned FA images to create a 4D mean FA image;

(iv) Thinning of the mean FA image to create a mean FA 'skeleton' representing the centre of all white matter tracts, and in this way removing partial-volume confounds

(v) Thresholding of the FA skeleton at FA 0.2 to suppress areas of extremely low mean FA and exclude those with considerable inter-individual variability.

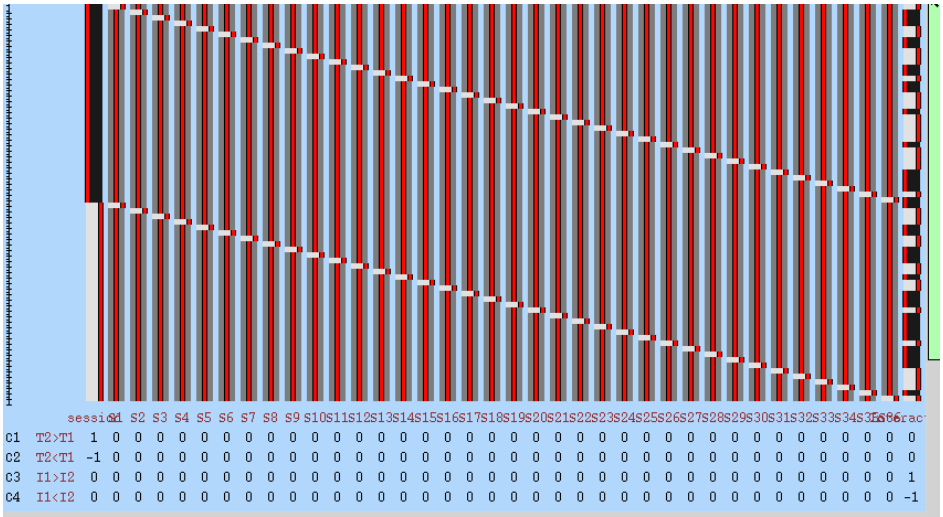
(vi) Non-parametric permutation-based statistics were employed using randomize with threshold-free cluster enhancement and 5000 permutations (Nichols & Holmes, 2002; S. M. Smith & Nichols, 2009)

(vii) A threshold of P<0.05 was then applied on the results, corrected for multiple comparisons.

2.3.6 Longitudinal TBSS analyses

An interaction design matrix was set up in a GLM framework and is depicted in **Figure 2-2**. This matrix was able to examine for an effect of time and an interaction on time of our chosen variable (e.g. IGF-I, GHD), but was unable to examine for an effect of group due to 'rank deficiency'. Covariates could not be added to this model for this reason also. To examine for an effect of group a subtraction design was set up whereby the two FA maps per subject were subtracted from one another and TBSS performed on the resulting image.

Figure 2-2 Longitudinal TBSS design matrix



2.4 Endocrine methods

The same method to diagnose pituitary dysfunction was used for chapter 3,4 and 5. The diagnostic algorithm and details of the endocrine tests used can be found in **3.2.4 Tests for pituitary dysfunction page 102.** These tests were performed as part of routine clinical care under the supervision of Dr Tony Goldstone (Consultant Endocrinologist). Pituitary hormone deficiencies were treated when appropriate. GH treatment was administered by the patients themselves following education from our endocrine specialist nurse, Debbie Peters, at Charing Cross Hospital. Patients were followed up in the multidisciplinary TBI clinic at either Charing Cross or St Mary's Hospitals.

2.5 Methods for assessing insulin resistance (IR) and the metabolic syndrome

2.5.1 Criteria for diagnosing the metabolic syndrome in this study

The method for diagnosing the metabolic syndrome in this study was using the criteria from the International Diabetes Federation (Alberti, Zimmet, & Shaw, 2006). I chose this criteria as it has ethnic specific cut-offs for waist circumference. Criteria are displayed in **Table 2-2**.

Table 2-2 IDF criteria for diagnosing the metabolic syndrome

Central obesity (defined as waist circumference* with ethnicity specific values)				
plus any two of the following four factors:				
Raised triglycerides	≥ 150 mg/dL (1.7 mmol/L)			
	or specific treatment for this lipid abnormality			
Reduced HDL cholesterol	< 40 mg/dL (1.03 mmol/L) in males			
	< 50 mg/dL (1.29 mmol/L) in females			
	or specific treatment for this lipid abnormality			
Raised blood pressure	systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg			
	or treatment of previously diagnosed hypertension			
Raised fasting plasma gluc	(FPG) ≥ 100 mg/dL (5.6 mmol/L),			
	or previously diagnosed type 2 diabetes			

** If BMI is >30kg/m2, central obesity can be assumed and waist circumference does not need to be measured.*

Cut-offs for ethnic specific waist circumference are displayed in **Table 2-3**.
For patients who I did not have a waist circumference measurement (Chapter 5), I calculated a 'metabolic score', assigning a single point (out of 4) for each of the categories in **Table 2-2** excluding BMI and waist measurement.

Table 2-3 Table showing cut offs for waist circumference for different ethnic groups

Country/Ethinc Group	Waist circumference
Eurpoids	Female ≥ 80 cm Male ≥ 94 cm
South Asians	Female ≥ 80 cm Male ≥ 90 cm
Chinese	Female ≥ 80 cm Male ≥ 90 cm
Japanese	Female ≥ 80 cm Male ≥ 90 cm
Ethnic South and Central Americans	Use South Asian recommendations until more specific data are available
Sub-Saharan Africans	Use European data until more specific data are available
Eastern Mediterranean and Middle East (Arab)	Use European data until more specific data are available

2.5.2 Sex-hormone binding globulin (SHBG)

SHBG is a specific steroid-binding globulin which is mainly derived from the liver (Selby, 1990). It binds testosterone with high affinity and oestrogens with lower affinity. Its function is a modulate androgen delivery to tissues. Insulin appears to have a modulatory effect on SHBG levels and therefore diseases characterised by IR affect serum concentrations of SHBG. For example lower SHBG level has been reported in obesity (Pasquali et al., 1991) and polycystic ovarian syndrome (PCOS) (Meirow et al., 1995). In addition, low SHBG levels are an independent risk factor for developing type 2 diabetes (Lindstedt et al., 1991). SHBG was measured on a single blood samples on all participants as part of a metabolic assessment.

2.5.3 The Homeostasis Model Assessment (HOMA)

The gold standard for measuring insulin resistance is the hyperinsulinaemic euglycaemic clamp which measures the amount of infused glucose require to maintain euglycaemia in a state of excess insulin (DeFronzo, Tobin, & Andres, 1979). However this method is time-consuming and not practical to perform in large clinical populations. However the HOMA, which estimates steady state beta cell function

(%B) and insulin sensitivity (%S) correlates well with the gold standard and is therefore widely accepted as a measure of IR (HOMA IR is the reciprocal of %S). In order to calculate HOMA-IR, a fasting insulin and glucose sample was taken on participants. I downloaded the model from (https://www.dtu.ox.ac.uk/homacalculator/) used this to calculated a HOMA IR value for each individual.

2.5.4 C-peptide

C-peptide is the connecting molecule of the insulin A and B chain in the proinsulin molecule. Measurements of this peptide were taken in the fasted state in the morning. 20/(fasting blood glucose x c-peptide) was calculated; a model which has been shown to be an effective measure of IR (Ohkura et al., 2013)

2.5.5 Glycosylated Haemoglobin (HbA1c)

Non-fasted blood measurements were taken of HbA1c to test for type 2 diabetes (>48 mmol/mol) and to provide a measure of dysglycaemia.

2.5.6 % body and % visceral fat

Where possible, measurements of body fat composition were taken on a BC-418 Segmental Body Composition Analyser (Tanita).

2.6 PET Screening Visit (Chapters 6 and 7)

For this initial visit patients arrived fasted at the Clinical Research Facility (CRF) on the Hammersmith campus. In summary, the following would be performed:

i) Signing of consent form
ii) Detailed history and clinical examination
iii) BP, waist circumference, height, weight, % body fat, electrocardiogram (ECG)

iv) Screening bloods for FBC, clotting, renal profile, HbA1c, lipids, sex-hormone binding globulin (SHBG), glucose, insulin, c-peptide

v) Bloods for TSPO genotyping.

vi) Structured Clinical Interview for DSM-IV Axis 1 disorders (SCID-I) to screen for current psychiatric disorders.

vii) Allen's test to test for patency of the ulnar artery

2.6.1 TSPO Genotyping

All patients had TSPO genotyping at screening visits to establish number of alleles for the Ala147Thr polymorphism. Venepuncture was performed and a single blood sample was sent to the pathology laboratory at Hammersmith Hospital. DNA was extracted using the Qiagen QIAmp DNA blood mini kit and genotyping was performed using a TaqMan Allelic Discrimination assay. Those individuals with two alleles for the Ala147Thr polymorphism, were labelled 'low-affinity binders' (LABs) and were excluded from the study. This is because we expected there to be minimal binding in LABs and therefore did not justify the radiation exposure for these individuals. Mixed affinity binders (MABs) had one allele for the polymorphism and high affinity binders (HABs) had none. These individuals were included in the study.

Analyses for chapter 6 and 7 were separated according to HAB or MAB status as we expected our TSPO tracer to show differentiation between the two such that MABs would show 50% binding compared to HABs (Owen et al., 2011; Owen et al., 2012).

2.7 PET scanning visit

Prior to PET scanning, participants had vital signs checked, venous cannula inserted and all females had a urinary pregnancy test. Blood samples are taken at this point for markers of inflammation, CRP, ESR, IL-6, TNF- α, CRP, HsCRP, MCP-1, IP-10, MIP 1- β, SB100β. An arterial line was inserted (where possible) to enable blood monitoring of the tracer. This was performed by external medical professionals trained in the procedure. A 10 cm line was inserted using 2% lidocaine (seldinger

technique). Verbal consent was taken separately for this procedure and recorded in the medical notes.

[18^F]GE-180 was manufactured at GE Healthcare's Amersham site and transported to the CIF according to local standard operating procedures (SOPs). It was used within 12 hours of manufacture.

2.8 Dynamic PET acquisition

Patients were positioned supine in the PET-CT scanner (Biograph 6). The arterial line was connected to continuous blood monitoring machine via 1.5 m tube after being flushed with saline. CT topogram was performing for positioning followed by low-dose CT scan for attenuation correction 5-10 minutes before tracer injection. PET scanner started at 30 seconds prior to injection to ensure machinery operation prior to injecting the tracer. Clocks were synchronised to the PET scanner clock and quality checks carried out in accordance with local SOPs. Approximately 185 MBq of [18^F]GE-180 (drawn up in 10mL of normal saline) was administered as a stat bolus followed by a 20 mL flush of normal saline at the same time as the 90 minute PET acquisition (list mode) started. Frames time are outlined in **Table 2-4** below:

Table 2-4 Frame times for the 90min dynamic PET acquisition

No.	Duration (sec)	Frames	Time (cumulative secs)
0	30	0	30
1-6	15	6	90
7-9	60	3	180
10-14	120	5	600
15-19	300	5	1500
20-24	600	5	3000

Patients were checked for head position every 15 minutes and remained in the scanner for the 90 minutes duration. At the end of the scan, the arterial line was removed and pressure applied for 20 minutes followed by a pressure dressing. The

venous cannula was also removed and vital signs taken before participant allowed to go home.

2.9 Acquisition Procedure-Blood and metabolites

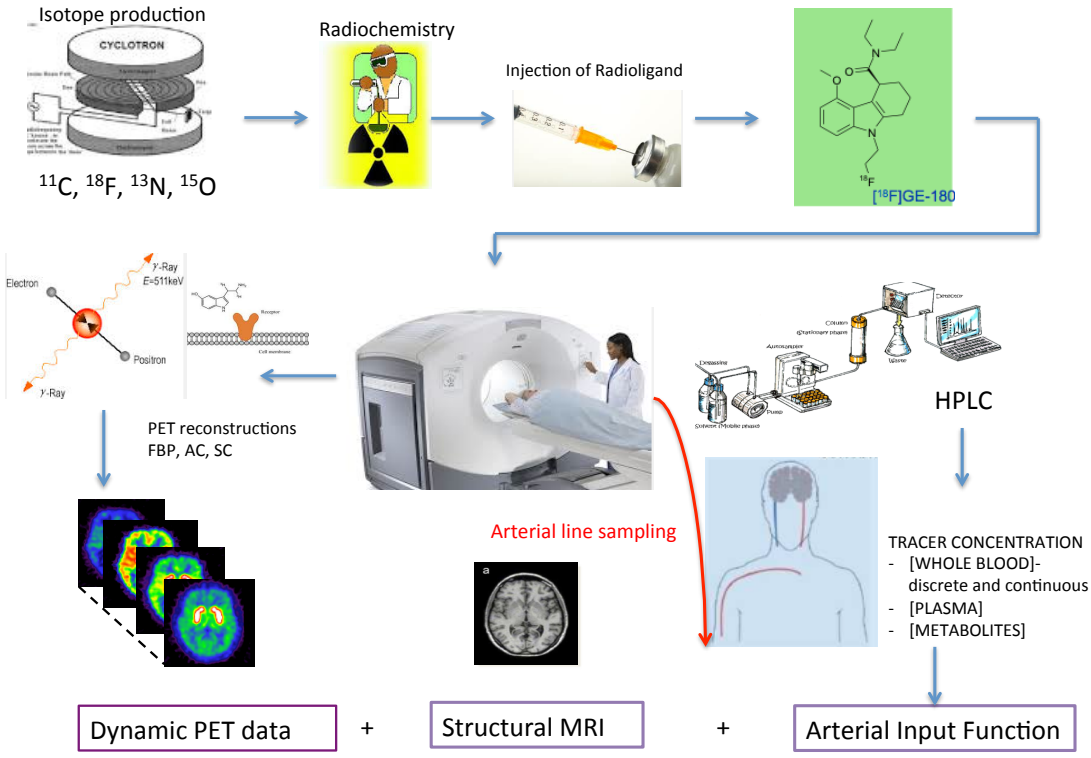
Continuous blood sampling was taken for the first 15 minutes (withdrawal rate 2.5 mL/min) and discrete blood samples taken at the following times following scan start: 0, 5, 10, 15, 30, 50, 70, 90 mins. Whole blood and plasma radioactivity readings were taken. The parent fraction of [18^F]GE-180 was measured by high-performance liquid chromatography (HPLC) of discrete plasma samples. Further details on these methods can be found in **6.2.5** Whole Blood, Plasma Activity and Parent Fraction of [^{18}F]GE-180 **page 181.**

2.10 PET data reconstruction

Data was reconstructed as 24 temporal frames. Filtered back projection (matric size 168x168, zoom 2.6, 5 mm Gaussian filter, pixel size 1.56 x 1.56, slice thickness 3 mm) was the method used for data reconstruction with and without attenuation correction. The standard corrections for scatter, decay and randoms were applied.

A diagrammatic overview of the processes involved in PET acquisition for this study is seen in **Figure 2-3**.

Figure 2-3 An overview of the processes involved in acquiring PET data



HPLC=High performance liquid chromatography

2.11 MRI acquisition

All participants had high resolution T1-weighted structural MRI. Participants were scanned on a Siemens 3T Verio system (Siemens Healthcare, Germany). An MPRAGE sequence was acquired with the following parameters: repetition time = 2300 ms; echo time = 2.98 ms ; flip angle = 9°; field of view = 25.6 cm x 24 cm; matrix = 256 x 240; number of slices = 160, slice thickness = 1 mm; yielding final voxel dimensions of 1 mm^3.

In addition to standard T1 imaging required for PET analysis, the following sequences were acquired; SWI, T2 Flair, arterial spin labelling (ASL), DTI, Magnetic Resonance Spectroscopy (MRS) and resting state fMRI. Three fMRI tasks were performed during acquisition, including a memory task where patients were shown abstract art pictures and tested on recall in the break, the Choice Reaction Time task (CRT) and a

Breath Hold task. Were possible a venous cannula was inserted and gadolinium contrast given. Delayed images were taken 5 minutes after injection.

2.12 PET Analysis-basic principles

A 'perfect' PET radioligand would be one where once injected into the circulation all of the free tracer is delivered to the brain and binds only to the receptor of interest and concentrates in the most receptor dense areas. In this situation it would not be of interest what was happening in the circulation and analysis could be performed using tissue counts only.

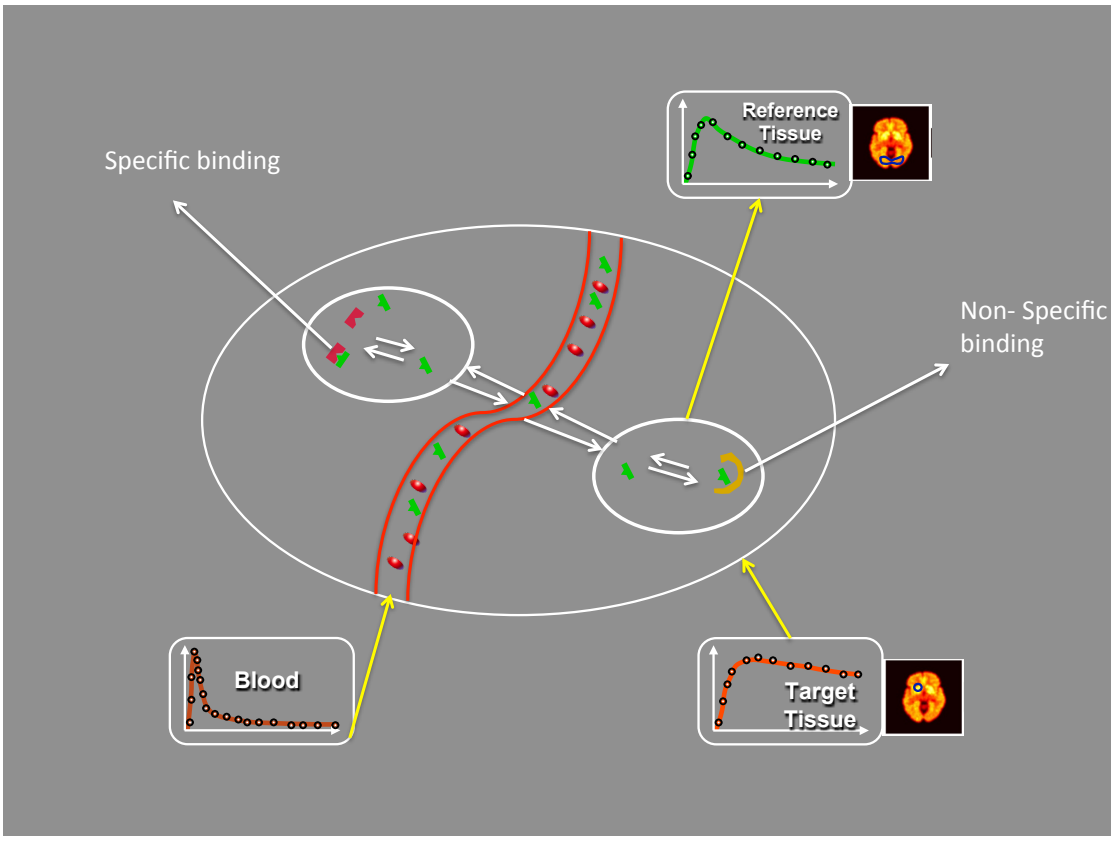
However this ideal scenario is never played out. Once a radioligand is injected into the circulation it undergoes metabolism, decay, it binds to plasma proteins and can enter into cells where it can become involved in a variety of cellular processes. When a proportion of the free tracer enters the brain it binds to the receptor of interest but also binds to non-specific receptors, so called specific and non-specific binding. **(Figure 2-4).**

Measurement of tracer metabolites, whole blood vs. plasma tracer and parent fraction (proportion of unchanged tracer in the circulation) is useful information as this determines how much tracer the brain 'sees'. Kinetic modelling explores the relationship seen in the blood vs. brain tissue. Mathematical equations are applied to describe the observed data.

Sometimes blood measurements are not required. For example, if there has been a lot of experience with a tracer, there may be a consensus on an analysis approach using a reference or pseudo-reference tissue approach. A reference tissue is a region where there are no receptors for the ligand of interest. A pseudo reference tissue is one where binding exists but is low and used consistently across analyses. Analysis approaches like this mean that blood measurements are not required and tissue data alone can be used. This has advantages in terms of patient comfort, resources,

cost and speed of analysis but can overlook issues such as variability in non-specific binding and radioactive metabolites.

Figure 2-4 Schematic overview of ligand-receptor binding model



In my PET studies, given that we were using a novel tracer, we decided to perform blood measurements and perform full kinetic modelling. This section will describe the common models used, define the outcome measures and describe the analysis pipeline used for chapters 7 and 8.

2.13 Compartmental Modelling (CM)

A compartment is an idealised container of a chemical substance and they may not be spatially distinct. CM is used in PET to describe the behaviour of the tracer. It enables quantification of the underlying physiological, biochemical and pharmacological processes. It also enables estimation of parameters at equilibrium when in reality most experiments cannot continue until that point. CMs can be

described in terms of linear, first-order, constant-coefficient, ordinary differential equations. However certain assumptions are made regarding the tracer:

i) TRACER: Labelled compound is in tracer amounts (i.e. so as not to perturb the physiology of the system)
ii) MIXING: Instant mixing i.e. homogeneous concentration in each compartment.
iii) EXCHANGE: The exchange rate of labelled compound among the compartments is related to the concentration within them.

By convention, the first compartment is the concentration of the tracer in the blood. This is known as the arterial input function and this is what drives the system.

2.13.1 Arterial Input Function

The arterial input function describes the concentration of the unchanged (non-metabolised) compound in arterial plasma as a function of time. It describes how much tracer is available for delivery to tissue over the duration of the scan. Broadly it can be generated by following these steps:

i) Continuous arterial blood sampling and/or discrete blood sampling from tracer administration to the end of the PET scan.
ii) Centrifugation of blood to separate plasma
iii) Plasma radioactivity measurement.
iv) Radioactivity divided by sample mass or volume.
v) Decay correction to the time of injection
vi) Metabolite correction
vii) Dispersion correction (where necessary)
viii) Time delay correction (to account for tubing delay and delay from brain to source of blood sample)

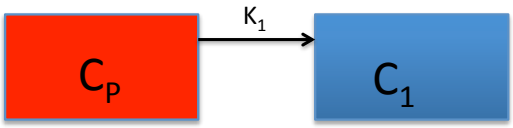
2.13.2 One tissue compartmental model (1TCM)

This is the simplest compartmental model and shown in

Figure 2-5. The first compartment is the arterial input function. The second compartment is the radioligand in tissue. Models can be irreversible of reversible. Irreversible model permit tracer extraction in one direction only (K1), whereas a reversible model allows exchange in the other direction and therefore has a second rate constant (K2). Both these models are described by differential equations.

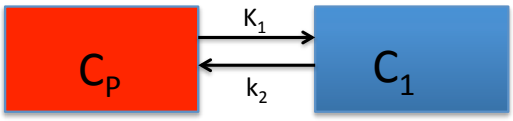
Figure 2-5 Schematic demonstrating 1TCM models (reversible and irreversible)

One compartment model (irreversible)



$$\frac{dC_1(t)}{dt} = K_1 C_P(t)$$

One compartment model (reversible)



$$\frac{dC_1(t)}{dt} = K_1 C_P(t) - k_2 C_1(t)$$

K_1= flow x extraction of tracer

k_2= second rate constant

C_P=concentration of tracer in plasma (arterial input function)

C_1=concentration of tracer in tissue (bound and unbound)

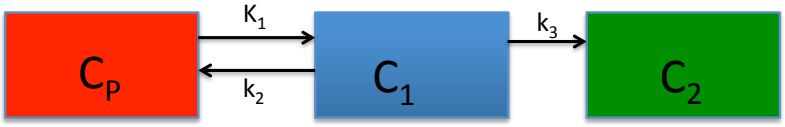
A 1TC model is the simplest model to apply to PET data but is often too simple to describe the behaviour of most tracers.

2.13.3 Two tissue compartment model (2TC)

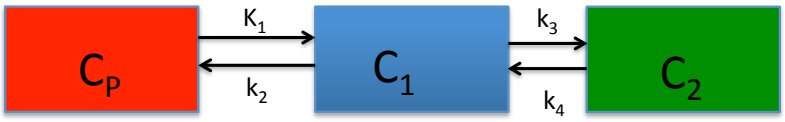
This is the commonest model used for PET tracers. The compartments generally refer to the concentration of the tracer in the plasma (as with 1TC), tracer that is specifically bound and tracer that is non-specifically bound. The amount of tracer in the blood volume within the tissue should be added as a fixed amount (5% in brain) or fitted blood volume could be used. Alternatively, blood volume in tissue could be considered as another compartment. Two differential equations are used to describe these models and again can be reversible of irreversible. The classic irreversible 2TC tracer is labelled Fludeoxyglucose (FDG). These models are shown **Figure 2-6**.

Figure 2-6 Schematic demonstrating 1TCM models (reversible and irreversible)

Two compartment model (irreversible)



Two compartment model (reversible)



Two compartment model (irreversible)

$$\frac{dC_1(t)}{dt} = K_1 C_P(t) - (k_2 + k_3) C_1(t)$$

$$\frac{dC_2(t)}{dt} = k_3 C_1(t)$$

Two compartment model (reversible)

$$\frac{dC_1(t)}{dt} = K_1 C_P(t) - (k_2 + k_3) C_1(t) + k_4 C_2(t)$$

$$\frac{dC_2(t)}{dt} = k_3 C_1(t) - k_4 C_2(t)$$

2.13.4 Volume of Distribution (V_T)

V_T is defined as the ratio of the radioligand concentration in tissue target region (C_T) to that in plasma (C_P) at equilibrium. The equation for this is simply C_T/ C_P. Equilibrium is when a steady state concentration of the tracer is achieved in tissue (i.e. flux into and out of tissue is the same). As a rule of thumb, V_T of 10 mL/cm^3 is being concentrated in the tissue at a ratio of 10:1.

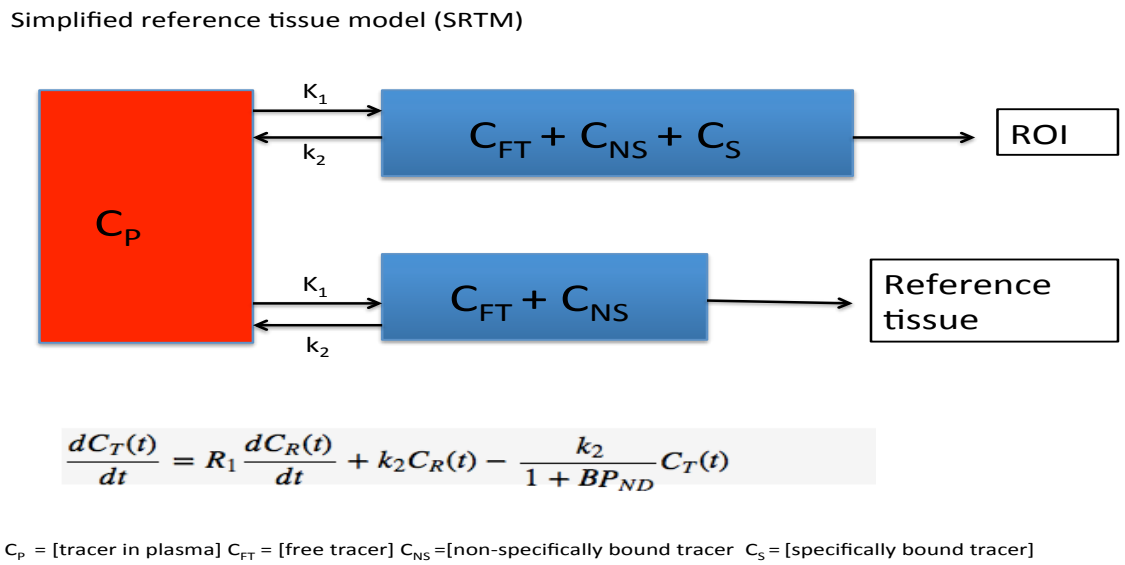
If a CM appropriately describes the tracer kinetics then is possible to calculate the volume of distribution (V_T) in a volume or interest or at a voxel level. V_T can be derived from any CM, as long as there is no irreversible uptake.

V_T is proportional to both receptor density and binding affinity of the tracer. V_T does not account for plasma protein binding (as this is not corrected for in the arterial input function) and does not differentiate between specific and non-specific binding. Therefore binding potentials are used differentiate between specific and non-specific binding. However in order to calculate this without a reference region approach then B_{max} (total density of receptors) and K_D (equilibrium dissociation constant) need to be known and this can only be confirmed with a 2nd receptor blocking PET study.

2.13.5 Simplified reference tissue model (SRTM)

This model can be used when a 1TC model could describe the kinetics of the tracer and a reference tissue (i.e. one with no specific binding) is known (Lammertsma & Hume, 1996). It does not require an arterial input function. Here BP_{ND} (i.e. specific binding) can be calculated by solving the differential equation below (**Figure 2-7**). It can also be solved by linearised methods which makes it possible to produce parametric images of model parameters.

Figure 2-7 Schematic demonstrating the simplified reference tissue model (SRTM)



$$\frac{dC_T(t)}{dt} = R_1 \frac{dC_R(t)}{dt} + k_2 C_R(t) - \frac{k_2}{1 + BP_{ND}} C_T(t)$$

C_P = [tracer in plasma] C_{FT} = [free tracer] C_{NS} =[non-specifically bound tracer C_S = [specifically bound tracer]

2.13.6 Akaike information criterion (AIC) (Akaike, 1974)

AIC is founded in information theory. It is a method of comparing model fits for a given set of data. The lower the number then the better the fit. In the case of two model fits being equally suitable to describe the data then the model with the least number of parameters is deemed to be most parsimonious.

2.13.7 Standardised uptake values (SUV)

SUV is another method of PET quantification that is used widely especially in clinical imaging. It is simply a ratio of measured radioactivity in tissue at certain time divided by body weight and injected dose of radioligand. It does not require dynamic PET acquisition, but relies on a single image, usually a 'summed' image at late time point. SUV calculations do not require arterial blood sampling. However, using tracer dose per unit of body weight is a crude approximation for tracer delivery to tissue. A

reference tissue can also be used to cancel out effect of non-specific binding and this is called SUVR.

2.13.8 Logan graphical analysis

Logan graphical analysis (or Logan plot) is a technique that simplifies the compartmental modelling approach by transforming the model equations into a linear equation evaluated at specific time points. This reduces the number of parameters to a slope and intercept. The slope is applied to the most linear portion of the curve. This method can be used for tracers with reversible uptake. For irreversible tracers, Patlak graphical analysis can be used.

2.13.9 Distribution volume ratios (DVR)

This is the ratio of the DV of a receptor-containing region to a non-receptor region. It usually requires an arterial input function to calculate but can also be derived from graphical analysis methods.

2.14 PET analysis pipeline

MIAKAT™ (Molecular Imaging And Kinetic Analysis Toolbox) was used for the PET analysis in this thesis. The first version was developed in 2015 and was used to do the analysis for the published study in Chapter 6. An updated version was used for the analysis in Chapter 7. It is freely available and was developed by Professor Roger Gunn, Graham Searle and Christopher Coello who work at Imanova and Imperial College, London.

MIAKAT™ contains software for the quantitative analysis of PET neuroimaging data an is presented in a pipeline format. The three main inputs to the pipeline are i) dynamic PET ii) structural MRI and ancillary blood data). Broadly speaking the pipeline follows the sequence below:

1) Brain Extraction

2) Brain Tissue Segmentation (GM/WM/CSF)
3) Motion Correction
4) Region of interest definition via a neuroanatomical atlas
5) Blood/plasma kinetic modelling
6) ROI tracer kinetic modelling
7) Parametric imaging

The outputs from this pipeline are quantitative outcome measures extracted from various CM on a regional or voxel-wise basis. The software uses code from the FMRIB Software Library (FSL) and Statistical Parametric Mapping (SPM). Matlab (with the Optimisation Toolbox) is required for running the pipeline. However there is also a graphical user interface (GUI).

2.14.1 Inputs to MIAKAT™

Dynamic PET data (attenuated and non-attenuated corrected) and structural T1 MRI image were required in nifti pair format. Blood data collected in the CIF was converted into ancillary file format by hand. This data file contained 15 minutes of continuous whole blood radioactivity readings, discrete plasma and whole blood readings and parent fraction. All data was decay corrected to the start of the injection time. A schematic of the inputs and outputs to the MIAKAT™ pipeline is shown in **Figure 1-1.**

An AnalysisManager data structure was used to gather the output of any given examination. This involved generating a binary .mat file from a template which contained information regarding i) the examinations and processes to be performed ii) configuration options for each process iii) progress of the analysis throughout the pipeline iv) paths to input/output data and v) references to all relevant analysis data structure mat-files. The template could be customised to include different CMs, reference models and different blood volume corrections.

2.14.2 Summary of structural MRI processing steps

Preprocess images

This step creates a back-up of the images, strips information in the header and converts the units of the dynamic PET AC image to kBq/ml.

Make isotropic

This step transforms a 3D volume with any voxel size to a 3 volume with isotropic voxel with a chosen size (default 2 mm).

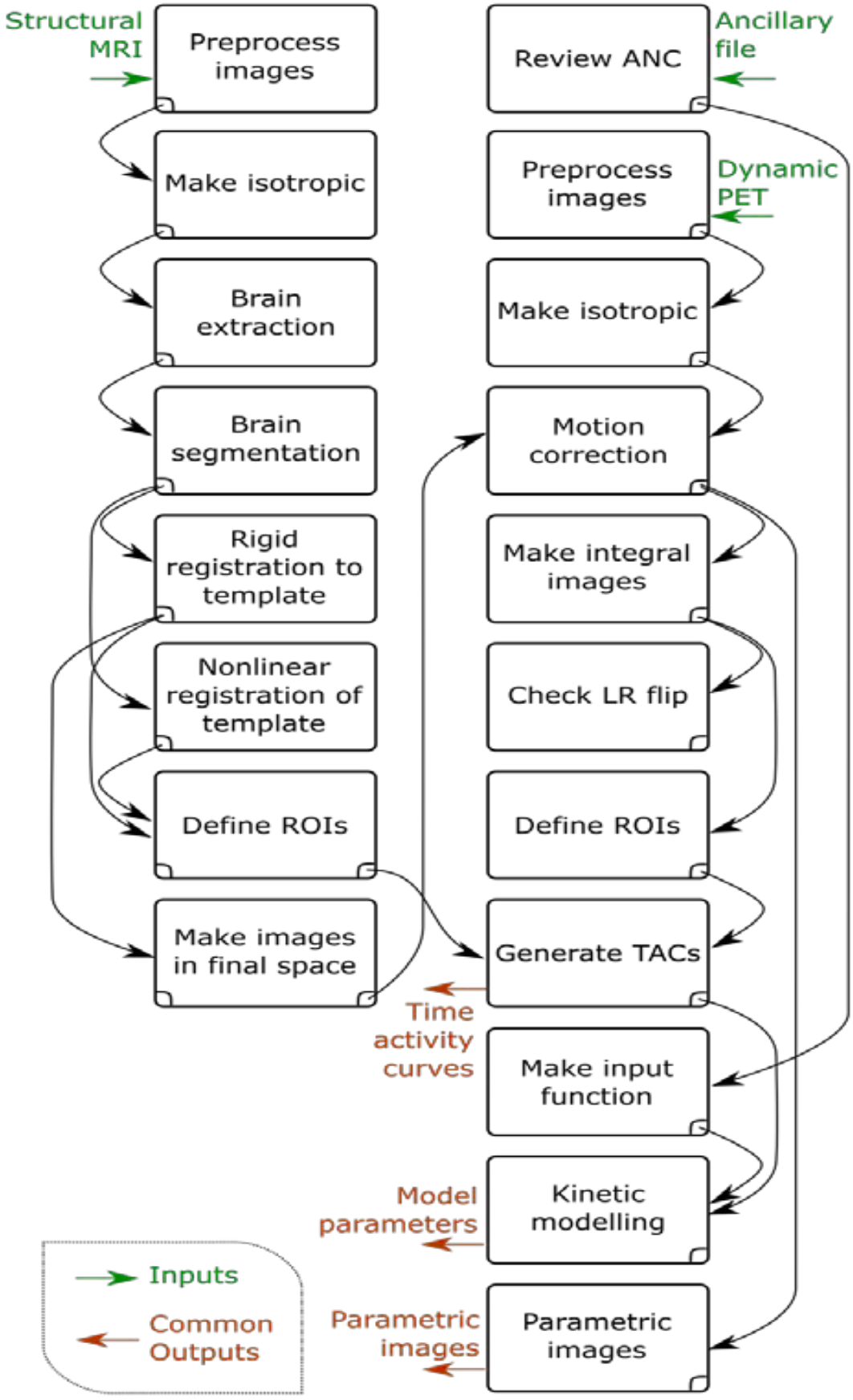
Brain extraction

This process uses the FSL toolbox (FSL Brain Extraction Tool) to perform brain extraction.

Brain segmentation

This process uses a specified tool (usually FSL or SPM) to segment the brain MRI creating a selection of output images: grey matter image, white matter image and csf image.

Figure 2-8 Overview of the PET analysis pipeline



Rigid registration to template

This step rigidly registers (6 degrees of freedom) the brain MRI to a template. The transformation matrix, is saved in the Analysis data structure and applied to the brain tissue probability maps from the previous steps. The default options are to use SPM co-registration and realignment functions.

Nonlinear registration of template

Using SPM as a default, this steps nonlinearly registers the template MRI to the subject's rigidly registered brain MRI and saves the deformation field.

Define ROIs

ROIs are defined during this process but not applied to this examination; they are applied to the PET pre-processing. The standard neuroanatomical atlas used is the CIC atlas version 2.0, defined on the nonlinear ICBM152 template and developed using the region of interest definitions defined by Tziorti *et al.* 2011 (Tziortzi et al., 2011).

Make into final space

This process reslices any specified volume to the final space. This means that they will be registered with PET dynamic image from which time activity curves (TACs) will be extracted.

2.14.3 Summary of PET pre-processing steps

Review ANC file

This process checks that this file is present and that the content matches pre-defined criteria.

Motion correction

This step realigns the dynamic PET data using a frame-to-frame rigid registration. One frame from the PET data is selected as reference and all other frames are registered to this. Frame 16 was chosen for these studies. Additionally this step

rigidly registers the dynamic PET to the MRI (brain-extracted MRI registered to MNI space) of the same subject.

Make integral images

This step makes one or several static or 'summed' images where each voxel is the average value of that voxel over a selected interval of time.

Check LR Flip

This step evaluates the left-right orientation match between PET and MRI images. If the cost function of the non-flipped is better than the flipped, the output is labelled 'probably OK'. If the cost function of the flipped is better than the non-flipped then the output is labelled 'Probably failed'. If they are similar the output is labelled 'Not determined'.

Generate time activity curves (TACs)

This process generates TACs for all regions of interest and stores them in the Analysis data structure.

Make input function

Continuous and discrete whole blood data are merged to form a whole blood TAC for the duration of the dynamic PET scan. A plasma-over-blood (POB model) is fitted to the ratios of discrete plasma and whole blood samples to generate a plasma TAC with the same temporal resolution as the whole blood curve. A parent fraction model is fitted to the parent fraction data and then applied to the plasma TAC to produce a parent in plasma TAC which serves as the arterial input function for the kinetic modelling stage.

Usually there will be a temporal delay between tissue TAC and parent in plasma TAC due to delay in tubing connecting to the continuous blood monitoring machine and delay in vasculature from brain to radial artery. In our examinations this delay was greater than usually expected due to the use of a 1mmx1.5m tube (withdrawal rate 2.5 mL/min). Therefore we applied a +30 second delay correction at this step.

Kinetic modelling

The principles of kinetic modelling have already been discussed on page 85 section 2.13. This step applies models that have been selected in the AnalysisManager. Models available are: Blood Volume, 1TC irreversible, 1TC reversible, 2TC irreversible, 2TC reversible, Logan, Patlak, simplified reference tissue model (SRTM), RefLogan, RefPatlak among others.

Parametric Images

Generation of parametric images involved fitting a model to a TAC for each voxel in the image.

Data Extraction

Individual parameters for rate constants and outcome measures were extracted using a matlab script enabling statistical analyses. Voxel-wise analyses using parametric maps were performed in FSL.

2.15 Data management

Neuroimaging data was stored on the Computational, Cognitive and Clinical Laboratory computer server based at Imperial College London. All patient identifiable information was stored on NHS computer that were password protected. No such data was stored on Imperial College or personal computer. Blood and genetic samples were anonymously coded. Each participant was assigned a file containing consent form, case report form and all documentation arising from the study. These files are stored in a lockable room in the C^3NL laboratory. Separate medical notes are stored in the ICCRF.

2.16 Statistical packages

Any statistical packages used for data analysis will be highlighted in each chapter. SPSS v21 and v22 were used consistently. Graphpad v 6.0 and MATLAB R2015b were used to generate figures in the results sections.

2.17 Ethical issues arising

Full ethical approval was obtained for all studies. Amendments were sought where necessary. Yearly reports were filed and sent back to the ethics committees. Reports of adverse events (n=1) were dealt with according to good clinical practice (GCP) and recorded in the medical notes. Incidental findings of abnormalities detected on clinical examination and blood work were communicated to the patient and details send to the general practitioner (n=2).

3 Pituitary Dysfunction after Blast Traumatic Brain Injury (bTBI)

3.1 Introduction

The prevalence of bTBI, from the use of improvised explosive devices (IEDs) has increased considerably since the Iraq and Afghanistan conflicts (Benzinger et al., 2009). Approximately 400 soldiers in the UK and 2,000 soldiers in the UK have been fatally injured by blast injuries since 2001 (SG, 2012). Injuries can result directly from the blast wave (primary), from missiles propelled by the blast force (secondary), or from impact with another object (tertiary) (Cernak & Noble-Haeusslein, 2010). Of those who have survived a blast impact, approximately 300,000 US soldiers have suffered at least a probable bTBI (Tanielian T, 2012). These individuals are usually young and of peak physiological fitness which means that the potential long term impact of subsequent physical, psychological and cognitive impairments is considerable. The mainstay of treatment is supportive; intensive rehabilitation can help, but there are no targeted pharmacological interventions available to improve prognosis for these individuals (Ruff & Riechers, 2012).

Pituitary dysfunction, especially growth hormone deficiency (GHD), is a well-recognised consequence of non-blast TBI (nbTBI). Initial prevalence studies reported pooled prevalence of hypopituitarism of 23-30% in the chronic phase following a TBI (Schneider, Kreitschmann-Andermahr, et al., 2007). However more recent studies report a consistently lower prevalence at approximately 5% (Kokshoorn et al., 2011). Reasons for this discrepancy include differences in the dynamic tests selected, number of testing points and differences in normal reference ranges (Kokshoorn et al., 2010).

Hypopituitarism can have metabolic consequences such as increased insulin resistance and reduction in bone mineral density as well as a deleterious effect on cognitive capabilities and psychological well being (Cherrier, 2009; Molitch, Clemmons, Malozowski, Merriam, & Vance, 2011). Therefore, growth hormone,

testosterone and cortisol replacement strategies offer welcome therapeutic options for these individuals (Cherrier, 2009; Molitch et al., 2011; Salvatori, 2005). What is not known however is the prevalence of pituitary dysfunction following bTBI.

Diffusion tensor imaging (DTI) is an advanced imaging technique, that can be used to study white matter damage following TBI (see Introduction: Diffusion tensor imaging (DTI) 1.3.6 page 35). By using diffusivity measures such as fractional anisotropy (FA). Even in mild, uncomplicated bTBI, marked DTI abnormalities have been seen in multiple brain regions (Mac Donald et al., 2011).

The primary objective of this study was to investigate the prevalence and associated features of pituitary dysfunction following moderate-severe bTBI compared to an age and gender matched control group of nbTBI. The secondary objective was to test the hypothesis that DTI would show differences between those soldiers with and without pituitary dysfunction. Finally, the third objective was to investigate whether certain cognitive domains were impaired in those soldiers with vs. those without pituitary dysfunction.

3.2 Methods

3.2.1 Participants

Nineteen UK soldiers who had sustained a moderate-severe bTBI in Afghanistan between December 2009-March 2012 were recruited by D Baxter using the Academic Department of Military Emergency Medicine (Birmingham, UK) trauma database. Research ethics approval was obtained from the Ealing and West London Research Ethics Committee (09/H0707/82) and informed consent was obtained from all individuals.

A control group of 39 patients with non-blast TBI was selected from a clinical database of all patients seen in the multidisciplinary TBI clinic at Imperial Healthcare NHS Trust from August 2009 though to March 2012. Patients were selected if they met the inclusion/exclusion criteria and if they fell within the same age group of the bTBI group.

All participants had a full clinical assessment to include recording of the following variables if available: time since injury, Injury Severity Score (ISS) (Baker, O'Neill, Haddon, & Long, 1974), Abbreviated Injury Scale (AIS) Glasgow Coma Score (GCS), post-traumatic amnesia (PTA), BMI, Limb amputation, skull/facial fractures, post-traumatic epilepsy and full medication history.

3.2.2 Inclusion/exclusion criteria for bTBI group

Inclusion*:* A primary moderate-severe brain injury caused by a single exposure to a blast.
Exclusion: 1) Requirement for massive blood transfusion indicating severe hypovolaemic shock at scene 2) Intracranial lesions causing mass effect 3) Post-traumatic stress disorder (PTSD)
(1)+2) so as to constrain our findings to that of a primary blast wave only and 3) as PTSD itself has been linked with endocrine disturbance (Pervanidou & Chrousos, 2010)).

3.2.3 Inclusion/exclusion criteria for bTBI and nbTBI group

Inclusion: 1) Males 2) >2 and <48 months from a single TBI 3) Moderate-severe injury based on the Mayo criteria (Malec et al., 2007) 4) Psychological or cognitive symptoms 5) Completion of all pituitary endocrine testing.

Exclusion: 1) Prior history of neurological and/or psychiatric disorder 2) Current or previous recreational drug use 3) Alcohol consumption above the recommended weekly amount of 21 units 4) Diabetes mellitus in case of interference with endocrine tests and potential effects on brain structure and function 5) Reversed sleep-wake cycle indicating disturbed diurnal rhythm with effects on hormonal pulsatility 5) Craniotomy following injury to avoid brain imaging registration difficulties from such gross changes in brain structure.

3.2.4 Tests for pituitary dysfunction

All patients had measurements of basal anterior pituitary hormone levels (GH, IGF-I (Immulite® 2000), TSH, free T4, free T3, LH, FSH, testosterone (Abbott Architect Ci8200), prolactin, ACTH, cortisol and sex hormone binding globulin (SHBG)). Free androgen index (FAI) was calculated as 100 x total testosterone/SHBG. All patients went on to have full dynamic endocrine testing. Criteria for diagnoses of pituitary dysfunction were as follows:

i) **Hyperprolactinaemia:** two consecutively raised prolactin readings (above the upper reference range) and negative macroprolactin which is an immunological artefact leading to artificially raised prolactin levels (T. P. Smith, Kavanagh, Healy, & McKenna, 2007). In subjects with confirmed biochemical hyperprolactinaemia, an MRI of the pituitary (with gadolinium contrast) was performed and reviewed by an experienced neuroradiologist to rule out an incidental pituitary tumour

ii) **Gonadotrophin deficiency** was defined as a low morning testosterone (<10 nmol/L) with low or non-elevated LH (NR 1.7-12.0 iu/L) and FSH (NR

1.7-8.0 iu/L) given only males were studied. As SHBG affects the bioavailability of circulating sex hormones, if the SHBG level was low (i.e. below lower reference limit of 15 nmol/L), then FAI needed to be <30 for the diagnosis. Primary hypogonadism was diagnosed using s standard approach of low morning testosterone (accounting for SHBG using FAI) with elevated FSH and/or LH.

iii) **GH deficiency** was diagnosed following failure of two dynamic pituitary tests carried out in the morning. The glucagon stimulation test (GST) was used as an initial screening test followed by either the GHRH-Arginine test or Insulin Tolerance Test (ITT)

iv) **ACTH deficiency** was diagnosed on the basis of failure of two dynamic pituitary tests carried out in the morning. All patients had a GST followed by an ITT or overnight Metyrapone Stimulation Test (MST). A Cortisol Day Curve (CDC) was used to help confirm the diagnosis where necessary and assess the need for maintenance hydrocortisone replacement.

Although the ITT is considered the 'gold standard' dynamic test for ACTH and GH deficiency it was not routinely performed here because of the high prevalence or absolute and relative contraindications in TBI populations. 10.5% of the bTBI group and 10.3% of the nbTBI group had an absolute contraindication (history of seizures, ischaemic heart disease/abnormal ECG/cardiac arrhythmias) and a further 21.1% and 53.8% had what we considered to be a relative contraindication (Intracranial post traumatic lesion) in the bTBI and nbTBI groups respectively. We therefore restricted the use of the ITT to those cases where there was an equivocal second test result e.g. difficultly adjusting for BMI in light of bilateral lower limb amputations and we had carefully excluded any contraindications that may have compromised patient safety.

Posterior Pituitary Function

Diabetes insipidus was screened for on the basis of symptoms (polyuria and polydipsia) and paired urine and plasma osmolalities. If thought to be clinically

indicated by the attending physician a water deprivation test was carried out, although this was not done routinely (n =1 in bTBI and n=6 in nbTBI group).

Oxytocin was not measured.

All dynamic endocrine testing was carried out either in a day ward at Charing Cross Hospital or St Mary's Hospital, London. A summary of the algorithm used to define pituitary dysfunction can be seen in **Table 3-1**.

Table 3-1 Summary table showing diagnostic algorithm for pituitary dysfunction

Pituitary Axis	1st Test	Confirmatory test
GH Deficiency	Glucagon Stimulation Test: peak GH < 5µg/L	GHRH-Arginine Test: GH < cut off based on age and BMI{Colao}; OR ITT: peak GH <3 µg/L
ACTH Deficiency	Glucagon Stimulation Test: peak cortisol <350 nmol/L (<12.7 µg/dL)	Metyrapone Test: 11-DOC <200 nmol/L (<6.9 µg/dL) OR if unavailable ACTH <60 ng/L despite cortisol < 200nmol/L (<7.2 µg/dL); OR ITT: peak cortisol <450 nmol/L (<16.3 µg/dL) Supported by AM cortisol <100 nmol/L (<3.62 µg/dL)
Hyperprolactinemia	Prolactin >375 mU/L (NR 75-375)	Repeat prolactin >375 mU/L AND negative Macroprolactin AND normal MRI pituitary with contrast
Gonadotrophin Deficiency	Random testosterone <10 nmol/L (<2.9 ng/mL) OR if SHBG low (<15 nmol/L) FAI <30, AND non-elevated LH (NR 1.7-12.0 IU/L) and FSH (NR 1.7-8.0 IU/L)	Repeat abnormal basal levels using morning (9-10am) sample
TSH Deficiency	Free T4 <9.0 pmol/L (<0.70 ng/dL) OR free T3 <2.5 pmol/L (<0.16 ng/dL), AND non-elevated TSH (NR 0.30-4.22 mU/L)	Repeat abnormal basal levels
ADH (Vasopressin) Deficiency (Diabetes Insipidus)	Symptoms of polyuria or polydipsia	Water Deprivation Test

Abbreviations: BMI (body mass index), FAI (free androgen index =(testosterone/SHBG) x 100, NR (normal range)

3.2.5 Glucagon Stimulation test (GST)

Following an overnight fast, basal blood samples were taken and Glucagon (GlucaGen™ Novo Nordisk Pharmaceuticals 1mg or 1.5 mg if weight >90kg) was administered by IM injection. Blood samples for glucose, serum cortisol and GH were taken at 90, 120, 150 and 180 minutes after glucagon was given via an IV cannula. The majority of subjects (89% bTBI and 70% nbTBI) also had samples taken at 210 and 240 minutes. "Failure" on the test was defined as a peak GH <5 µg/L and cortisol <350 nmol/L (Cegla et al., 2013)

During the course of data collection the assay for cortisol measurements changed in August 2010 from the Immulite®2000 to the Architect i2000. To ensure comparability, linear regression was performed (not shown) to show coefficients of variation of <10% for cortisol levels 83-967 nmol/L.

3.2.6 GHRH-Arginine Test

In the fasted state, patients had blood samples taken for GH and IGF-I measurement at 0 minutes. GHRH (Somatorelin, Ferring) 1 µg/kg was given as a bolus IV injection into one arm followed by the IV infusion 0.5g/kg L-arginine monohydrochloride (Stockport Pharmaceuticals) as a 10 % solution (30g/300mL up to a maximum of 30g) in normal saline over 30 minutes. Further blood samples for GH estimation were taken at +30, 60,90 120 and 150 minutes after the start of the arginine infusion. "Failure" on this test was defined using published age and BMI criteria (Colao et al., 2009). If BMI could not be calculated, in amputees for example, then BMI cut offs in the overweight range were used.

3.2.7 Insulin Tolerance Test (ITT)

In the fasted state, IV insulin Actrapid (NovoNordisk) was administered (0.1 U/kg) though an IV line by an endocrine specialist nurse or physician. Blood samples were taken for GH, cortisol and glucose at 0,30,60,90 and 120 mins. Once target hypoglycaemia of <2.2 mmol/L was achieved, hypoglycaemia was reversed with oral glucose. Abnormal cortisol response was defined as peak cortisol of <450 nmol/L. Severe GH deficiency was defined as a peak GH <3 µg/L (Molitch et al., 2011).

3.2.8 Other pituitary function tests

A cortisol day curve (blood samples of serum cortisol at 0900h, 1200h, 1500h, 1800h and 2100h) was carried in in cases of diagnostic uncertainty. The MST works on the principle that metyrapone causes inhibition of 11 β-hydroxylase (used in the conversion of 11-deoxycortisol to cortisol) which suppresses cortisol. Once a desired threshold of <200 nmol/L is reached, this is thought to stimulate an ACTH drive.

Where carried out, this test was conducted in line with accepted practice (Steiner, Bahr, Exner, & Oelkers, 1994). Subjects were considered to be ACTH sufficient if 11-DOC was >200 nmol/L or where 11-DOC unavailable, ACTH to above 60 ng/L. Where indicated a two stage water deprivation test was carried out in non-fasted subjects using widely accepted methodology (Vokes & Robertson, 1988).

3.2.9 Quality of Life (QoL) assessments

Quality of Life (QoL) assessments were carried out in all individuals in both the bTBI and nbTBI groups. These assessments included the Assessment of GH Deficiency in Adults (AGHDA scale), Beck Depression Inventory Score (BDI-II), Epworth Sleepiness Scale, Pittsburgh Sleep Index, Nottingham Health Profile (NHP) and the Short Form Survey (SF-36). Please see **Methods section** Quality of Life (QoL) assessments **2.3.1 page 73.** for more detailed information.

3.2.10 Neuropsychological Assessments

Each soldier completed a standardised battery of neuropsychological tests previously shown in our group to detect to cognitive impairment following TBI (Kinnunen et al., 2011). For more detailed explanation please see Neuropsychological assessment 2.3.2 page 74. In summary these tests included:

i) The Wechsler Test of Adult Reading (WTAR) to assess pre-morbid intelligence (green)
ii) The People test from the Doors and People Test (Baddeley 2010) to measure associative learning and memory
iii) The Trail Making Test to assess executive functions of set-shifting, inhibitory control and cognitive flexibility.
iv) Verbal and non-verbal reasoning ability via the Wechsler Abbreviated Scale of Intelligence Similarities and Matrix Reasoning subtests (Wechsler 1999).
v) The Colour-Word subtest and Letter fluency from the Delis-Kaplan Executive Function System.

vi) Median reaction time for accurate responses on a simple computerised choice reaction task.

3.2.11 Structural Imaging

Each soldier had standard high-resolution T1 and gradient-echo (T2*) (1.75x1.75x2mm^3) imaging to assess focal brain injury, evidence of microbleeds, superficial siderosis, presence and location of contusions and gross pituitary injury. All structural MR scans were reviewed by a single experienced consultant neuroradiologist.

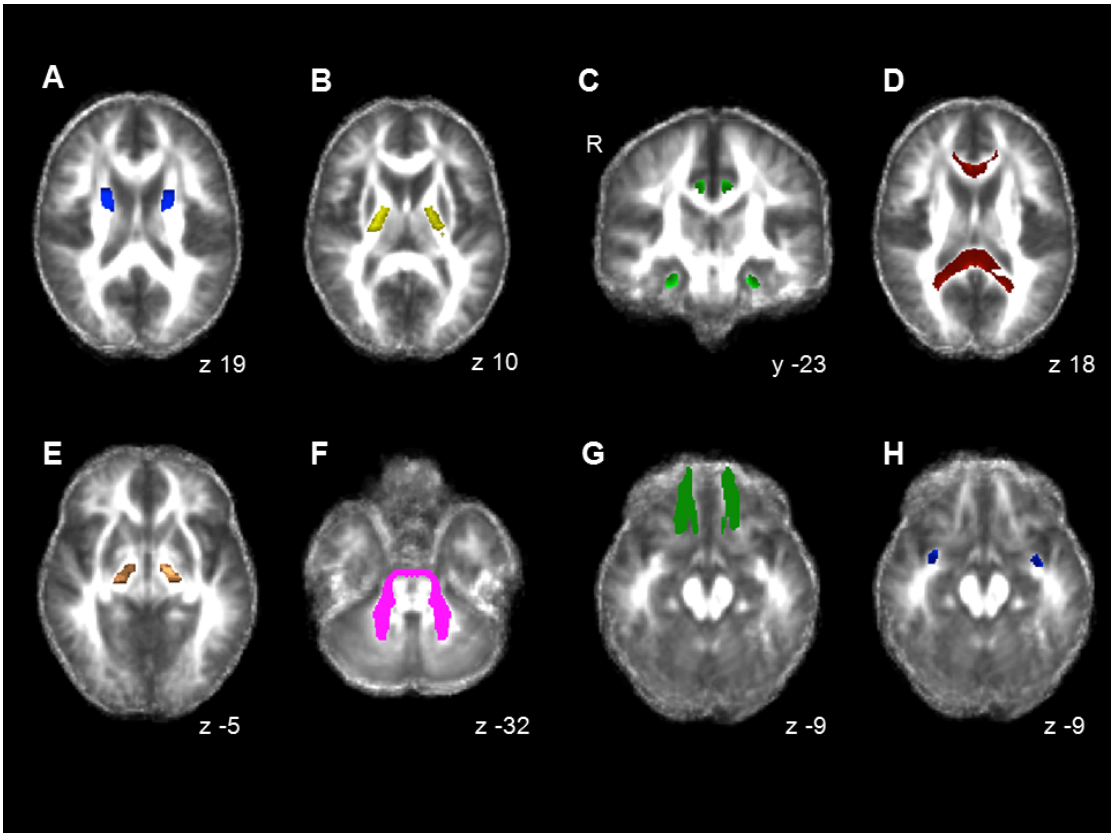
Contusion volume was calculated by converting the T1 images into standard 1mm MNI brain space using FLIRT (FMRIB, University of Oxford, UK) and manually drawing a mask in the z plane.

3.2.12 DTI Analysis

Using individual FA maps, TBSS (see Methods: Tract based spatial statistics (TBSS) 2.3.5 page 75) was used to look for whole brain difference between those with and those without pituitary dysfunction. An FA region of interest analysis was also carried out using the John Hopkins University (JHU) white matter atlas. Ten regions were selected that have previously been shown to be damaged in nbTBI (Kinnunen et al., 2011) as well as mild bTBI (Mac Donald et al., 2011).

These regions were: anterior and posterior internal capsules, cingulum, body/genu and splenium of the corpus callosum, cerebral peduncles, middle cerebellar peduncles, and uncinate fasciuli (**Figure 3-1**). In addition a cerebellum ROI mask was drawn manually and an orbitofrontal white matter ROI mask made using the Washington University, St Louis criteria from the standard MNI152 1mm T1 brain (Mac Donald et al., 2011). A repeated measures ANOVA was performed to assess the overall significance effect of pituitary dysfunction on FA, including group, ROI and group x ROI interaction as independent variables, with post-hoc 2-tailed t-tests for comparison of FA in individual ROIs between groups.

Figure 3-1 White matter tract regions of interest



Regions of interest (ROIs) used for determination of fractional anisotropy (FA) in soldiers after blast traumatic brain injury (bTBI). Individual color masks overlaid onto group average FA map for soldiers with bTBI (n=19) registered into standard MNI space (using MNI co-ordinates). ROIs are: (A) anterior internal capsule, (B) posterior internal capsule, (C) cingulum, (D) corpus callosum, (E) cerebral peduncles, (F) middle cerebellar peduncles, (G) orbitofrontal white matter, (H) uncinate fasciculi. FA was sampled from areas within a white matter skeleton (not shown) produced by tract based spatial statistics (TBSS). (Figure produced by D. Baxter)

3.2.13 Statistical Analyses

Comparisons between groups (nbTBI vs. bTBI; and bTBI with pituitary dysfunction vs. bTBI without pituitary dysfunction) were made using Fisher's exact test for prevalence data and unpaired Student *t* test (FA and neurocognitive variables) or Mann-Whitney U test (other variables) for continuous data (SPSS v 19.0). Significance was defined as $p<0.05$. A group x ROI repeated measure ANOVA was performed to assess the overall effect of pituitary dysfunction on FA.

3.3 Results

3.3.1 Patient Characteristics

All soldiers with bTBI had sustained their injuries by proximity to IED exposure in Afghanistan. All had been wearing full personal protective equipment. In the control nbTBI group, injuries were secondary to road traffic accidents (RTAs; 34%) assaults (32%), falls (23%) and sporting injuries (2%). There was a history of previous mild TBI in the nbTBI group, but not in the bTBI group.

The bTBI and nbTBI groups were generally well-matched in age, gender, ISS whole body injury severity, presence of skull or facial fractures (15.8 vs. 15.4%) and post-traumatic seizures (10.5 vs. 7.7%). The bTBI group had a significantly longer post-traumatic amnesia duration (PTA median 5.5 days vs. 0.5 days, $p = 0.01$); more injuries requiring surgery to or loss of function of major extracranial organs (57.9 vs. 7.7%, $p = 0.002$); more amputations (36.8 vs. 0%, $p < 0.001$); and greater use of strong prescription opiates (47.3 vs. 7.7%, $p < 0.001$). The time from TBI to endocrine testing was significantly longer in the bTBI group (median 15.2 vs. 5.8 months, $p < 0.001$). Demographic data are displayed in **Table 3-2**.

Table 3-2 Patient Characteristics

	units / max score	All nbTBI	All bTBI	P value	bTBI: No Pituitary Dysfunction	bTBI: Pituitary Dysfunction	P value
n		**39**	**19**		**13**	**6**	
Age at TBI	yr.	31.3 [22.5-35.7]	26.7 [26.1-30.9]	0.40	26.6 [24.6-30.6]	29.3 [25.8-36.6]	0.48
		17.2 - 44.8	19.0 - 43.5		19.0 - 36.3	25.0 - 43.5	
Age at testing	yr.	32.3 [23.1-36.7]	28.3 [26.8-32.2]	0.40	28.0 [25.3-31.4]	30.3 [27.4-38.3]	0.32
		19.9 - 45.1	19.6 - 44.7		19.6 - 37.6	26.3 - 44.7	
Time since TBI	mo	5.8 [3.1-11.0]	15.2 [10.8-19.3]	**0.001**	15.2 [8.8-16.6]	17.6 [12.3-20.2]	0.32
		1.9-41.2	4.1 - 23.7		4.1-23.7	4.9-21.9	
ISS	75	25.0 [16.0-32.0]	33.0 [20.0-45.0]	0.17	24.0 [14.5-40.5]	35.5 [27.0-51.3]	0.24
		1-75	9-70		9-45	9-70	
AIS Head	6	5.0 [4.0-5.0]	4.0 [3.0-5.0]	**0.04**	4.0 [2.5-4.0]	5.0 [3.0-5.3]	0.06
		1-6	0-6		0-5	0-6	
AIS Chest	6	0 [0-0]	0 [0-2]	0.11	0 [0-3]	0.5 [0-2.3]	0.83
		0-6	0-4		0-4	0-3	
AIS Abdomen	6	0 [0-0]	0 [0-2]	**0.02**	0 [0-2]	0 [0-2.3]	0.97
		0-3	0-3		0-2	0-3	
GCS	15	14.0 [6.0-14.0] [a]	3.0 [3.0-14.5] [b]	0.24	14.0 [3.0-15.0] [c]	3.0 [3.0-3.0] [d]	0.19
		3-15	3-15		3-15	3-3	
PTA	days	0.5 [0-7.3] [e]	5.5 [0.8-22.8]	**0.01**	3.0 [0-19.3]	15.5 [6.3-31.5]	0.10
		0-42	0-84		0-84	4-42	
PTA>24 hrs		20 (51.3%)	13 (68.4%)	0.27	7 (58.3%)	6 (100%)	0.11
BMI	kg/m^2	24.7 [22.4-29.4]	26.7 [24.5-28.9]	0.28	26.6 [24.5-28.7] [f]	25.5 [22.4-32.0] [g]	0.79
		17.0-33.4	21.7-33.7		23.6-29.4	21.7-33.7	
Limb amputation		0 (0%)	8 (42.1%)	**<0.001**	6 (46.1%)	2 (33.3%)	1.00
Major organ damage		3 (7.7%)	11 (57.9%)	**<0.001**	7 (53.9%)	4 (66.7%)	1.00
Skull/facial fracture		6 (15.4%)	3 (15.8%)	1.0	0 (0%)	3 (50.0%)	**0.02**
Opiate use		3 (7.7%)	9 (47.3%)	**0.001**	6 (46.2%)	3 (50.0%)	1.00
Anti-depressant use		5 (12.8%) [h]	10 (52.7%) [i]	**0.003**	7 (53.8%) [j]	3 (50.0%) [k]	1.00
Seizures post TBI		3 (7.7%) [l]	2 (10.5%) [m]	1.0	1 (7.7%) [n]	1 (16.7%) [o]	1.00
Primary hypogonadism		1 (2.6%) [p]	4 (21.1%) [q]	**0.04**	4 (30.8%) [q]	0 (0%) [q]	0.26

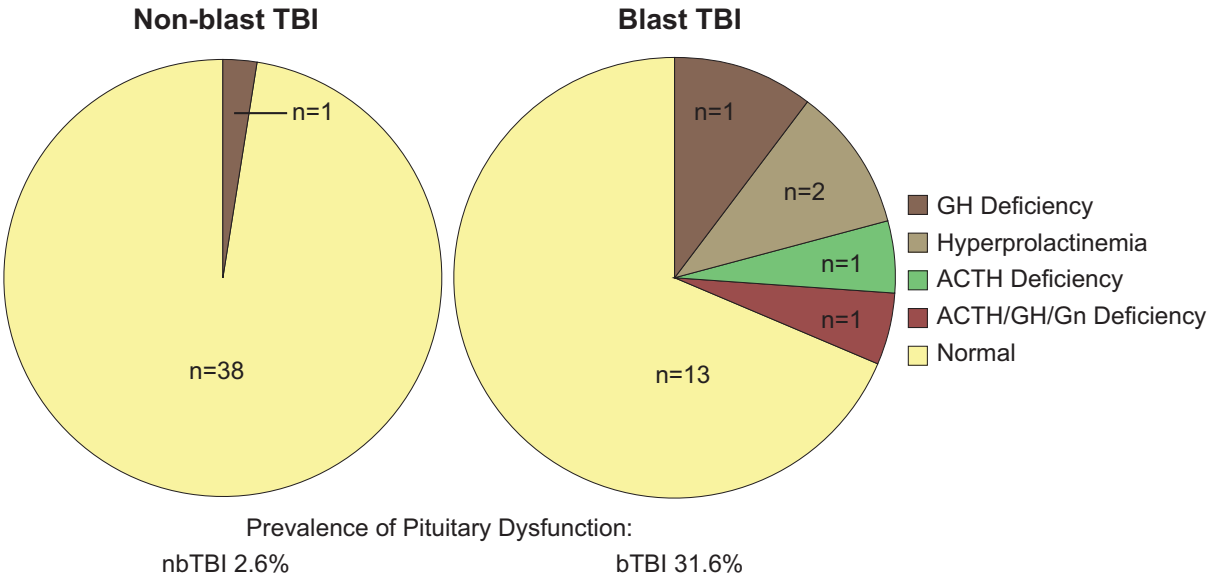
Data are expressed as median [interquartile range], range, or No. (%). Probability values are from Mann–Whitney U test or Fisher exact test between groups. [a]Statistically significant; $p < 0.05$. Data available for [b]n 5 16, [c]n 5 9, [d]n 5, [e]n 5 4, [f]n 5 38, and due to amputations: [g]n 5 7, [h]n 5 4. For analgesic purposes only in: [i]n 5 5 (12.8%), [j]n 5 6 (31.6%), [k]n 5 4 (30.8%), [l]n 5 2 (33.3%). For depression itself in: [i]n 5 0 (0%), [j]n 5 4 (21.1%), [k]n 5 3 (23.1%), [l]n 5 1 (16.7%). On antiepileptic drugs in [m]n 5 3, [n]n 5 1, [o]n 5 0, [p]n 5 1. [q]Not due to trauma. [r]Due to perineal

trauma. AIS 5 Abbreviated Injury Score; BMI 5 body mass index; bTBI 5 blast traumatic brain injury; GCS 5 Glasgow Coma Scale; ISS 5 Injury Severity Score; nbTBI 5 nonblast TBI; PTA 5 post-traumatic amnesia.

3.3.2 Prevalence of Pituitary Dysfunction in bTBI

Six of 19 soldiers with bTBI (31.6%) had confirmed anterior pituitary dysfunction. Of these, two soldiers (10.5%) had hyperprolactinaemia, 2 (10.5%) had isolated GH deficiency, 1 (5.3%) had isolated ACTH deficiency and 1 (5.3%) had combined ACTH, GH and gonadotrophin deficiencies. Within the nbTBI control group, only 1 of 39 subjects had pituitary dysfunction (isolated GH deficiency) (p=0.004 in bTBI vs. nbTBI). No patient in either group had TSH deficiency or diabetes insipidus. (**Figure 3-2**).

Figure 3-2 Pie Chart showing the prevalence of pituitary dysfunction in bTBI vs. nbTBI



The 3 soldiers with GH deficiency had IGF-I levels in the low normal range (**Table 3-3**) and the 2 soldiers with ACTH deficiency had normal early morning cortisol levels on initial assessment of 287 to 292 nmol/L. However, on subsequent cortisol day curves, both subjects with ACTH deficiency had low cortisol levels consistent with the diagnosis that was then confirmed with a second-line confirmatory test (**Table 3-4**).

None of the soldiers with ACTH deficiency had any history of hypotension, hypoglycaemia or hyponatraemia.

Additional endocrine findings included the finding of primary hypogonadism in 4 out of 19 soldiers with bTBI (21.2%). None of these individuals had pituitary dysfunction and the primary nature of the deficiency was likely due to perineal/testicular blast injury. They were already on testosterone at the time of testing (**Table 3-5**).

3.3.3 Comparison of soldiers with and without Pituitary Dysfunction.

There was no significant difference in age, time since injury, ISS, abdominal AIS, BMI (but could not be adequately assessed in n=8 who had limb amputations), major organ damage, prevalence of amputations, seizures and use of opiates or antidepressants (**Table 3-6**). The medications recorded for each soldier at time of research visit are displayed in **Table 3-7**.

There were trends for the AIS head injury scores to be higher (p=0.06) and duration of PTA to be longer (15.5 vs. 3.0 days, p=0.10) in those soldiers with pituitary dysfunction after bTBI than in those without.

There was a significantly higher presence of skull/facial fractures in the soldiers with pituitary dysfunction compared to those without (50 vs. 0%, p=0.02) (**Table 3-6**).

Table 3-3 Growth hormone-IGF-I axis in blast traumatic brain injury

		GROWTH HORMONE / IGF-1 AXIS										
		Glucagon Stimulation Test					GHRH-Arginine Test				Insulin Tolerance Test	
ID	Summary of Pituitary Dysfunction	IGF-I	IGF-I age related NR	IGF-I median of NR	IGF-I ratio to median	Peak GH	IGF-I	BMI	GH cut off	Peak GH	IGF-I	Peak GH
	Units	nmol/L	nmol/L	nmol/L	n/a	µg/L	nmol/L	kg/m^2	µg/L	µg/L	nmol/L	µg/L
	Normal range					>5						>5
No Pituitary Dysfunction n=13												
M02	Nil	22.6	14.2-36.9	22.9	0.72	6.55	n/a	n/a	n/a	n/a	23.5	52.10
M04	Nil	58.0	15.2-42.8	25.5	1.84	13.10	n/a	n/a	n/a	n/a	n/a	n/a
M05	Nil	19.9	15.2-42.9	25.5	0.63	11.60	n/a	n/a	n/a	n/a	n/a	n/a
M09	Nil	29.0	18.3-62.8	33.9	0.62	0.98	18.7	n/a	11.7	17.20	n/a	n/a
M11	Nil	31.9	16.5-55.1	30.2	1.01	1.56	31.9	26.6	11.7	37.80	n/a	n/a
M12	Nil	38.1	15.2-42.8	25.5	1.21	0.64	38.1	n/a	8.1	13.50	n/a	n/a
M13	Nil	74.5	15.1-46.5	26.4	2.37	0.69	ND	n/a	11.7	17.30	n/a	n/a
M15	Nil	27.4	15.2-42.8	25.5	0.87	2.84	30.0	n/a	8.1	23.30	n/a	n/a
M16	Nil	31.3	14.2-36.9	22.9	0.99	3.17	29.8	28.7	8.1	27.00	n/a	n/a
M17	Nil	19.9	15.2-42.8	25.5	0.63	8.66	n/a	n/a	n/a	n/a	n/a	n/a
M18	Nil	17.9	15.2-42.8	25.5	0.57	2.65	19.9	n/a	8.1	10.00	n/a	n/a
M19	Nil	29.6	15.2-42.8	25.5	0.94	5.96	n/a	n/a	n/a	n/a	n/a	n/a
M20	Nil	17.2	15.0-39.9	24.4	0.55	7.96	n/a	n/a	n/a	n/a	n/a	n/a
	Median [IQR]	29.0 [19.9-35.0]			0.87 [0.63-1.11]	3.17 [1.27-8.31]	29.9 [19.6-33.5]		17.30 [13.50-27.00]			
	Range	17.2-74.5			0.55-2.37	0.64-13.10	19.0-38.0			10.0-38.0		
Pituitary dysfunction n=6												
M01	PRL	21.6	15.2-42.8	25.5	0.69	6.97	n/a	n/a	n/a	n/a	n/a	n/a
M03	ACTH	26.7	15.2-42.8	25.5	0.85	0.19	32.1	31.6	8.1	15.40	n/a	n/a
M07	GH	23.7	15.0-39.9	24.4	0.75	2.66	26.1	26.1	5.5	3.43	n/a	n/a
M08	ACTH/GH/Gn	16.1	13.1-34.7	21.3	0.66	0.08	16.6	n/a	8.1	4.26	ND	0.18
M10	PRL	18.9	15.2-42.8	25.5	0.60	2.78	29.4	24.3	8.1	25.80	n/a	n/a
M14	GH	18.2	15.2-42.8	25.5	0.58	0.05	21.8	33.7	5.5	2.70	n/a	n/a
	Median [IQR]	20.3 [17.7-24.5]			0.68 [0.60-0.78]	1.43 [0.07-3.83]	26.1 [19.2-30.8]			4.26 [3.07-20.6]		
	Range	16.1-26.7			0.58-0.85	0.05-6.97	16.6-32.1			2.70-25.80		
	P value	0.07			0.28	0.09	0.54			0.11		

Abnormal values in grey shading

Table 3-4 ACTH-cortisol axis in blast traumatic brain injury

		ACTH CORTISOL AXIS									
		Glucagon Stress Test			Day Curve	Metyrapone Test (post levels)			Insulin Tolerance Test		
ID	Summary of Pituitary Dysfunction	Basal Cortisol [a]	Peak Cortisol [a]	Basal ACTH	Cortisol [a]	Cortisol [a]	ACTH	11-DOC [b]	Basal Cortisol [a]	Peak Cortisol [a]	CBG
	Units	nmol/L	nmol/L	ng/L	nmol/L	nmol/L	ng/L	nmol/L	nmol/L	nmol/L	µg/L
	Normal Range	100-500	>350	<50	09, 12, 15, 18, 21h	<200	>60	>200	100-500	>500	27.1-52.3
No Pituitary Dysfunction n=13											
M02	Nil	230	230	6.0	304, 208, 226, 295, ND	n/a	n/a	n/a	544	636	48.9
M04	Nil	283	378	27.4	395, 153, 136, 72, 54	123	247	244.5	n/a	n/a	n/a
M05	Nil	309	494	39.2	n/a	n/a	n/a	n/a	n/a	n/a	n/a
M09	Nil	335	389	15.3	n/a	n/a	n/a	n/a	n/a	n/a	n/a
M11	Nil	323	473	38.0	n/a	n/a	n/a	n/a	n/a	n/a	n/a
M12	Nil	192	478	17.2	n/a	n/a	n/a	n/a	n/a	n/a	n/a
M13	Nil	363	386	24.6	n/a	n/a	n/a	n/a	n/a	n/a	n/a
M15	Nil	376	497	ND	n/a	n/a	n/a	n/a	n/a	n/a	n/a
M16	Nil	427	427	92.7	n/a	n/a	n/a	n/a	n/a	n/a	n/a
M17	Nil	207	626	20.0	n/a	n/a	n/a	n/a	n/a	n/a	n/a
M18	Nil	409	533	27.0	n/a	n/a	n/a	n/a	n/a	n/a	n/a
M19	Nil	220	435	19.7	n/a	n/a	n/a	n/a	n/a	n/a	n/a
M20	Nil	420	420	28.6	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	Median [IQR]	323.0 [225.0-392.5]	435.0 [387.5-495.5]	25.8 [17.8-35.7]							
	Range	192-427	230-626	6.0-92.7							
Pituitary dysfunction n=6											
M01	PRL	606	606	164.0	n/a	n/a	n/a	n/a	n/a	n/a	n/a
M03	ACTH	292	292	23.2	67, 50, <20, 28, <20	38	22	87.1	n/a	n/a	63.0
M07	GH	419	419	24.8	n/a	n/a	n/a	n/a	n/a	n/a	n/a
M08	ACTH/GH/Gn	287	287	18.7	204, 86, 100, 44, 22	n/a	n/a	n/a	110	268	74.0
M10	PRL	109	350	20.6	422, 333, 200, 260, 79	ND	207	200.0	n/a	n/a	55.7
M14	GH	88	445	32.2	200 at 13h	n/a	n/a	n/a	n/a	n/a	n/a
	Median [IQR]	289.5 [103.8-465.8]	384.5 [290.8-485.3]	24.0 [20.1-65.2]							
	Range	88-606	287-606	18.7-164.0							
	P value	0.70	0.28	0.75							

Abnormal values in grey shading

Table 3-5 Pituitary-gonadal axis, pituitary-thyroid axis and prolactin in blast traumatic brain injury

		GONADOTROPHIN / TESTOSTERONE AXIS						THYROID			PROLACTIN
ID	**Summary of Pituitary Dysfunction**	**LH**	**FSH**	**Testosterone**	**SHBG**	**Free Androgen Index**	**Primary Hypogonadism**	**Free T4**	**Free T3**	**TSH**	**Prolactin**
	Units	IU/L	IU/L	nmol/L	nmol/L			pmol/L	pmol/L	mU/L	mU/L
	Normal range	2.0-12.0	1.7-8.0	10.0-30.0	15-55	30-150		9.0-26.0	2.5-5.7	0.3-4.2	75-375
No Pituitary Dysfunction n=13											
M02	Nil	2.4	1.1	23.7	55.0	43.1	No	15.6	4.4	0.46	97
M04	Nil	4.7	3.9	21.0	33.0	63.6	No	12.2	5.7	2.11	146
M05	Nil	4.4	2.5	15.5	18.0	86.1	No	15.3	3.2	1.78	118
M09	Nil	1.2	4.8	39.5	18.0	219.4	Yes	13.4	5.8	1.04	219
M11	Nil	1.6	0.7	12.5	18.0	69.4	No	14.0	4.4	1.43	285
M12	Nil	18.5	36.4	6.2	10.0	62.0	Yes	15.5	3.5	1.21	177
M13	Nil	5.8	9.6	13.1	10.0	131.0	No	13.2	5.2	1.33	240
M15	Nil	40.3	60.3	11.6	18.0	64.4	Yes	14.0	6.6	0.77	136
M16	Nil	5.5	4.9	22.3	24.0	92.9	No	13.4	4.4	0.96	200
M17	Nil	4.7	3.3	25.2	39.0	64.6	No	16.9	5.2	1.13	131
M18	Nil	0.0	0.1	12.3	19.0	64.7	Yes	18.5	4.8	1.25	312
M19	Nil	2.3	1.5	22.0	28.0	78.6	No	15.0	4.7	2.06	183
M20	Nil	3.8	3.6	28.8	32.0	90.0	No	13.2	4.6	0.70	330
	Median [IQR] or n (%)	4.4 [2.0-5.6]	3.6 [1.3-7.3]	21.0 [12.4-24.5]	19.0 [18.0-32.5]	69.4 [64.0-91.5]	4 (30.8%)	14.0 [13.3-15.6]	4.7 [4.4-5.5]	1.21 [0.87-1.61]	183 [134-263]
	Range	0-40.3	0.1-60.3	6.2-39.5	10.0-55.0	43.1-219.4		12.2-18.5	3.2-6.6	0.46-2.11	97-330
Pituitary dysfunction n=6											
M01	PRL	1.8	2.5	21.7	27.0	80.4	No	17.3	4.6	4.18	619
M03	ACTH	1.5	1.2	23.8	35.0	68.0	No	16.0	5.5	1.11	126
M07	GH	3.7	2.4	13.1	24.0	54.6	No	14.4	4.9	1.81	172
M08	ACTH/GH/Gn	1.3	1.8	2.0	18.0	11.1	No	15.5	4.3	1.19	199
M10	PRL	2.6	1.3	22.8	26.0	87.7	No	10.8	4.7	2.29	439
M14	GH	6.5	3.9	22.4	33.0	67.9	No	12.9	4.0	0.90	216
	Median [IQR] or n (%)	2.2 [1.5-4.4]	2.1 [1.3-2.9]	22.1 [10.3-23.1]	26.5 [22.5-33.5]	68.0 [43.7-82.2]	0 (0%)	15.0 [12.4-16.3]	4.7 [4.2-5.1]	1.50 [1.06-2.76]	208 [161-484]
	Range	1.3-6.5	1.2-3.9	2.0-23.8	18.0-35.5	11.1-87.7		10.8-17.3	4.0-5.5	0.90-4.18	126-619
	P value	0.37	0.28	0.97	0.42	0.47	0.26	0.90	0.70	0.31	0.47

Abnormal values in grey shading

Table 3-6 Characteristics of soldiers with blast TBI

Subjects	Age at TBI	Age at GST	Time since TBI	ISS	AIS Head	AIS Chest	AIS Abdo	GCS	PTA	BMI at GST	PTA >24 hrs	Limb amputation	Major organ damage	Skull/facial fracture	Opiate use	Antidepressant use	Seizures
Units / Maximum score	Years	Years	Months	75	6	6	6	15	Days	kg/m^2							
No pituitary dysfunction (n=13)																	
M02	36.3	37.6	15.2	20	4	0	2	3	1	25.4	No	No	No	No	Yes	Yes	No
M04	26.4	27.6	14.2	24	0	4	0	n/a	4	27.7	Yes	No	Lung/eye	No	No	No	No
M05	27.3	28.6	15.2	24	0	4	0	n/a	28	24.5	Yes	No	No	No	Yes	No	No
M09	19.0	19.6	6.7	45	4	0	2	n/a	4	n/a	Yes	Yes	Perineum	No	Yes	Yes *	No
M11	19.3	20.9	16.6	25	5	0	0	3	84	26.6	Yes	No	No	No	No	No	Yes
M12	30.2	30.5	4.1	33	2	0	2	n/a	0	n/a	No	Yes	Perineum	No	Yes	Yes *	No
M13	22.8	23.7	10.8	45	4	0	0	n/a	21	n/a	Yes	Yes	Eye/Skin	No	No	Yes *	No
M15	26.4	26.8	4.1	45	4	0	2	15	0	n/a	No	Yes	Eye/Skin/perineum	No	Yes	Yes *	No
M16	34.7	36.7	23.7	24	4	2	0	15	0	28.7	No	No	No	No	Yes	Yes	No
M17	26.6	28.0	16.6	9	3	0	0	14	0	23.6	No	No	No	No	No	No	No
M18	26.7	28.3	20.2	36	4	4	2	n/a	14	n/a	Yes	Yes	Lung/colon/perineum	No	No	No	No
M19	26.6	27.7	13.6	9	3	0	0	n/a	n/a	n/a	n/a	Yes	Skin	No	No	No	No
M20	30.9	32.2	15.4	9	3	0	0	n/a	2	29.4	Yes	No	No	No	No	Yes	No
median	26.6	28.0	15.2	24.0	4.0	0	0		3.0	26.6	7 (58.3%)	6 (46.1%)	7 (53.9%)	0 (0%)	6 (46.2%)	7 (53.8%)	1 (7.7%)
IQR	[24.6-30.6]	[25.3-31.4]	[8.8-16.6]	[14.5-40.5]	[2.5-4.0]	[0-3]	[0-2]		[0-19.3]	[24.5-28.7]							
Pituitary dysfunction (n=6)																	
M01 (PRL)	30.0	30.4	4.9	33	5	0	0	3	4	21.7	Yes	No	No	Yes	Yes	Yes *	Yes #
M03 (ACTH)	25.0	26.3	15.9	70	6	0	3	3	17	n/a	Yes	Yes	Spleen/Liver	Yes	No	Yes *	No
M07 (GH)	34.3	36.2	21.9	38	5	3	0	3	7	26.7	Yes	No	Lung	No	No	No	No
M08 (ACTH/GH/Gn)	43.5	44.7	14.7	45	4	2	0	n/a	42	n/a	Yes	Yes	No	No	Yes	No	No
M10 (PRL)	28.5	30.1	19.3	33	5	0	2	n/a	28	24.3	Yes	No	Eye/Liver/lung	Yes	No	No	No
M14 (GH)	26.1	27.7	19.6	9	0	1	0	3	14	33.7	Yes	No	Skin	No	Yes	Yes	No
median	29.3	30.3	17.6	35.5	5	0.5	0		15.5	25.5	6 (100%)	2 (33.3%)	4 (66.7%)	3 (50.0%)	3 (50%)	3 (50%)	1 (16.7%)
IQR	[25.8-36.6]	[27.4-38.3]	[12.3-20.2]	[27.0-51.3]	[3.0-5.3]	[0-2.3]	[0-2.3]		[6.3-31.5]	[22.4-32.0]							
P value	0.48	0.32	0.32	0.24	0.06	0.83	0.97		0.10	0.79	0.11	1.00	1.00	**0.02**	1.00	1.00	1.00

All data expressed as median [interquartile range] or n (%)

* for analgesia only, # on anti-epileptic drug

Abbreviations: AIS: Abbreviated Injury Score, BMI: bodymass index, GCS: Glasgow Coma Scale, GST: Glucagon stimulation test, ISS: Injury Severity Score, n/a: not available, PTA: Post traumatic amnesia.

Table 3-7 Medications used by soldiers with blast TBI

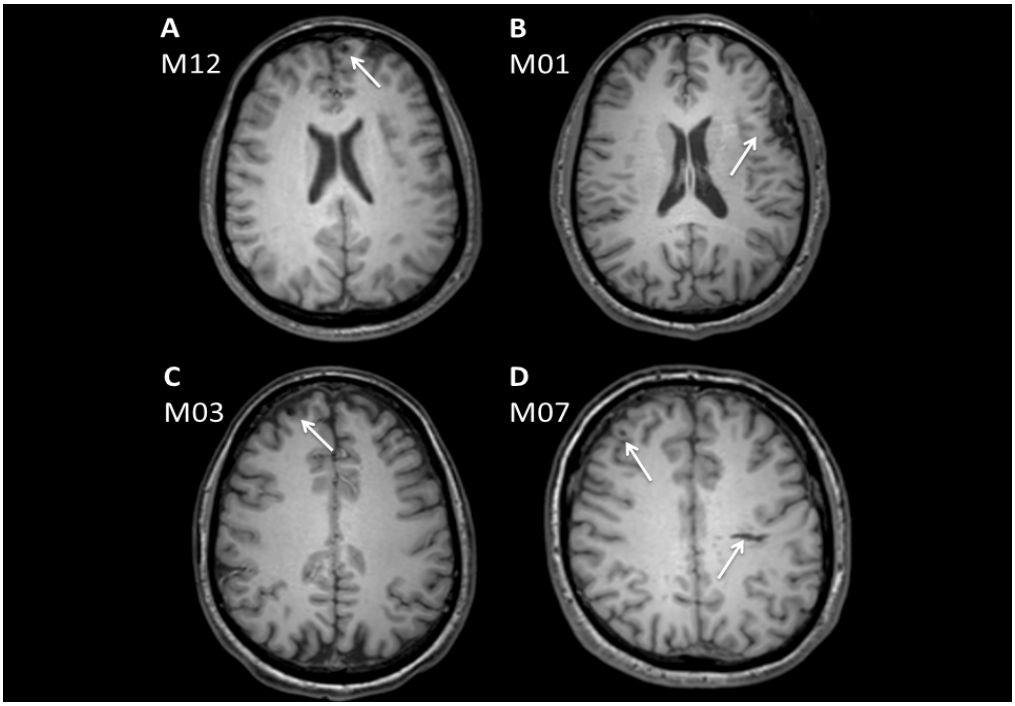
Subjects	Medications
No pituitary dysfunction (n=13)	
M02	Diclofenac, Sertraline, Tramadol
M04	Co-codamol
M05	Diclofenac, Tramadol
M09	Amitriptyline, MST, Nebido, Pregabalin
M11	None
M12	Amitriptyline, Diclofenac, Nebido, Pregabalin, Ranitidine, Sildenafil, Tramadol
M13	Amitriptyline, Baclofen, Pregabalin
M15	Amitriptyline, Nebido, Pregabalin, Tramadol
M16	Mirtazepine, Paracetamol, Pregabalin, Tramadol, Zopiclone
M17	None
M18	Nebido
M19	Diclofenac, Pregabalin, Ranitidine
M20	Sertraline, Zopiclone
Pituitary dysfunction (n=6)	
M01 (PRL)	Amitriptyline, Diclofenac, MST, Phenytoin
M03 (ACTH)	Amitriptyline, Erythromycin, Gabapentin
M07 (GH)	None
M08 (ACTH/GH/Gn)	Diclofenac, Lansoprazole, MST, Paracetamol, Pregabalin, Tramadol
M10 (PRL)	Betnovate ointment, Co-codamol
M14 (GH)	Amitriptyline, Diclofenac, Fluoxetine, Mirtazepine, MST, Paracetamol, Pregabalin, Salbutamol inhaler, Temazepam, Zopiclone

Abbreviations: MST morphine sulphate

3.3.4 Neuroimaging Results

Three of the 5 (50.0%) soldiers with pituitary dysfunction, compared to only 1 of the 13 (7.7%) soldiers without pituitary dysfunction, had contusions on brain MRI scans (p=0.07). However, overall, contusion volume was small (<10 cm^3). (**Figure 3-3**).

Figure 3-3 Intracerebral contusions following bTBI



High resolution T1 brain scans (axial sections) in subject space showing contusions (arrows) in soldiers after blast TBI (A) without pituitary dysfunction, and (B-D) with pituitary dysfunction. Total contusion volumes for these patients were: (A) 0.2, (B) 9.1, (C) 0.6, (D) 1.0 cm3. Produced by D Baxter

No hypothalamic-pituitary abnormalities were seen on MRI brain scans in any soldiers in the bTBI group. There were also no abnormalities detected on the 4/6 soldiers with pituitary dysfunction who had dedicated contrast-enhanced MRI pituitary scans. The prevalence of structural abnormalities in soldiers with and without pituitary dysfunction are displayed in **Table 3-8**.

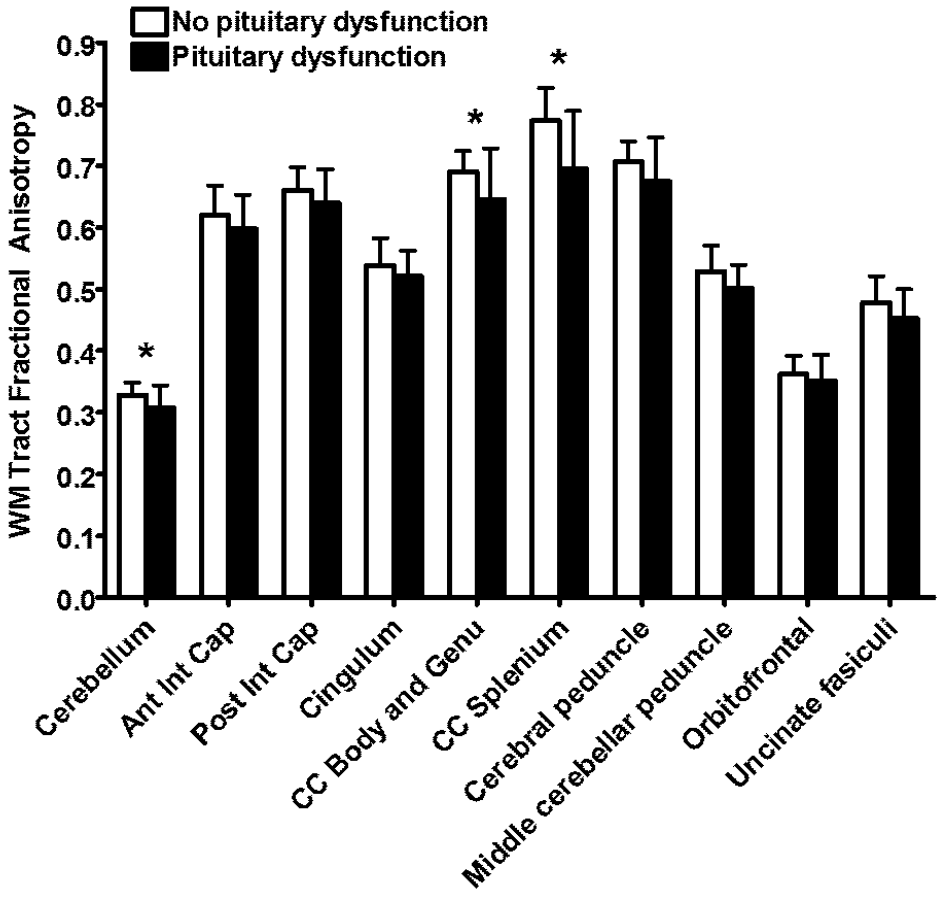
Table 3-8 Prevalence of structural abnormalities in soldiers with and without pituitary dysfunction

	No pituitary dysfunction	Pituitary dysfunction	P
n	13	6	
Acute CT brain			
Extra-dural hemorrhage	0 (0%)	0 (0%)	n/a
Sub-dural hemorrhage	0 (0%)	0 (0%)	n/a
Traumatic sub-arachnoid or intra-ventricular hemorrhage	0 (0%)	0 (0%)	n/a
Diffuse swelling	1 (7.7%)	0 (0%)	1.00
Study MRI brain			
Contusion	1 (7.7%)	3 (50.0%)	0.07
Siderosis	3 (23%)	1 (16.6%)	1.00
Microbleeds	7 (53%)	3 (50%)	1.00
Gliosis	0 (0%)	1 (16.6%)	0.32
Hypo-pituitary damage	0 (0%)	0 (0%)	n/a
MRI pituitary with contrast	ND	4 normal, 2 ND	n/a

Data given as n (%)
Abbreviations: n/a: not applicable, ND: not done

DTI FA analysis showed a reduction in FA depending on the ROI, indicating greater white matter damage, in those soldiers with pituitary dysfunction after bTBI compared to those without ($p=0.14$ effect of group, $p=0.02$ group x ROI interaction). Planned post hoc analysis showed significantly lower FA values for those soldiers with pituitary dysfunction within the cerebellum ($p<0.05$), and body/genu ($p < 0.05$) and splenium ($p=0.01$) of the corpus callosum (**Figure 3-4**).

Figure 3-4 Pituitary dysfunction and FA measurements in various WM tracts



*Lower fractional anisotropy was seen in a priori white matter tract regions of interest in soldiers with pituitary dysfunction after blast traumatic brain injury (black, n 5 6) compared to those without pituitary dysfunction (white, n 5 13). Data are expressed as mean+ standard deviation. *p < 0.05 (unpaired t test). Ant=anterior; CC=corpus callosum; Cap=capsule; Int=internal; Post=posterior;WM=white matter. Produced by D Baxter*

3.3.5 Quality of Life and Cognitive Function

Soldiers with bTBI appeared to have worse scores for physical activity and daily living problems than those who had a nbTBI. There were trends for higher reporting of pain and deleterious change in health in the soldiers vs. civilians. However there were no differences in mood or sleep disturbance between the groups.

Within the soldier group there was a trend towards lower mood and higher AGHDA scores (consistent with lower mood) in those subjects with pituitary dysfunction vs. those without. There were also trends towards worse measures of QoL and symptom scores in several domains relating to social and emotional functioning and fatigue **Table 3-9**.

The bTBI subjects with pituitary dysfunction had significantly worse average current verbal intellectual ability than those without pituitary dysfunction and significantly worse cognitive impairment in the domains of visual/naming/reading/processing speed, verbal fluency, and information processing. This was despite there being no difference in premorbid intelligence between the two groups as measured by the Wechsler Test of Adult Reading **Table 3-10.**

Table 3-9 Quality of life and symptom questionnaires in non-blast and blast traumatic brain injury

Quality of Life / Symptom Assessment		nbTBI	All bTBI	P value nbTBI vs. bTBI	bTBI: No Pituitary Dysfunction	bTBI: Pituitary Dysfunction	P value no pit dys vs. pit dys
n		38	18 [h]		12 [h]	6	
Assessment of GH Deficiency in Adults (AGHDA)		9.5 [5.8-14.5] [a]	16.0 [4.0-18.5] [b]	0.22	14.0 [3.0-17.0]	17.5 [16.0-19.5]	0.10
Beck Depression Inventory Score (BDI-II)		11.0 [7.0-20.0] [c]	20.5 [4.0-24.5]	0.30	11.5 [1.8-21.8]	24.5 [20.3-26.3]	0.08
Epworth Sleepiness Scale		7.0 [2.0-12.0] [d]	7.0 [2.5-11.0]	0.89	6.0 [1.5-10.5]	10.0 [3.0-16.5]	0.25
Pittsburgh Sleep Index		ND	10.0 [2.8-16.0]		4.5 [2.0-16.3]	12.0 [8.0-15.5]	0.37
NHP	Energy Levels	39.0 [0-100] [e]	49.0 [18.0-100]	0.63	49.0 [0-94.0]	68.5 [24.0-100]	0.39
NHP	Pain	0 [0-48] [e]	28.5 [9.7-52.5]	0.08	24.0 [14.0-51.0]	36.0 [0-54.5]	0.96
NHP	Emotional Reactions	20.0 [10.0-47.0] [e]	32.5 [6.7-55.5]	0.71	15.0 [0-46.2]	54.0 [25.0-67.7]	0.10
NHP	Sleep	22.0 [0-73.0] [e]	55.0 [0-100]	0.20	30.0 [0-93.2]	64.0 [31.7-100]	0.29
NHP	Social Isolation	0 [0-45.0] [e]	21.0 [0-59.0]	0.52	0 [0-48.5]	29.0 [21.2-69.0]	0.13
NHP	Physical Activity	0 [0-21.8] [e]	26.5 [11.0-42.0]	**0.02**	26.5 [13.2-42.0]	27.0 [0-60.7]	0.96
NHP	Average	22.0 [5.4-41.0] [e]	41.5 [15.5-55.2]	0.09	28.5 [9.5-55.0]	48.5 [38.7-55.2]	0.25
NHP	Daily Living Problems (0-7)	2.0 [0-5.0] [f]	5.0 [3.5-6.0]	**0.04**	4.5 [2.3-5.8]	4.5 [3.8-6.3]	0.62
SF-36	Physical functioning	85.0 [60.0-95.0] [e]	52.5 [27.5-81.3]	0.21	52.5 [41.3-58.8]	60.0 [27.5-88.8]	0.82
SF-36	Role limitations due to physical health	12.5 [0-62.5] [g]	12.5 [0-75.0]	0.94	25.0 [0-93.8]	0 [0-18.8]	0.10
SF-36	Role limitations due to emotional problems	67.0 [0-100] [g]	67.0 [24.8-100]	0.90	83.5 [33.0-100]	50.0 [0-100]	0.49
SF-36	Energy/Fatigue	50.0 [35-60] [e]	42.5 [33.8-66.3]	0.92	52.5 [36.3-70.0]	37.5 [26.3-47.0]	0.15
SF-36	Emotional well being	64.0 [52.0-80.0] [e]	60.0 [48.0-81.0]	0.57	68.0 [53.0-83.0]	58.0 [31.0-66.3]	0.34
SF-36	Social functioning	63.0 [38.0-75.0] [e]	50.0 [38.0-75.0]	0.66	56.5 [41.0-84.8]	44.0 [22.0-63.0]	0.18
SF-36	Pain	55.0 [33.0-88.0] [e]	45.0 [33.0-70.5]	0.68	68.0 [35.5-75.5]	33.0 [23.0-58.8]	0.21
SF-36	Health change	50.0 [38.0-75.0] [e]	32.5 [25.0-50.0]	0.06	25.0 [25.0-50.0]	45.0 [25.0-56.3]	0.49
SF-36	General health	50.0 [25.0-75.0] [e]	50.0 [25.0-61.3]	0.49	50.0 [27.5-63.8]	40.5 [18.8-61.3]	0.55

Table 3-10 Cognitive impairment in soldiers with pituitary dysfunction vs. those without pituitary dysfunction

Cognitive domain	Cognitive variable	No pituitary dysfunction	Pituitary dysfunction
		n=13	n=6
Pre-morbid intelligence: reading ability	WTAR raw score	35.9 ± 11.7	34.7 ± 14.6
Intellectual ability	WASI similarities (verbal)	32.6 ± 6.2	27.0 ± 4.1 *
	WASI matrix reasoning (non-verbal)	24.4 ± 7.5	24.2 ± 6.0
Memory: associative memory	People test immediate recall	22.6 ± 8.1	25.0 ± 7.8
Processing speed: visual search/complex	Trail Making Test Trail A (s)	23.1 ± 5.7	28.7 ± 5.2 *
	Trail Making Test Trail B (s)	47.9 ± 14.5	53.8 ± 12.2
Processing speed: naming/reading	Stroop Colour Naming (s)	32.5 ± 9.1	51.0 ± 29.7 *
	Stroop Word Reading (s)	24.3 ± 6.7	37.2 ± 13.6 **
Executive function: alternating-switch cost	Trail Making Test Trail B minus A (s)	24.8 ± 13.5	25.2 ± 9.0
Executive function: cognitive flexibility	Color Word Stroop Inhibition/switching (s)	70.5 ± 24.2	86.3 ± 30.8
	Inhibition/switching minus a baseline of	30.0 ± 18.8	26.5 ± 8.5
	color naming and word reading (s)		
Word generation fluency	DKEFS Letter Fluency F+A+S total	40.1 ± 12.9	28.8 ± 3.6 *
Information processing	Choice reaction task median reaction time (ms)	413 ± 38	473 ± 31 *

*Worse cognitive function in soldiers after blast TBI with pituitary dysfunction (n=6) compared to those without pituitary dysfunction (n=13). Data expressed as mean ± SD. *P<0.05, **P<0.005 (unpaired t-test). Abbreviations: DKEFS: Delis-Kaplan Executive Function System, WASI: Wechsler Abbreviated Scale of Intelligence Similarities and Matrix Reasoning subsets, WTAR: Wechsler Test of Adult Reading. Table produced by D Baxter*

3.4 Discussion

In this study, we report that nearly one third of soldiers who had sustained a bTBI had evidence of pituitary dysfunction, compared to only 2% of patients who had a nbTBI. The most common pituitary abnormality was GH deficiency, followed by hyperprolactinaemia, ACTH and gonadotrophin deficiency. One soldier had three hormone deficiencies (GH, ACTH and LH/FSH).

We used conservative and stringent methods for diagnosing pituitary dysfunction for both groups and followed identical protocols for each. Our algorithm included two stimulation tests rather than one for the diagnosis of GH and ACTH deficiency (taking into account age and BMI in the cut offs for GH deficiency (Colao et al., 2009), we also ruled out the presence of macroprolactin in the case of hyperprolactinaemia and considered the effect of SHBG on the diagnosis of gonadotrophin deficiency.

The prevalence of pituitary dysfunction in the nbTBI group (1/39) appears to be consistent with other studies that have also reported a low prevalence (Kokshoorn et al., 2011; Kokshoorn et al., 2010). We therefore feel confident that we have neither under- or over-diagnosed pituitary abnormalities.

We could not find any clear differences between the patient characteristics of the two groups that may explain the difference in prevalence of pituitary dysfunction. There was a higher use of antidepressant and opiate use in the bTBI group overall but not between those with and without pituitary dysfunction. The time to endocrine testing was longer in the bTBI group mainly because they were referred to us as a tertiary rather than secondary referral. However this factor is not likely to account for an increased prevalence of pituitary dysfunction as we might expect pituitary abnormalities to recover with time (Aimaretti et al., 2005).

PTA was longer in bTBI vs. nbTBI hinting at more severe head injury as a group. It is possible however that PTA duration could have been complicated by the use of conscious sedation. Soldiers did have greater polytrauma including amputations and

major organ damage although it is not clear how this may lead to an increased rate of pituitary dysfunction when compared with civilians and there was no difference in these factors between those with and without endocrine abnormalities.

Blast appears to produce a specific pattern of TBI (Mac Donald et al., 2011), although the mechanisms leading to this are not completely clear as blast injury is often a combination of primary, secondary and tertiary causes (Cernak & Noble-Haeusslein, 2010). In terms of the effect of blast injury on the hypothalamus and/or pituitary this could be explained by a number of potential mechanisms, including direct damage to cells bodies, hypophyseal vessels , local superficial siderosis, local/generalised inflammation and hypovolaemia/ischaemia. There was no evidence radiographically however of these type of injuries at the hypothalamus-pituitary.

There were however important differences between those soldiers with and without pituitary dysfunction. We found lower FA in several white matter tracts (including the body, genu and splenium of the corpus callosum), more skull/facial fractures and a trend towards more cerebral contusions and PTA in those soldiers with pituitary dysfunction vs. those without. It is possible that these factors taken together are simply more indicative of a more severe injury overall rather than a specific pattern of injury that predisposes to hypothalamic-pituitary damage.

Our focus here was on bTBI that was defined as moderate-severe in severity and caused by a single blast. We found another study reporting high prevalence of pituitary abnormalities in soldiers following repeated, mild bTBI (Wilkinson et al., 2012). Although the methods for diagnosing pituitary dysfunction were considerably different from that reported here, it is interesting nonetheless to see concordance with our results even in milder cases of bTBI.

We have alluded to some of the consequences of pituitary dysfunction in this study. The trend for worse fatigue, mood, social and emotional problems in those soldiers with and without pituitary dysfunction may be a direct cause of the pituitary deficiencies. These symptoms are well-recognised features of GHD and fatigue is also

commonly seen in cortisol and testosterone deficiencies (Cherrier, 2009; Salvatori, 2005; Webb et al., 2012). The greater cognitive impairment in soldiers with pituitary dysfunction may also be a direct consequence of the hormone deficiency which is well reported with GH (van Dam, 2005). However it is also important to bear in mind that these soldiers may have worse QoL and cognitive impairment due to greater brain injury/axonal damage (Kinnunen et al., 2011).

In the clinical setting, the diagnosis of treatable pituitary deficiency in these individuals has led to substantial changes in their clinical care. All patients with GHD are being treated with daily subcutaneous recombinant GH. This treatment was started >1 year from TBI in line with national recommendations and has been continued due to symptomatic improvement at 6-9 months. The one case of testosterone deficiency is being treated with depot testosterone every 3 months and the two soldiers with ACTH deficiency are now on daily hydrocortisone replacement. The cases of hyperprolactinaemia have not required treatment due to absence of secondary hypogonadism. Most of these patients have been referred back to their local endocrinologist for follow-up as many of these soldiers do not live in the locality of our clinic.

In conclusion, this was the first study to report a significantly higher prevalence of pituitary dysfunction following moderate-severe bTBI which was associated with worse QoL and cognitive function. It is not clear whether the mechanism for this higher prevalence is due to greater axonal injury overall or whether blast can specifically predispose to pituitary dysfunction via another unknown mechanism. We recommend that all individuals who have had a moderate-severe bTBI should have full dynamic pituitary function tests as part of their routine post TBI care. The diagnosis of pituitary dysfunction may warrant hormone replacement which is likely to have diverse benefits for these patients.

In my next chapter, I will be examining the effect of GH and its downstream mediator, IGF-I on white matter recovery following TBI.

4 Association of serum IGF-I with white matter, neuropsychological and cognitive recovery following traumatic brain injury

4.1 Introduction

Hypopituitarism is a recognised consequence of TBI (Agha & Thompson, 2006). The reported prevalence of anterior pituitary dysfunction is wide-ranging and this is likely to be due to differences in patient populations including severity of injury, time since injury and methodology used for diagnosis (Bavisetty et al., 2008; Kokshoorn et al., 2010). More recent larger studies using gold-standard dynamic testing have reported a prevalence of the most common endocrinopathy, growth hormone deficiency, (GHD) of 2.7-11.8% in patients more than 1 year after TBI (Bavisetty et al., 2008; Klose et al., 2014; Kokshoorn et al., 2011). As described in the previous chapter, blast TBI appears to be a particular strong risk factor for developing post-traumatic hypopituitarism. We found an overall prevalence of pituitary dysfunction of 32.0% with GHD accounting for nearly half these cases (15.8%) (Baxter et al., 2013).

GH has several targets within the brain (Lobie et al., 1993; Nyberg & Hallberg, 2013), with effects either direct or mediated through its downstream hormone insulin-like growth factor-I (IGF-I). In non-TBI related GH deficiency (GHD), there is impaired cognitive function, and lower FA in the corpus callosum and corticospinal tract in children (Webb et al., 2012), and GH replacement increases serum IGF-I and improves attention, memory, and psychological well-being(Arwert, Deijen, Muller, & Drent, 2005; Lasaite, Bunevicius, Lasiene, & Lasas, 2004; Oertel, Schneider, Stalla, Holsboer, & Zihl, 2004). Variations in serum IGF-I concentrations are also related to brain structure and cognitive function (Nyberg & Hallberg, 2013). In stroke, higher serum IGF-I concentrations at baseline are associated with better neurological and functional outcomes (N. D. Aberg, Brywe, & Isgaard, 2006; De Smedt et al., 2011). Mechanistically, GH/IGF-I deficiency reduces oligodendrocyte turnover in the corpus callosum (Hua, Forbes, Lichtenwalner, Sonntag, & Riddle, 2009), whereas GH/IGF-I

administration increases the formation of new neurons in the hippocampus (D. Aberg, 2010; N. D. Aberg et al., 2006; De Smedt et al., 2011)

In TBI-related GHD, GH replacement also improves cognition, psychological function and quality of life. Improvement in WM tract recovery after TBI might be a mechanism by which GH replacement and associated increases in serum IGF-I achieve such effects. However the effects of GHD/IGF-I status on WM recovery following TBI are unknown.

TBI is a disabling disease with an uncertain prognosis, although a significant proportion of individuals improve over time (McMillan et al., 2012; McMillan et al., 2011). DTI studies have previously demonstrated reductions in FA throughout several WM tracts after TBI that correlate with functional cognitive deficits, including memory impairment with damage in the fornices, and non-sustained attention with damage in the cingulum bundle (Bonnelle et al., 2011; Kinnunen et al., 2011; Sharp & Ham, 2011; Sidaros et al., 2008). Furthermore, dynamic changes in FA of the splenium of the corpus callosum (SPCC) and posterior limb of the internal capsule (PLIC) have been described following TBI in a longitudinal study (Sidaros et al., 2008).

It is currently unclear what inter-individual factors may contribute to the variability in improvement over time of clinical outcome and WM tract damage after TBI. Taking into account the prevalence of post-traumatic hypopituitarism, It is possible that abnormal hormone levels could influence brain repair following TBI. GH replacement can improve cognition, psychological function and quality of life in TBI-related GHD (Gardner et al., 2015; High et al., 2010; Maric et al., 2010; Moreau et al., 2013; Reimunde et al., 2011). Improvement in WM tract recovery after TBI may be a mechanism by which GH replacement and associated increases in serum IGF-I achieve such effects. However the effects of GHD/IGF-I status on WM recovery following TBI are unknown.

The aim of this chapter was to investigate the impact of GH/IGF-I status on WM recovery following TBI. We hypothesised that IGF-I status would impact on recovery

after TBI such that improvement in WM tract FA, neuropsychological and cognitive measures over time would be greater in those with a higher serum IGF-I at baseline. Therefore DTI scans were performed at two time points along with neuropsychological and cognitive testing in a longitudinal study of patients after TBI, and improvements compared by GH/IGF-I status.

4.2 Methods

4.2.1 Participants

Thirty-nine patients with persistent neuropsychological symptoms were recruited in the post-acute phase following a TBI (>6 weeks). All patient had the following data recorded: age, gender, BMI, mechanism of injury, date and severity of TBI using the Mayo classification (Malec et al., 2007). Research ethics committee approval (Ealing and West London REC 09/H0707/82) and informed written consent were obtained, in accordance with the Declaration of Helsinki. Participants had standard structural brain MRI, and DTI at baseline and follow-up at least 6 months later. Thirty-five age and gender-matched healthy volunteers underwent an identical neuroimaging visit at a single time point as a control group.

4.2.2 Endocrine assessment

Patients had full endocrine testing at baseline as part of their routine clinical care (see **Table 3-1** page 104). Measurements of anterior pituitary hormones (serum IGF-I, prolactin, TSH, free thyroxine, LH, FSH, testosterone/oestrogen, SHBG) were taken in the late morning/early afternoon in the non-fasted state. Serum IGF-I was measured by immunoassay using the Immulite 2500 assay (Siemens, Erlangen, Germany), with reference ranges given in **Table 4-1**.

Table 4-1 Age-related reference ranges for serum IGF-I assay

Age	IGF-I median	IGF-I lower	IGF-I upper
yr	nmol/L	nmol/L	nmol/L
18	40.0	21.2	75.9
19	33.9	18.3	62.8
20	30.2	16.5	55.1
21-25	26.4	15.1	46.5
26-30	25.5	15.2	42.8
31-35	24.4	15.0	39.9
36-40	22.9	14.2	36.9
41-45	21.3	13.1	34.7
46-50	20.0	12.2	32.8
51-55	18.7	11.3	30.9
56-60	17.6	10.5	29.3
61-65	16.4	9.8	27.6
66-70	15.3	9.0	26.0
71-75	14.3	8.3	24.4
76-80	13.3	7.7	23.0
81-90	12.4	7.2	21.6

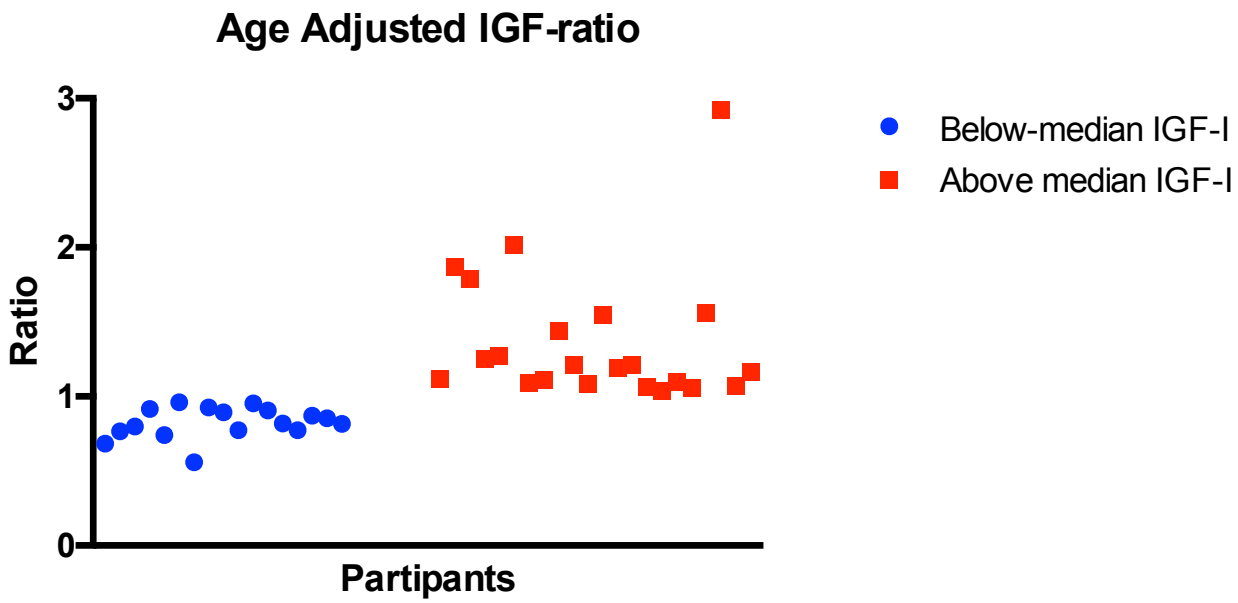
Median, lower (<5% CI) and upper (>95% CI) values normal range for serum IGF-I assay (Immulite 2500) from Dept. of Biochemistry, University College London Hospital, London, UK.

Subsequently, patients had initial screening for GH (and ACTH) deficiency using the glucagon stimulation test (Gardner et al., 2015). The diagnosis of GHD was confirmed using the GHRH-Arginine test, with age and BMI-adjusted cut-offs (Colao et al., 2009) and/or insulin tolerance test, in all patients. The protocol for diagnosis of other pituitary dysfunction e.g. ACTH or gonadotropin deficiency, was as described in Chapter 3. Although limited by the low prevalence, an exploratory sub-group analysis of FA recovery was made for those patients after TBI with (n=4) versus without (n=35) confirmed GHD. However since only 2 subjects with GHD completed neuropsychological and cognitive testing at both time points, these endpoints were not analysed by GHD status.

4.2.3 Serum IGF-I status

To account for the fact that IGF-I level decline naturally with age, an age-adjusted IGF-I ratio was calculated for each patient. Absolute IGF-I was divided by the median of the age-related reference range **Table 4-1.** Patients were then assigned to an 'above median' or 'below median' IGF-I group if this ratio was >=1 or <1 respectively. Figure shows a plot of IGF-I ratio values for those in above and below median IGF-I groups

Figure 4-1 Scatter plot of age-adjusted IGF-I ratios in above and below median IGF-I groups



4.2.4 Structural neuroimaging

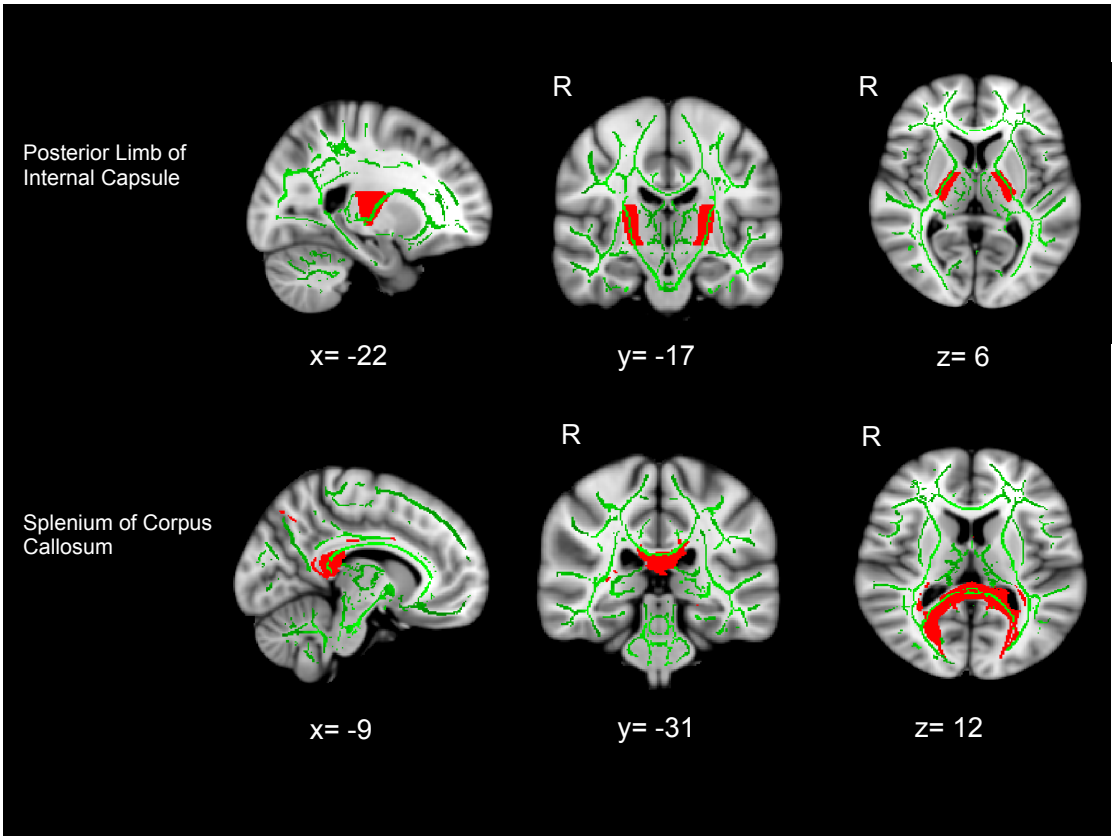
Each patient had standard high-resolution T1 and gradient-echo T* magnetic resonance imaging to assess focal brain injury and presence of microbleeds, on a Phillips 3T Achieva scanner (Phillips Medical Systems, Netherlands) using an 8-channel head coil. For DTI, diffusion-weighted volumes with gradients applied in 16 non-collinear directions were collected in four runs, with a total of 64 directions, using the following parameters: 73 contiguous slices, slice thickness 2mm, field of

view 224mm, matrix 128 × 128 (voxel size 1.75×1.75×2mm^3), *b*-value 1000s/mm^2, four images with no diffusion weighting (*b*0). DTI images were registered to the *b*0 image by affine transformations, then brain-extracted and FA maps generated using the FMRIB Software Library image processing toolbox. FA diffusion maps were analysed using both whole brain and region-of-interest voxelwise analysis approaches to compare those with vs. without GHD, and with low vs. high serum IGF-I concentrations.

4.2.5 DTI and FA analysis

FA was analysed specifically in the two *a priori* WM tract ROIs that have previously shown differences in FA over time after TBI, namely the SPCC and the PLIC (Sidaros et al., 2008). Bilateral ROI masks were defined using the John Hopkins University WM tractography atlas with a cut-off threshold >20% (Figure 4.1), averaging mean FA values in left and right ROIs. FA differences and changes over time were also analysed across the whole brain using tract based spatial statistics (TBSS) in the FMRIB Software Library using threshold-free cluster enhancement (TFCE) to correct for multiple comparisons at cluster-wise threshold P<0.05 (see **Methods:** Tract based spatial statistics (TBSS) **2.3.5 page 75).**

Figure 4-2 Posterior limb of internal capsule and splenium of corpus callosum white matter tract regions of interest.



Group average FA skeleton (green, threshold >0.2) from all patients after TBI (n=39) and healthy controls (n=35), overlaid onto standard MNI152 1mm T1 MRI brain scan. White matter (WM) tract defined using the JHU white matter tractography atlas with a cut-off threshold of 20% indicated in red. Mean fractional anisotropy (FA) values in posterior limb of internal capsule and splenium of corpus callosum WM tract regions of interest were calculated from the average of the voxels indicated by the overlap of the green skeleton and red mask. Co-ordinates given in Montreal Neurological Institute (MNI) standard space. R indicates right.

4.2.6 FA comparisons by group

Analyses and design matrices were constructed to compare FA: (i) between patients after TBI (baseline scan 1) versus controls: to examine effects of TBI (ROIs, whole brain); and in patients only: (ii) between follow-up scan 2 vs. scan 1: to examine overall recovery over time after TBI (ROI, whole brain); (iii) using a longitudinal interaction design analysis: to examine the effect of GHD and IGF-I status at baseline and FA recovery over time (ROIs); (iv) in a cross-section design between groups at scan 1 only to examine the effects of GHD and IGF-I status at baseline only (whole

brain); and (v) using the subtracted FA difference between scan 2 and scan 1: to examine the interaction between GHD and IGF-I status at baseline and recovery in FA over time (whole brain).

4.2.7 Cognitive and Neuropsychological Assessment

Where possible, all patients underwent detailed cognitive testing, previously found to be sensitive to impairments after TBI (Baxter et al., 2013; Bonnelle et al., 2012; Kinnunen et al., 2011): (i) subsets of Weschsler Memory Scale-Third edition (WMS-II) to test immediate and delayed verbal recall (Logical Memory I and II) and working memory (Digit Span); (ii) People test from Doors and People test battery to measure associative learning and recall; (iii) verbal and letter fluency and Colour–Word (Stroop) tests from Delis-Kaplan Executive Function System to assess cognitive flexibility, inhibition and set-shifting; (iv) Trail Making Test to assess executive function; (v) Wechsler Test of Adult Reading and Wechsler Abbreviated Scale of Intelligence Similarities and Matrix-Reasoning subsets to assess pre-morbid verbal and non-verbal reasoning ability at the first visit only. Please **see Methods:** Neuropsychological assessment **2.3.2 page 74** for more detailed information.

In addition patients completed the following psychological and quality of life (QoL) questionnaires at both visits that we have used previously in TBI (Baxter et al., 2013): Beck Depression Inventory Score (BDI-II), Epworth Daytime Sleepiness Scale and Assessment of GH Deficiency in Adults (AGHDA) and Short Form 36 Health Survey (SF-36) QoL questionnaires. Please see **Methods:** Quality of Life (QoL) assessments **2.3.1 page 73** for more detailed information.

4.2.8 Statistical analyses

Analysis of demographics and ROI FA values was performed using SPSS (v21, IBM). Comparison between groups used Mann-Whitney U test, chi-squared test, 2-way unpaired or paired Student t-test, and one-way ANCOVA or one-way repeated measures ANCOVA with post-hoc Fisher LSD test, as appropriate with statistical threshold $P<0.05$.

Comparison of FA in ROIs between controls and TBI patients at baseline using ANCOVA included age and gender as co-variates. Comparison of FA in ROIs between groups in TBI patients at baseline using ANCOVA included age, gender, TBI severity, time since TBI as co-variates. Longitudinal analyses were performed both without, and with addition of the following co-variates that might influence WM tract recovery over time: age, gender, TBI severity, time since TBI, and time between DTI scans. Repeated measures ANCOVA examined improvement in FA in ROIs over time in TBI patients, and differences between groups to establish effects of group, time and group x time interaction. Time variables were $\log_{10}$-transformed as they were not normally distributed.

4.3 Results

4.3.1 Patient characteristics

Thirty-three of 39 patients (84.6%) were male with median age 30.5y (interquartile range (IQR) 24.5-47.0). Thirteen patients (33.3%) had sustained their TBI as a result of a blast exposure from an improvised explosive device, 10 (25.6%) road traffic accident, 7 (17.9%) assault, 6 (15.4%) fall and 3 (7.7%) through a sporting injury. Thirty-four patients (87.2%) had a moderate-severe TBI by Mayo classification 35. The median time between TBI and first scan was 16.3 months (IQR 3.6-24.5). There was no significant difference in prevalence of blast TBI or moderate-severe TBI, or time since TBI between GH status or IGF-I groups. Further demographic data is given in **Table 4-2**.

There were no significant differences in the prevalence of contusions, microbleeds, superficial siderosis and diffuse axonal injury between IGF-I groups. Structural abnormalities in the baseline MRI are summarised in Table 1. Although dedicated pituitary MRI imaging was only performed in those with GHD, there was no evidence of hypothalamic-pituitary damage in any patient. No patients had craniotomy as these subjects had been excluded from the study.

Thirty-five healthy controls were studied with the following demographics: 23 (65.7%) male (P=0.11 vs. TBI), and median age 30.7y (IQR 25.9-38.2, P=0.22 vs. TBI). Further demographic data is given in **Table 4-2**.

4.3.2 GHD and pituitary dysfunction

Four (10.2%) patients after TBI had confirmed untreated GHD. Overall, 7/39 (17.9%) had pituitary dysfunction (3 isolated GHD, 1 ACTH deficiency, 2 hyperprolactinemia, 1 combined ACTH/GH/gonadotropin deficiencies). Six were soldiers who had sustained blast TBI(Baxter et al., 2013). None of these patients had yet started any hormone replacement therapy at the time of their first or second scan. There were

no significant differences in age, gender, TBI severity, time since injury, time between scans, prevalence of blast TBI, or prevalence of structural MRI abnormalities at baseline, between patients with and without GHD (**Table 4-3**). Absolute serum IGF-I and age-adjusted IGF-I ratio tended to be lower (P=0.11) in those patients with vs. without GHD, with 75% of GHD subjects having serum IGF-I below the age-related median **Table 4-1**.

Table 4-2 Patient characteristics by IGF-I group

	All Group	Above Median IGF-I	Below Median IGF-I	P Above vs Below[a]
n	39	22	17	
Age at first scan (yrs)	30.5 [24.5-47.0]	30.2 [24.3-47.6]	34.5 [25.2-48.8]	0.43
Range	19.6-66.9	19.6-62.2	21.3-66.9	
Male Sex	33 (84.6%)	17 (77.3%)	16 (94.1%)	0.21
Moderate-Severe Severity	34 (87.2%)	20 (90.6%)	14 (82.4%)	0.67
Time since Injury (months)	16.3 [3.6-24.5]	12.5 [4.-25.9]	17.2 [3.1-33.5]	0.89
Range	1.47-571.0	1.5-571.0	1.5-102.9	
Time between scans (months)	13.3 [12.1-14.9]	12.35 [10.81-13.4]	14.4 [13.1-15.1]	0.08
Range	6.3-24.6	6.3-24.6	8.87-17.8	
Absolute IGF-1 nmol/L[b]	23.8 [19.2-29.5]	28.2 [23.3.-35.1]	19.9 [16.8-23.7]	**0.02**
Range	12.8-74.5	19.2-74.5	12.8-29.0	
Age-Adjusted IGF-1 Ratio	1.1 [0.9-1.2]	1.2 [1.1-1.5]	0.8 [0.8-0.9]	**<0.001**
Range	0.6-3.0	1.0-2.9	0.6-1.0	
GHD	4 (10.2%)	1 (4.5%)	3 (17.6%)	0.30
Any Pituitary Deficiency	7 (17.9%)	3 (13.6%)	4 (23.5%)	0.68
Blast Injury	13 (33.3%	8 (36.3%)	5 (29.4%)	0.74
Any structural brain abnormality	30 (76.9%)	19 (86.4%)	11 (64.7%)	0.14
≥ 1 contusion	20 (51.3%)	11 (28.2%)	9 (52.9%)	1.00
Average no. of contusions*	1.7	2.8	2	
≥ 1 microbleed	10 (25.6%)	8 (36.4%)	2 (11.8%)	0.14
Superficial siderosis	13 (33.3%)	6 (27.3%)	7 (41.2%)	0.50
Diffuse axonal injury (DAI)	5 (12.8%)	3 (13.6%)	2 (11.8%)	1.00
BMI	25.3 [23.1-29.8]	23.6 [19.6-29.0]	25.3 [24.4-30.2]	1.00
	17.0-35.0	17.0-32.3	21.3-35.0	
Post menopausal women	3 (7.7%)	2 (9.1%)	1 (5.9%)	1.00

Data given as n (%) or median [IQR] and range (minimum-maximum).

[a] P value for comparison high versus low serum IGF-I group using chi-squared test for categorical variables and Mann-Whitney U test for continuous variables. [b] To convert nmol/L to ng/mL divide by 0.131.

Table 4-3 Patient characteristics by GH deficiency status

	All	No GHD	With GHD	P with vs without GHD[a]
n	39	35	4	
Age at first scan (yrs)	30.5 [24.5, 47.0]	30.4 [24.1, 49.2]	39.4 [29.7, 44.4]	0.50
Range	19.6-66.9	19.6-66.9	27.8-44.7	
Male Sex	33 (84.6%)	29 (82.9%)	4 (100%)	1.00
Moderate-Severe Severity	34 (87.2%)	30 (85.7%)	4 (100%)	1.00
Time since Injury (months)	16.3 [3.6, 24.5]	11.4 [3.2, 25.7]	26.8 [7.7-34.3]	0.38
Range	1.5-571.0	1.5-571.0	3.6-34.5	
Time between scans (months)	13.3 [12.1, 14.9]	13.1 [12.1, 14.5]	14.6 [10.3-15.1]	0.58
Range	6.3-24.6	6.3-24.6	9.1-15.1	
Absolute IGF-1 nmol/L[b]	23.8 [19.2-29.5]	24.2 [19.5, 29.5]	19.6 [16.7, 23.2]	0.11
Range	12.8-74.5	12.8-74.5	16.5-23.7	
Age-Adjusted IGF-1 Ratio	1.1 [0.9, 1.2]	1.1 [0.9, 1.3]	0.9 [0.8-1.0]	0.11
Range	0.6-3.0	0.6-2.9	0.8-1.0	
Blast Injury	13 (33.3%	10 (28.6%)	3 (75.0%)	0.10
Any structural brain abnormality	30 (76.9%)	28 (80.0%)	2 (50.0%)	0.22
≥ 1 contusion	20 (51.3%)	19 (54.3%)	1 (25.0%)	0.34
Average no. of contusions*	1.7	2.5	n/a	
≥ 1 microbleed	10 (25.6%)	10 (28.6%)	0 (0.0%)	0.56
Superficial siderosis	13 (33.3%)	11 (31.4%)	2 (50.0%)	0.59
Diffuse axonal injury (DAI)	5 (12.8%)	4(11.4%)	1 (25.0%)	0.44
BMI	25.3 [23.1-29.8]	25.0 [21.7-28.6]	29.3 [21.7-28.2]	0.10
Range	17.0-35.0	17.0 -25.0	25.3-33.7	
Post menopausal women	3 (7.7%)	3 (8.6%)	0 (0.0%)	1.00

Data given as n (%) or median [IQR] and range (minimum-maximum).
[a] P value for comparison high versus low serum IGF-I group using chi-squared test for categorical variables and Mann-Whitney U test for continuous variables. [b] To convert nmol/L to ng/mL divide by 0.131.

4.3.3 IGF-I status

Seventeen (43.6%) patients were in the below median age-adjusted IGF-I group and 22 in the above median IGF-I group. In the below median IGF-I group 1/17 (5.6%) had

a serum IGF-I <5th percentile (age-adjusted normal range), and in the above median IGF-I group 4/22 (18.2%) had a serum IGF-I >95th percentile. There were no significant differences in age, gender, TBI severity, time since injury, prevalence of blast TBI, of structural MRI abnormalities at baseline or of post-menopausal females, or BMI, between IGF-I groups (Table 4.1). There was a trend towards a greater length of time between the two scans in the below median IGF-I group compared to the above median IGF-I group (median 14.4 vs. 12.5 months, P=0.08).

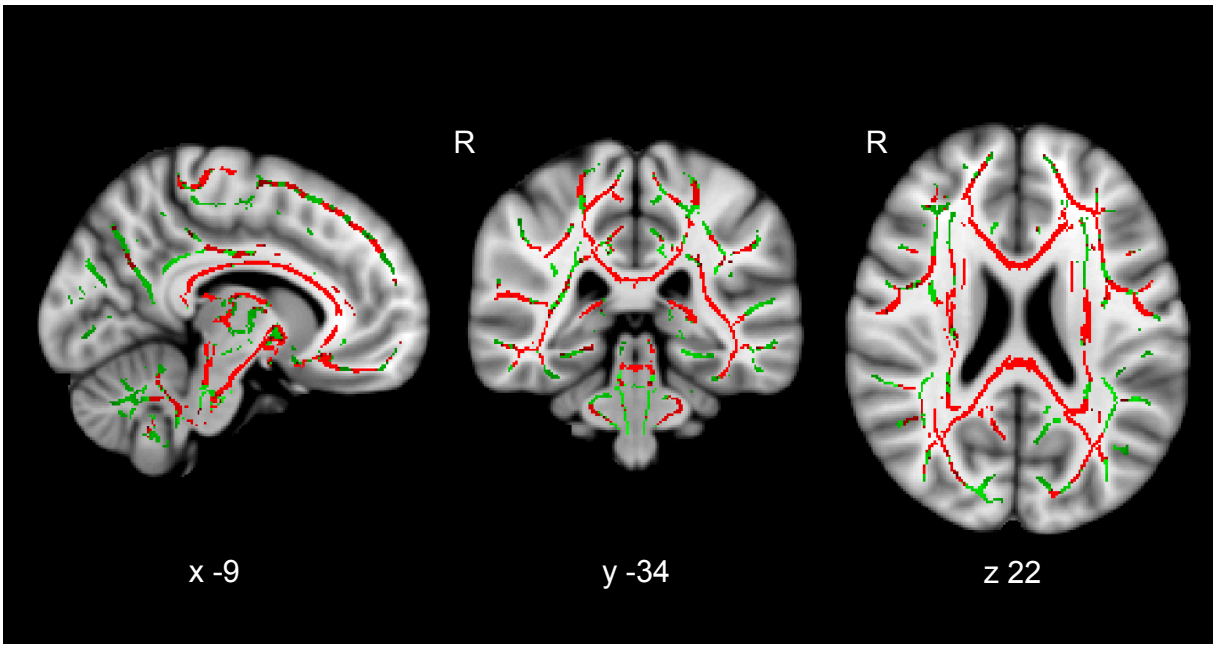
Only 3/17 (17.6%) of patients in the below median IGF-I group had GHD confirmed on dynamic testing, compared to 1/22 (4.5%) in the above median IGF-I group (P=0.15). Of the 4 patients with confirmed GHD, none had serum IGF-I <5th percentile, 3 had IGF-I between 5th percentile and median, and one had IGF-I between median and 95th percentile.

4.3.4 Effect of TBI on WM tracts

(i) ***TBI patients show evidence of axonal injury*****:** In whole brain analysis, FA was significantly lower in many WM tracts including splenium, body and genu of corpus callosum, and the PLIC, in patients following TBI at baseline scan 1 (n=39), compared to healthy controls (n=35) (**Figure 4-3**).

As expected, compared to the healthy control group, the TBI group overall had significantly lower FA in the *a priori ROIs* SPCC (effect size mean ± SEM -0.021 ± 0.009, 95% CI [-0.003, -0.040], t(72)=-2.26, P=0.027) and PLIC (effect size -0.026 ± 0.006, [-0.039, -0.013], t(72)=-3.90, P<0.001) (**Figure 4-5**).

Figure 4-3 Lower white matter tract FA in patients after TBI



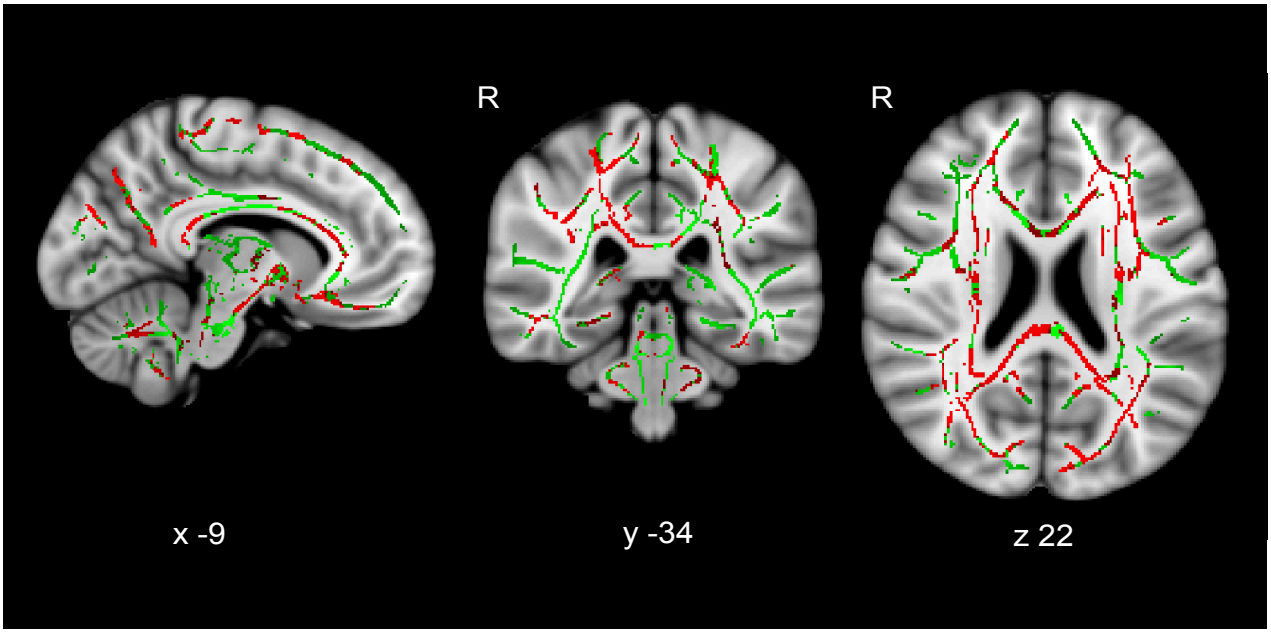
Voxels displaying lower fractional anisotropy (FA) in patients with TBI (n=39) compared to healthy controls (n=35) displayed in red (TFCE P<0.05 corrected and correcting for age and gender), overlaid on combined whole group FA skeleton (green), displayed on standard MNI125 1mm T1 MRI brain scan. Co-ordinates given in Montreal Neurological Institute (MNI) standard space. R indicates right.

4.3.5 Effect of time on WM tract recovery over time

(ii) ***Increased FA over time suggest axonal recovery following TBI:*** Within the TBI patients, FA increased over time from scan 1 to follow-up scan 2, suggesting recovery of axonal over time, in a number of WM tracts including splenium, body and genu of corpus callosum, subcortical regions, right frontal region and widespread ventral regions (**Figure 4-4**).

As expected, in the TBI group there were significant increases in FA between scans in SPCC (effect size 0.015 ± 0.007, [0.001, 0.281], t(38)=2.2, P=0.035), and PLIC (effect size 0.016 ± 0.004 [0.003, 0.020], t(38)=3.1, P=0.005). However there were no significant effects of age (P=0.35-0.51), gender (P=0.14-0.60), TBI severity (P=0.11-0.23), time since TBI (P=0.33-0.73), or time between scans (P=0.58-0.90) on the change in FA over time (co-variate x time interaction), when including each factors separately as a within subject co-variate in a repeated measures ANCOVA model.

Figure 4-4 White matter tract FA improvement between scans.



Regions displaying higher fractional anisotropy (FA) in TBI patients at follow-up scan (in red) compared to baseline scan overlaid on group FA skeleton (green) using TFCE P<0.05 corrected and correcting for age, time since injury, gender, TBI severity and time between scans. Results overlaid on standard MNI125 1mm T1 MRI brain scan. Co-ordinates given in Montreal Neurological Institute (MNI) standard space. R indicates right.

4.3.6 Effect of IGF-I group on WM tract FA

(v) ***No cross-sectional influence of IGF-I group on FA at baseline:*** Within the TBI group, there was no significant effect of IGF-I group on FA at baseline in whole brain analysis, with or without inclusion of co-variates.

(vi) ***Influence of IGF-I group on recovery in FA over time:*** Within the TBI group, there was a significantly greater increase in FA in SPCC over time in the above median than below median IGF-I group (IGF-I group x time interaction F(1,37)=4.62 P=0.038, above median IGF-I group effect size +0.027 ± 0.008, 95% CI [0.009, 0.044], F(1,37)=9.82, P=0.03; below median IGF-I group effect size -0.001 ± 0.100, [-0.021, 0.190], F(1,37)=0.01, P=0.92). Similar results were seen when including co-variates in the repeated measures ANCOVA (IGF-I group x time interaction F(1,32)=5.66, P=0.023, above median IGF-I group effect size +0.030 ± 0.009, [0.011, 0.048],

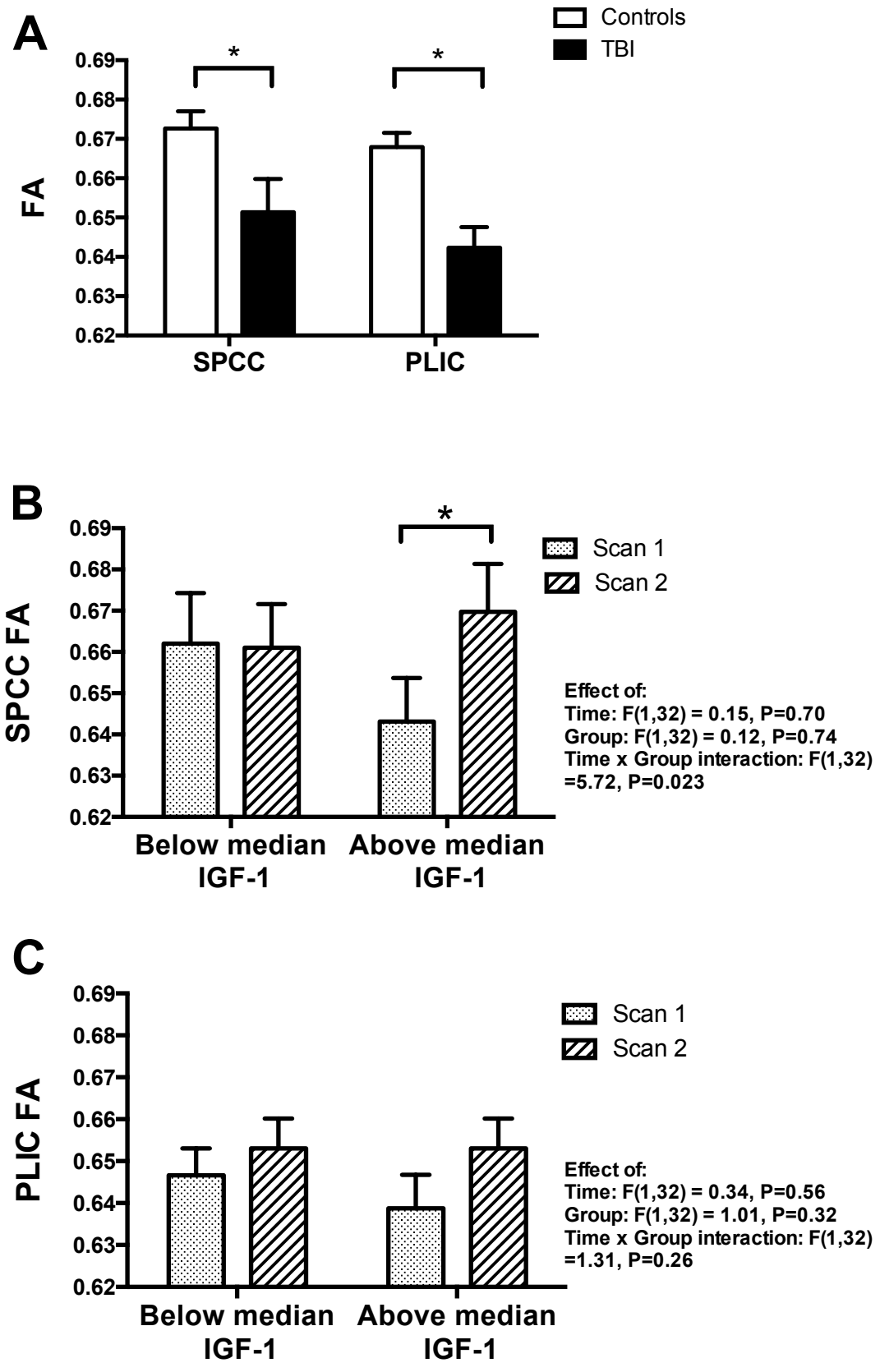
F(1,32)=10.60, P=0.003; below median IGF-I group effect size 0.011 ± 0.010, [-0.026, 0.017], F(1,32)=0.21, P=0.65 (**Figure 4-5B**). There was no significant difference in SPCC FA between IGF-1 groups at baseline.

A similar interaction was seen for FA improvement in SPCC over time when excluding 4 subjects without moderate-severe TBI (IGF-I group x time interaction (F(1,29)=3.74, P=0.063), and when excluding 2 subjects with a prolonged time since TBI >100 months (IGF-I group x time interaction (F(1,31)=5.06, P=0.032, including age, gender, severity and time between scans).

However, there was no significant effect of IGF-I group on increase in FA over time in PLIC either without any covariates (IGF-I group x time interaction F(1,37)=0.57, P=0.46), or when including co-variates (IGF-I group x time interaction F(1,32)=1.31, P=0.26) (**Figure 4-5C**).

In whole brain subtraction analysis, there was no significant effect of IGF-I group on the change in FA between scans 2 and 1, with or without inclusion of co-variates. A similar lack of effect of IGF-I group was also seen when restricting the skeleton in whole brain analysis to only those voxels showing a significant increase in FA between scans (**Figure 4-4**).

Figure 4-5 White matter tract FA in regions of interest in patients after TBI at baseline and recovery over time by IGF-I group



*(A) Lower FA in splenium of corpus callosum (SPCC) and posterior limb of internal capsule (PLIC) ROIs in patients after TBI (black bar) compared to controls (white bar) at baseline scan, adjusting for age and gender (*P<0.05, ANCOVA with post-hoc Fisher LSD test).*
*(B) Greater improvement in FA in SPCC, but (C) no difference in improvement in FA in PLIC, between scan 1 (dotted bar) and scan 2 (striped bar) in above median IGF-1 group compared to below IGF-I group, adjusting for age, gender, TBI severity, time since TBI and time between scans (*P<0.05, repeated measures ANCOVA with post-hoc Fisher LSD test).*
All data given as mean ± SEM.

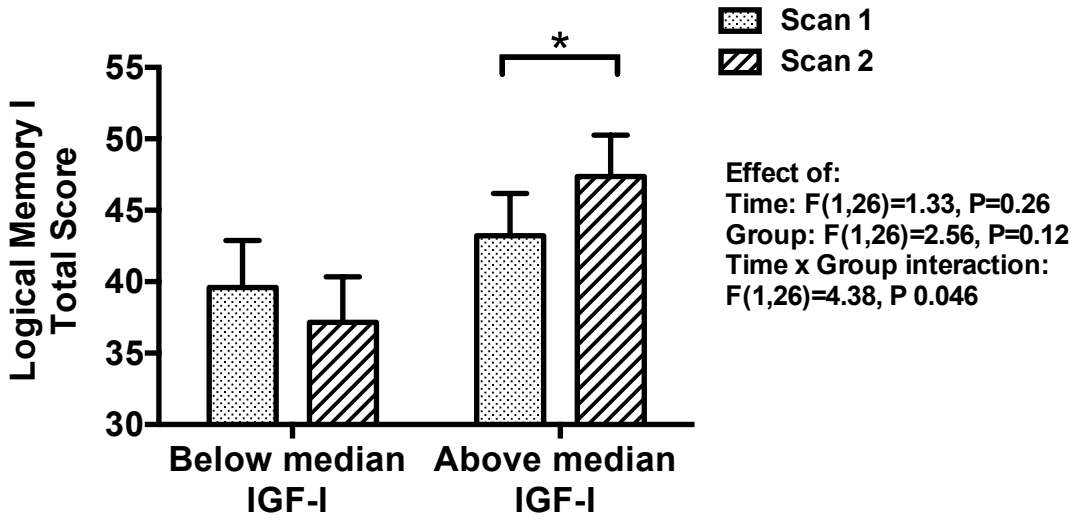
4.3.7 Effect of IGF-I group on neuropsychological and cognitive variables

Within the TBI group, there was a significantly greater increase in the logical memory I total score (measure of immediate recall) over time in the above median than below median IGF-I group (IGF-I group x time interaction F(1,26)=4.38 P=0.046, above median IGF-I group effect size +4.19 ± 2.01, 95% CI [0.06, 8.31], F(1,26)=4.34, P=0.47; below median IGF-I group effect size -2.42 ± 2.22, [-0.699, 2.15], F(1,26)=1.19, P=0.29), when including covariates (age, gender, severity, time since injury and time between scans) in the repeated measures ANCOVA, despite scores being similar between groups at baseline (F(1,26)=0.60, P=0.45) **(Figure 4-6).**

There was no significant correlation between the improvement in SPCC FA over time and the improvement in logical memory I total score over time in patients after TBI, either alone (r=0.20, P=0.28), or when correcting for age, gender, severity, time since injury and time between scans (partial r=0.26, P=0.18).

There was no significant IGF-I group x time interaction for any of the other cognitive measures, or symptom (BDI-II, Epworth) or quality of life (SF-36, AGHDA) questionnaire scores (**Table 4-4**). There were however significant improvements over time in associative memory (People Test) and cognitive flexibility (Color Word Stroop) independent of IGF-I group.

Figure 4-6 Recall memory in patients after TBI at baseline and recovery



*Greater improvement in **Logical Memory I (LMI) total score (immediate recall)** in above median IGF-1 group compared to below IGF-I group, adjusting for age, gender, TBI severity, time since TBI and time between scans (*P<0.05, repeated measures ANCOVA with post-hoc Fisher LSD test). Significant increase in LMI score between scan 1 (dotted bar) and scan 2 (striped bar) for IGF-I group above but not below median at baseline, and no significant difference between IGF-I groups at baseline. Data given as mean ± SEM.*

Table 4-4 Neuropsychological outcome measures in patients after TBI at baseline and recovery over time by IGF status.

Symptom Domain		n	Max score	First Visit	n	Second visit	Effect of Time*	Effect of IGF Group*	Time*Group Interaction*
Assessment of GH Deficiency in Adults (AGHDA-QoL)[a]		25	25	9 [2.5-16.5] 0-23	25	7.0 [1-12.5] 0-22	0.99	0.71	0.89
Beck Depression Inventory Score (BDI-II)		25	63	13.0 [4.0-23.0] 0-27	25	11.0 [2.5-18.5] 0-32	0.46	0.75	0.63
Epworth Sleepiness Scale		22	24	3.0 [0.9.5] 0-19	15	4.0 [1.0-10.0] 0-14	0.69	0.45	0.53
SF-36 QoL	Physical functioning	16	100	70.0 [22.5-98.8] 0-100	24	87.5 [62.3-95.0] 0-100	0.32	0.82	0.53
	Role limitations due to physical health	17	100	75.0 [0-100] 0-100	24	50.0 [6.25-100] 0-100	0.58	0.37	0.58
	Role limitations due to emotional problems	16	100	100 [66.7-100] 0-100	24	100 [41.7-100] 0-100	0.88	0.59	0.77
	Energy/Fatigue	16	100	45.0 [45.0-70.0] 15-85	24	67.5 [47.5-83.8] 5-100	0.41	0.41	0.12
	Emotional well being	16	100	70.0 [54-83.0] 28.0-92.0	24	80.0 [61.0-92.0] 24.0-100	0.52	0.75	0.96
	Social functioning	17	100	62.5 [50.0-75.0] 12.5-100.0	24	81.3 [50.0-100] 25-100	0.22	0.77	0.87
	Pain	17	100	45.0 [39.0-83.8] 22.5-100	24	77.5 [55.6-100] 42.5-100	0.74	0.82	0.93
	General health	17	100	60.0 [42.5-80.0] 20-100	24	65.0 [45.0-91.3] 15-100	0.27	0.59	0.18
Cognitive domain	Cognitive variable								
Pre-morbid intelligence: reading ability	WTAR raw score	32		41.0 [35.3-45.0] 0-50	32	42.0 [31.8-46.8] 9-50	0.88	0.83	0.60
Intellectual ability	WASI similarities (verbal)	33		36.0 [32.5-38.0] 21-46	33	39.0 [35.5-41.0] 23-46	0.61	0.96	0.42
	WASI matrix reasoning (non-verbal)	32		26.5 [20.5-30.0] 9-34	32	28.5 [25.3-30.8] 12-35	0.08	1.00	0.20
Memory: associative memory	People test (total score)	33		22.0 [14.5-29.0] 4-38	33	27.0 [18.0-31.0] 6-38	**0.010**	0.19	0.78
Immediate recall	Logical memory I Total	33		28.0 [20.5-32.0] 10-40	33	43.0 [33.0-51.5] 19-62	0.26	0.12	**0.046**
	Logical memory II Total	33		26.0 [18.5-32.5] 0-42	33	27.0 [19.5-33.0] 10-43	0.63	0.20	0.57
Delayed Recall	Logical memory retention	32		86.7 [78.9-94.3] 33.3-107.7	33	90.3 [82.9-98.0] 45-190	0.41	0.34	0.93
Processing speed: visual search/complex	Trail Making Test Trail A (s)	32		26.0 [21.0-32.0] 14-67	33	22.0 [16.5-28.5] 13-87	0.82	0.60	0.65
	Trail Making Test Trail B (s)	32		52.0 [43.3-68.5] 22-192	33	47.0 [30.5-71.0] 25-166	0.62	0.32	0.81
Processing speed: naming/reading	Stroop Colour Naming (s)	31		32.0 [28.0-41.0] 20-101	33	32.0 [30.0-35.0] 19-51	0.56	0.90	0.96
	Stroop Word Reading (s)	31		23.0 [19.0-32.0] 15-62	32	23.0 [20.0-25.8] 14-43	0.53	0.25	0.34
Executive function: alternating-switch cost	Trail Making Test Trail B minus A (s)	32		22.5 [13.0-38.8] 4-125	33	19.0 [15.0-42.5] 7-102	0.57	0.24	0.62
Cognitive flexibility	Color Word Stroop Inhibition/switching (s)	32		66.0 [55.5-76.5] 38-128	33	57.0 [51.5-66.5] 37-119	**0.003**	0.33	0.50
Word generation fluency	DKEFS Letter Fluency F+A+S total	33		36.0 [27.0-45.0] 11-65	33	41.0 [35.5-48.5] 15-60	0.15	0.57	0.59

* = correction for all covariates (time since injury, time between scans, gender, age at first scan, TBI severity)

Data displayed as median, [IQR], range.

Note: AGHDA, BDI-II, Epworth: higher scores equals worse symptoms; SF-36: lower score equals worse quality of life, WTAR, Similarities, Matrix reasoning, People test, Logical memory: higher score = better performance; Trail-making, Stroop, letter fluency; lower score=better cognitic

Abbreviation: SF-36=Short Form 36 Health Survey, WTAR =Wechsler Test of Adult Reading, WASI=Wechsler Adult Intelligence Scale; DKEFS=Delis-Kaplan executive function system, (s)=seconds

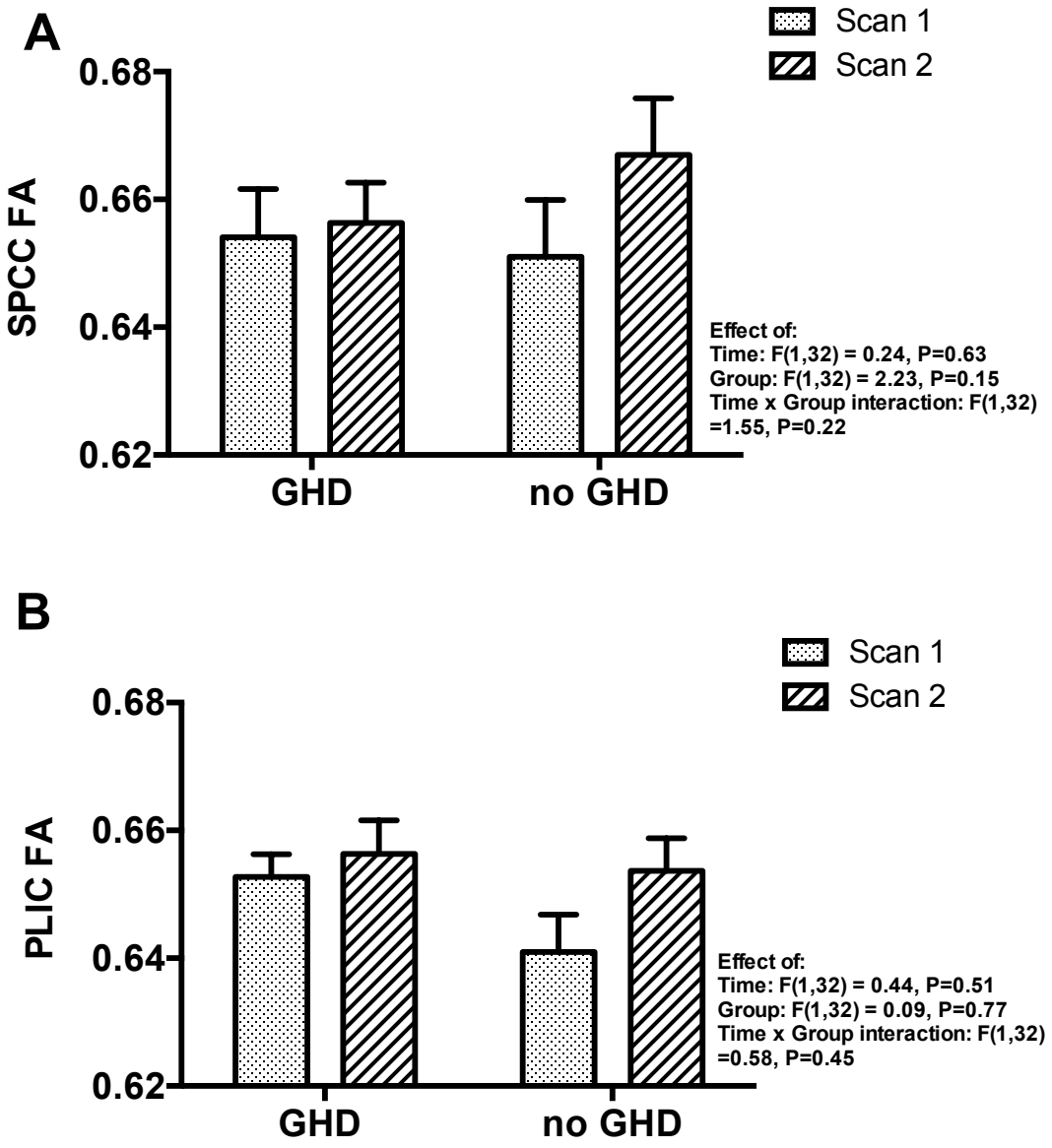
4.3.8 Effect of GHD on WM tract FA

(iii) ***No cross-sectional influence of GHD on FA at baseline:*** Within the TBI group, there was no significant effect of GHD on FA at baseline in whole brain analysis, with or without inclusion of co-variates (age, gender, TBI severity, time since TBI).

(iv) ***No longitudinal influence of GHD status on change in FA over time:*** Within the TBI group, there was no significant effect of GHD on the increase in FA over time in either the SPCC or PLIC ROIs (GHD status x time interaction P=0.29-0.45). This remained non-significant when including the co-variates: age, gender, TBI severity, time since TBI, and time between DTI scans (GHD status x time interaction P=0.22-0.45) (**Figure 4-7**).

Similarly in whole brain subtraction analysis, there was no significant effect of GHD on the change in FA between scans 2 and 1, with or without inclusion of co-variates. A similar lack of effect of GHD was also seen when restricting the skeleton in whole brain analysis to only those voxels showing a significant increase in FA between scans (**Figure 4-4**).

Figure 4-7 White matter tract FA in regions of interest in patients after TBI at baseline and recovery over time by GHD status.



No significant difference in improvement in FA in either (A) splenium of corpus callosum (SPCC) or (B) posterior limb of internal capsule (PLIC) between scan 1 (dotted bar) and scan 2 (striped bar) in patients after TBI between those without (n=35) and with (n=4) GH deficiency (GHD), adjusting for age, gender, TBI severity, time since injury and time between scans.

4.4 Discussion

This study suggests that the recovery of diffuse axonal injury after TBI, measured using DTI, appears dependent on serum IGF-I at baseline. A greater increase in FA over time in the SPCC, indicative of WM recovery, was seen in patients who had IGF-I concentrations above the median for their age, compared to those with IGF-I below median. This supports a neuroendocrine effect on brain recovery after TBI that may have important therapeutic implications. GHD at baseline did not influence increase in FA in either ROI, although these results should be interpreted with caution since only 4 patients had GHD in this cohort.

Human serum IGF-1, a 70 amino acid polypeptide is thought to mediate many of the trophic effects of GH through both hepatic and local production. The effects of both systemic IGF-I and GH on the brain are not completely clear or disentangled, however both peptides are able to penetrate the blood brain barrier and peripheral IGF-I and GH administration can improve spatial learning and memory in animal studies, suggesting that similar pathways mediate these effects (Le Greves et al., 2006; Nyberg & Hallberg, 2013).

At a mechanistic level, GH receptors are ubiquitous throughout the brain and GH/IGF-I can have a direct neurogenic effect on proliferation of neurons, oligodendrocytes and new blood vessels (N. D. Aberg et al., 2006). Deficiency of either hormone can also decrease neuronal survival time suggestive of a neuroprotective effect (Le Greves et al., 2006). GH also influences the hippocampal NMDA receptor system with effects on memory acquisition. GH/IGF-I may also promote brain repair through the local induction and release of a number of neurotrophic factors such as VEGF, EGF and BDNF (Lichtenwalner, Forbes, Sonntag, & Riddle, 2006). Other putative mechanisms may be directly through reduction of neuroinflammation or improvement in peripheral metabolic factors such as metabolic syndrome.

GHD following TBI is a well-recognised post-traumatic phenomenon but appears not be as common as initially reported. More recent larger studies have reported a prevalence of GHD of 2.7-11.8% of 112 patients in a Dutch cohort (using insulin tolerance test, or GHRH-arginine test if contra-indicated) (Kokshoorn et al., 2011) and 4.5-11.8% of 439 patients in a Danish cohort (using insulin tolerance test or GHRH-arginine/GHRH-pyridostigmine test) (Klose et al., 2014), both including patients more than 1 year after TBI. We have found a prevalence of GHD in 5.7% of 163 patients after TBI (median 0.47 years) in our clinic with diagnosis requiring inadequate GH response to both glucagon, and GHRH-Arginine testing or insulin-induced hypoglycaemia (Carmen Tenorio Jimenez, 2012). In this study we found only 4 of 39 patients (10.3%) had GHD using these criteria, a prevalence enhanced by one third of patients having blast TBI, which is associated with a particularly high risk of pituitary dysfunction (Baxter et al., 2013). The small number of patients with GHD in our study means that we are likely to have significant type II errors in examining the effects of WM tract recovery.

We therefore chose to also study serum IGF-I as an additional biomarker given the potential convergence of the GH/IGF-I axis on brain recovery. Indeed serum IGF-I is the biomarker used to titrate GH replacement therapy in subjects who are GHD, with a target IGF-I in the upper half of the age-related reference range being the usual target for dose adjustment (Drake, Howell, Monson, & Shalet, 2001). We were not powered to look for effects of extremely low or high serum IGF-I concentrations since only 5 of 39 patients (12.8%) had serum IGF-I outside the 95% CI for their age (1 above, 4 below). Another limitation of our study is that we did not measure serum IGF-binding proteins that may alter free IGF-I concentrations, though none of our patients were malnourished.

We chose to focus on FA as a measure of WM tract recovery as this is a reliable measure of WM integrity (Bonnelle et al., 2012; Kinnunen et al., 2011). Given DTI measures water diffusion in WM tracts and FA is a measure of diffusion directionality, we cannot be certain that this specifically relates to underlying WM damage and repair in these patients. However we do show a striking reduction in FA

across the whole brain in patients after TBI compared to controls and that FA increases over time after TBI. We have previously shown that after TBI FA measurements in particular WM tracts relate to multiple neuropsychological domains (Bonnelle et al., 2012; Bonnelle et al., 2011; Kinnunen et al., 2011) and diffusivity measures in the SPCC negatively correlated with functional connectivity of the posterior cingulate cortex (Sharp, Scott, & Leech, 2014). Lower FA in the corpus callosum also predicts worse verbal memory, attention, speed of information processing, and executive function over five years in patients with multiple sclerosis (Bodini et al., 2013). Future studies will need to address whether the greater increase in SPCC FA seen over time in high IGF-I group are also associated with greater improvements in neuropsychological function, including domains such as memory, attention, planning, mood, and whether other neuroimaging markers such as brain atrophy are also influenced by IGF-I status.

Our ROI analysis was restricted to areas expected to see a change in FA based on previous studies (Sidaros et al., 2008; Sidaros et al., 2009). Whole brain analyses using TBSS showed a striking effect of time on FA recovery across many WM tracts. However we did not find any significant interaction with IGF-I group. This may be due to a number of factors including small numbers given the need for robust statistical correction for multiple comparisons in whole brain analysis, and variability in the time since TBI and time between scans in our cohort, though these did not appear to be important factors influencing FA recovery in ROI analysis. Future studies should utilise larger cohorts with more consistent time periods.

In conclusion it appears that higher concentrations of serum IGF-I for age in adults following a TBI enhances WM recovery in the SPCC, irrespective of the presence of GHD. As there are limited treatments to improve recovery from TBI and relatively little is know about what predicts the prognosis for patients, this raises the possibility that GH treatment to raise serum IGF-I into the upper part of the age-related reference range might aid WM recovery, and hence neuropsychological function, following TBI, regardless of whether GHD is present using dynamic tests.

5 The effect of GH replacement therapy on the recovery from TBI

5.1 Introduction

In the last chapter, I investigated the effect of GHD and serum IGF-I on white matter recovery following TBI. I was not able to study the effect of GHD alone as at the time of the study we found only 4/39 individuals with confirmed GHD. This was lower than we had originally expected but is consistent with other multi-centre studies that also report low prevalence (Kokshoorn et al., 2011). Instead we focussed on the principal downstream mediator of GH i.e. IGF-I and found that higher levels of this hormone were associated with greater WM recovery in the splenium of the corpus callosum (SPCC) and greater improvement in logical memory scores.

However there are several limitations of this finding. IGF-I deficiency is not generally considered a clinical entity in adults, although in children the diagnosis is sometimes made and treatment with recombinant IGF-I is initiated (Cohen et al., 2014). Serum IGF-I can also be affected by nutritional status and other factors which makes it an unreliable diagnostic test for GHD although useful in the titration of GH treatment (Drake et al., 2001). GHD however is a clinical entity which is usually diagnosed with dynamic function testing and is amenable to treatment with daily GH injections. GHD is treated in adults if there is evidence of impaired quality of life, usually assessed by the AGHDA-QoL (QoL-AGHDA). Reduced bone density and evidence of impaired adipocyte metabolism, reduced lean muscle mass and other markers of insulin resistance can also support the use of growth hormone replacement (GHR) (Moller & Jorgensen, 2009; Rosen, Hansson, Granhed, Szucs, & Bengtsson, 1993).

Several small studies have reported a positive effect of GHR on cognition and quality of life following TBI ((Gardner et al., 2015; High et al., 2010; Maric et al., 2010; Moreau et al., 2013; Reimunde et al., 2011). However it is not clear what effect GH

has on microstructural brain recovery following TBI in cases of GHD. This could have important therapeutic opportunities.

The purpose of this chapter is to investigate the effect GHR on cognition WM recovery and metabolism. My hypotheses are as follows:

1) GHR improves WM recovery in the SPCC and other regions that relate to areas of greater density of GH receptors in the brain, namely the anterior thalamic radiculus and the cingulum hippocampus (Lobie et al., 1993).
2) GHR improves cognitive functions such as working memory and also improves quality of life compared to those patients who had not received GH injections following a TBI.
3) GHD patients exhibit greater insulin resistance compared to those without.
4) GHR improves insulin sensitivity.

5.2 Methods

5.2.1 Participants

Ten patients with confirmed GHD were recruited following a TBI from the multidisciplinary TBI clinic at Imperial College Healthcare NHS Trust. All patients had the following data recorded: age, gender, date of TBI, severity of TBI (Mayo classification). Research ethics committee approval was obtained (Ealing and West London REC 09/H0707/82). All patients gave their informed consent. Patients had a full endocrine and metabolic assessment to include dynamic function testing, a dedicated pituitary MRI and bone densitometry imaging (DEXA). Participants had standard structural imaging and a cognitive and psychological assessment following diagnosis of GHD and approximately after one year of GH replacement.

The treated GHD group was compared against 23 age and gender-matched TBI patients without GHD and who had not received GH treatment. They underwent identical imaging and cognitive assessment at two timepoints.

35 age and gender matched healthy volunteers underwent an identical neuroimaging visit at a single time point, but no cognitive assessment.

5.2.2 Endocrine assessment

Patients had full endocrine testing at baseline as part of their routine clinical care (**Table 3-1**). Measurements of anterior pituitary hormones (serum IGF-I, prolactin, TSH, free thyroxine, LH, FSH, testosterone/oestrogen, SHBG) were taken in the late morning/early afternoon in the non-fasted state. Subsequently, patients had initial screening for GH (and ACTH) deficiency using the glucagon stimulation test and confirmed using the GHRH-Arginine test (with age and BMI-adjusted cut-offs). A careful medication history was taken to exclude any confounding factors in the interpretation of these tests. If these tests confirmed GHD sooner than one year

after TBI, then a further test was performed after 1 year to ensure that GHD had not recovered spontaneously. Please refer to section **3.2.4** Tests for pituitary dysfunction on page 102.

Following diagnosis of GHD, a gadolinium-enhanced pituitary MRI was obtained (where possible) to exclude any other cause of GHD such as a pituitary adenoma. Given that GHD can have deleterious effects on bone health, and given that this is an indication for GH replacement, a DEXA scan was performed at baseline. Markers of insulin resistance and dyslipidaemia were also measured before and after treatment and these included fasting insulin, c-peptide, glucose, HbA1c, fasting cholesterol, LDL, HDL and triglyceride levels and BMI. Please see section **2.5** Methods for assessing insulin resistance (IR) and the metabolic syndrome on page 77. Serum IGF-I measurements were taken at baseline and follow-up. An age-adjusted IGF-I ratio was calculated using the median of age-adjusted reference ranges, see section **4.2.3** Serum IGF-I status on page 131 for more details.

If patients met NICE criteria for GH replacement (i.e. >1 yr. from TBI and symptom score on AGHDA of 11/25 or above ((NICE), 2003) then patients were commenced on 0.2 mg of recombinant GH (Omnitrope® or Genotropin ®) by daily subcutaneous injection. Choice of GH depended on local prescribing rules. Each individual had a target IGF-I in the upper third of the normal reference range. Dose of GH was titrated every month for the first three months to achieve an IGF-I in the desired range. Patients were reviewed approximately every three months for the first year to review symptoms, document any side effects and alter dose if necessary. Following a year of treatment, patients had full basal pituitary and metabolic profile, a follow-up structural MRI visit and cognitive/psychological/quality of life assessment.

5.2.3 Structural neuroimaging

Each patient had standard high-resolution T1 and gradient-echo T2* magnetic resonance imaging to assess focal brain injury and presence of microbleeds, on a Philips 3T Achieva scanner (Philips Medical Systems, Netherlands) using an 8-channel head coil. For DTI, diffusion-weighted volumes with gradients applied in 16 non-collinear directions were collected in four runs, with a total of 64 directions, using the following parameters: 73 contiguous slices, slice thickness 2mm, field of view 224mm, matrix 128 × 128 (voxel size 1.75×1.75×2mm^3), *b*-value 1000s/mm^2, four images with no diffusion weighting (*b*0). DTI images were registered to the *b*0 image by affine transformations, then brain-extracted and FA maps generated using the FMRIB Software Library image processing toolbox. FA diffusion maps were analysed using both whole brain and region-of-interest voxel-wise analysis approaches to compare those pre- vs. post- GH replacement. The 23 TBI controls (without GHD) had identical imaging visits designed to be 1 year apart.

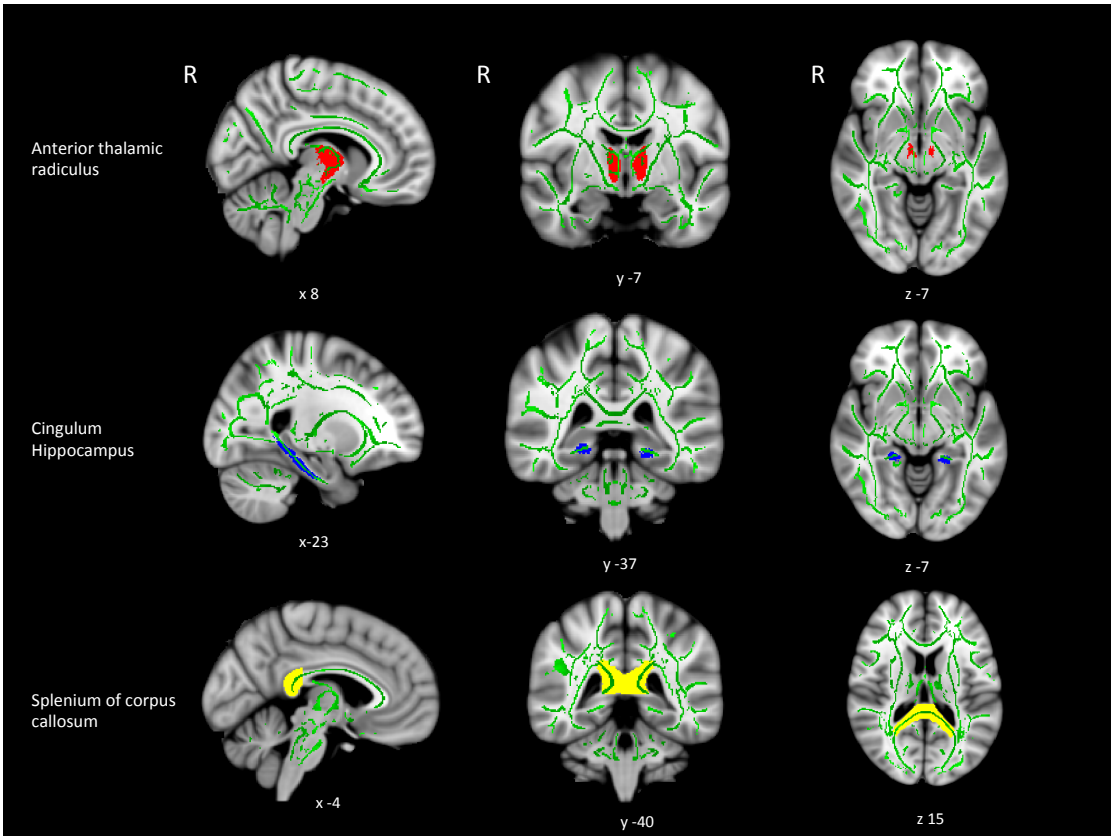
5.2.4 DTI Analysis

FA was the principal outcome measurement. For details on DTI analysis and TBSS please refer to 2.3.4 Generation of FA maps on page 75 and **section 2.3.5** Tract based spatial statistics (TBSS) also on page 75. Identical design matrices were used for chapter 4 (i.e. an interaction and subtraction design). Here instead of IGF-I groups patients were separated into GHD (n=10) and no GHD (n=23) groups. Please refer to section **4.2.5** DTI and FA analysis **on page 132** for more details.

Three region of interest were selected. The SPCC was selected as this region is commonly affected following a TBI and baseline serum IGF-I appears to predict recovery in the region, as seen in **Chapter 4** Association of serum IGF-I with white matter, neuropsychological and cognitive recovery following traumatic brain injury**.** I also selected two other *a priori* regions from the JHU atlas that relate to region of increased GH receptor density in the brain, namely the namely the anterior

thalamic radiculus and the cingulum hippocampus (Lobie et al., 1993). These regions can be seen below in **Figure 5-1**.

Figure 5-1 Anterior thalamic radiculus, cingulum hippocampus and splenium of corpus callosum white matter tract regions of interest.



Group average FA skeleton (green, threshold >0.2) from all patients after TBI (n=33) and healthy controls (n=35), overlaid onto standard MNI152 1mm T1 MRI brain scan. White matter (WM) tract defined using the JHU white matter tractography atlas with a cut-off threshold of 20% indicated in red/blue/yellow. Mean fractional anisotropy (FA) values in anterior thalamic radiculus, cingulum hippocampus and splenium of corpus callosum WM tract regions of interest were calculated from the average of the voxels indicated by the overlap of the green skeleton and red/blue/yellow mask. Co-ordinates given in Montreal Neurological Institute (MNI) standard space. R indicates right.

5.2.6 Neuropsychological Assessment

Each patient with GHD completed a standardised battery of neuropsychological tests previously shown in our group to detect to cognitive impairment following TBI (Kinnunen et al., 2011). For more detailed explanation and references please section **2.3.2** Neuropsychological assessment on page 74. In summary these tests included:

i) The Wechsler Test of Adult Reading (WTAR) to assess pre-morbid intelligence
ii) The People test from the Doors and People Test to measure associative learning and memory
iii) The Trail Making Test to assess executive functions of set-shifting, inhibitory control and cognitive flexibility.
iv) Verbal and non-verbal reasoning ability via the Wechsler Abbreviated Scale of Intelligence Similarities and Matrix Reasoning subtests.
v) The Colour-Word subtest and Letter fluency from the Delis-Kaplan Executive Function System.
vi) Median reaction time for accurate responses on a simple computerised choice reaction task.

5.2.7 Quality of Life (QoL) assessments

Quality of Life (QoL) assessments were carried out in subjects at each neuroimaging visit and periodically during GH treatment. These assessments included the Assessment of GH Deficiency in Adults (AGHDA scale), Beck Depression Inventory Score (BDI-II), Epworth Sleepiness Scale, Pittsburgh Sleep Index, Nottingham Health Profile (NHP) and the Short Form Survey (SF-36). Please see section **2.3.2** Neuropsychological assessment **on page 73** for more detailed information and references.

5.2.8 Statistical analyses

Analysis of demographics, ROI FA values and cognitive variables was performed using SPSS (v22, IBM). Comparison between groups used Mann-Whitney U test or chi-squared test and repeated measures ANCOVA with post-hoc Fisher LSD test, as appropriate with statistical threshold $P<0.05$.

Comparison of FA in ROIs between controls and TBI patients at baseline using ANCOVA included age and gender as co-variates. Comparison of FA in ROIs between groups in TBI patients at baseline using ANCOVA included age, gender, TBI severity, time since TBI as co-variates. Longitudinal analyses were performed both without, and with addition of the following co-variates that might influence WM tract recovery over time: age, gender, TBI severity, time since TBI, and time between DTI scans. Repeated measures ANCOVA examined improvement in FA in ROIs over time in TBI patients, and differences between groups to establish effects of group, time and group x time interaction.

Analysis of metabolic variables and QoL before and after GHR treatment was performed in SPSS (v22, IBM) using paired Student t-tests with a statistical significance threshold of <0.05.

5.3 Results

5.3.1 Patient characteristics

Thirty-three patients in total were studied of whom 10 (30.3%) had confirmed GHD followed by GHR.

5.3.1.1 TBI group who received GHR at baseline

Nine out of 10 patients (90%) were male with a median age 41.7 (IQR) 22.7-56.7 years. All patients has suffered a moderate-severe severity TBI with the exception of the one female participant who had a mild injury. Median time since injury was 23.5 (IQR) 20.5-67.9 months and median time between scans 17.1 (IQR) 13.9-18.9 months.

Seven patients (70%) sustained their TBI via a road traffic accident, 2 (20%) from a blast explosion, 1 (10%) from a fall and 1 (10%) following an assault.

At the time of GHR initiation, 4 patients (40%) had post-traumatic epilepsy, 1 (10%) had type 2 diabetes mellitus and 1 patient (10%) also had confirmed ACTH and gonadotrophin deficiency in addition to GHD. This patient had been started and stabilised on testosterone treatment prior to GHR initiation. The medications recorded at the start of GHR treatment and other demographics data pertaining to the GHR group are displayed in **Table 5-1** and the whole group displayed in **Table 5-2**.

Median absolute IGF-I was 20.9 nmol/L (IQR) 19.2-23.1 and age adjusted ratio 1.1 (IQR) 0.9-1.2. GHD was diagnosed using a glucagon stimulation test (GST) and either a GHRH-arginine test or insulin tolerance test (ITT) as a confirmatory test. For patients within one year of TBI, a 3rd confirmatory test was performed after one year to confirm the persistence of GHD. All patients were started on 0.2 mg of synthetic

GH, irrespective of IGF-I level and once stable titrated at 3 monthly intervals throughout treatment.

On standard structural imaging 7 (70%) of patients had evidence of structural lesions, although structural burden was low and no patient had more than one contusion. Nine patients also had dedicated pituitary imaging on confirmation of GHD and two of these had reported abnormalities e.g. small volume gland and hypoplastic stalk.

5.3.1.2 TBI group without GHR at baseline

Twenty of the 23 patients without GHD who did not receive GHR were male (87%) with median age 30.5 (IQR) 26.8-47.0 years. 22/23 had sustained a moderate to severe TBI using the Mayo classification score. Time since injury was 19.2 months (IQR) 4.4-23.0 and time between scans 11.8 months (IQR) 9.0-14.8. Median absolute IGF-I was 23.0 (IQR) 15.7-26.7 nmol/L and age-adjusted ratio 0.9 (IQR) 0.8-1.0. All of these patients also received identical endocrine assessment including a screening and confirmatory test for GHD. Testing was complete before enrolment in the study and were documented to have no evidence of GHD in the clinical notes.

There was no difference in the age at first scan, gender, severity and time since injury between the group that did and did not receive GHR (P 0.21-0.87). The time between scans was significantly lower in the group who did not receive GHR (11.8 vs. 17.1 months P=0.04). There was a trend towards a lower absolute IGF-I in the GHR group at baseline (20.9 vs. 23.0 nmol/L, P=0.08), but no difference in the age-adjusted IGF-I ratio. Demographic data are displayed in **Table 5-2**.

5.3.1.3 GHR group at follow-up

All patients self-administered their GHR subcutaneously throughout the duration of treatment and all attended their regular follow-up visits at the multidisciplinary TBI

clinic. Median duration of GHR (i.e. time from GH start to 2nd scan) was 14 months (range 7-20). However of note, two patients (patient 2 and 9, median duration 18 and 20 months respectively, **Table 5-1**) had an interruption to treatment due to possible allergic reaction and patient discomfort (patient 2) and another was asked to temporarily stop treatment to confirm ACTH deficiency and re-initiate hydrocortisone treatment (patient 9).

GHR was well tolerated by all patients. There were no reports of headache or arthralgia on starting treatment. Patient 2 intermittently stopped his GHR due to experiencing pruritus. However, following review by an experienced endocrine consultant it was felt that GH injections were not responsible for this symptom.

5.3.2 Effect of TBI on WM tracts

In whole brain analysis, FA was significantly lower in several WM tracts including the superior longitudinal fasciculus, superior region of the internal capsule and cerebellar peduncles in patients following TBI at baseline scan (n=33), compared to healthy controls (n=35) **(Figure 5-2A).**

5.3.3 Effect of time on WM recovery

Within the TBI group as a whole, FA increased over time from scan 1 to scan 2 in a number of WM tracts including the splenium and body of corpus callosum and posterior limb of the internal capsule and other subcortical regions (n=33) **(Figure 5-2B).** There was a trend towards an effect of time in the anterior thalamic radiculus $F(1,31)=0.39$, $P=0.06$ **Figure 5-3**.

Table 5-1 Baseline characteristics of the 10 patients who had confirmed GHD and went on to receive GHR

PARTICIPANT	MODE OF INJURY	SEVERITY (MAYO)	GENDER	MEDICATIONS AT INITIATION OF GHR	OTHER PITUITARY DEFICIENCES/DIABETES/EPILEPSY	STRUCTURAL MRI LESIONS	PITUITARY MRI
1	cyclist	moderate-severe	male	levetiracetam, escitalopram, venlafaxine, rampril, vitamin D	epilepsy	siderosis left parietal lobe	small vol of pituitary tissue
2	RTA-pedestrian	moderate-severe	male	carbamazepine, fluoextine, quetiapine, omeprazole	epilepsy	nil	hypoplastic stalk
3	RTA-pedestrian	moderate-severe	male	phenytoin, temazepam, sertralline, quietapine, lansoprazole, codydramol, ramipril	epilepsy	cystic encephalomalacia	normal
4	assault	moderate-severe	male	vitamin D	nil	left frontal haemorrhagic contusion	normal
5	RTA-motorbike	moderate-severe	male	citalopram, modafinil, omeprazole	nil	contusion	normal
6	Fall	moderate-severe	male	carbamezpine, fluoextine, lorazepam, zopiclone	epilepsy	gliosis, microbleed	not performed
7	RTA-car	mild	female	metformin, lisinopril, rosuvastatin, gliclazide, bendroflumethiazide, atenolol, aspirin	type 2 diabetes	nil	normal
8	RTA-cyclist	moderate-severe	male	vitamin D	nil	microhaemorrhages, contusion, siderosis	normal
9	blast	moderate-severe	male	nebido, pregabalin, amitriptyline, sodium docusate, hydrocortisone	ACTH and gonadotrophin	nil	normal
10	blast	moderate-severe	male	fluxetine, morphine sulphate, amitryptilline, zopiclone, mirtazapine, sodium docusate	nil	microbleed	normal

Table 5-2 Patient characteristics and markers of insulin resistance at baseline

	n	No GHR	With GHR	P with vs. without GHR [a]
n	33	23	10	
Age at first scan (yrs)	33	30.5 [26.8-47.0]	41.7 [26.8-45.4]	0.62
Range		23.1-55.0	22.7 -56.7	
Male Sex	33	20 (87.0%)	9 (90%)	0.74
Moderate-Severe Severity	33	22 (95.7%)	9 (90%)	0.87
Time since Injury (months)	33	19.2 [4.4-23.0]	23.5 [20.5-67.9]	0.21
Range		1.5-25.5	17.6-292.8	
Time between scans (months)	33	11.8 [9.0-14.8]	17.1 [13.9-18.9]	**0.04**
Range		6.3-16.4	11.5-19.4	
Absolute IGF-1 nmol/L [b]	33	23.0 [15.7-26.7]	20.9 [19.4-23.1]	0.08
Range		12.8-27.0	15.9-31.7	
Age-Adjusted IGF-1 Ratio	33	0.9 [0.8-1.0]	1.1 [0.9-1.2]	0.21
Range		0.7-1.1	0.7-1.3	
Blast Injury	33	9 (39.1%)	2 (20%)	0.50
Any structural brain abnormality	33	10 (43.4%)	6 (60%)	0.62
BMI (kg/m^2)	27	24.9 [21.6-29.5]	28.8 [27.5-33.1]	0.12
Range		20.9-31.6	26.1-33.7	
Fasting blood glucose	32	5.2 [4.7-5.2]	5.2 [4.9-7.0]	0.70
Range		4.5-5.4	4.0 -17.0	
HbA1c	32	32.5 [30.3-35.2]	38.0 [32.0-43.5]	0.25
Range		4.4-39.0	29.0-53.0	
Fasting C-peptide	31	665.0 [381.5-818.3]	861.0 [526.-1167.0]	0.20
Range		247.0-923.0	217.0-2552.0	
Fasting insulin	29	6.0 [3.8-8.7]	10.0 [8.5-13.7]	**0.02**
Range		0.8-83.3	2.5-44.3	
HOMA-IR	24	0.9 [0.5-1.1]	1.4 [1.1-1.9]	**0.002**
Range		0.4-2.0	1.0-5.9	
SHBG	33	25.5 [18.2-39.5]	25.0 [14.0-35.5]	0.97
Range		18.0 - 91.0	11.0 - 38.0	
Total cholesterol (mmol/L)	33	5.1 [4.4-5.5]	4.9 [4.1-5.7]	0.97
Range		4.1-7.2	3.2-6.2	
Low density lipoprotein (LDL) mmol/L	32	3.2 [2.9-3.7]	3.0 [2.0-3.5]	0.70
Range		2.6-5.3	1.4-4.0	
High density lipoprotein (HDL) mmol/L	32	1.1 [0.9-1.4]	1.0 [0.9-1.3]	0.70
Range		0.8-1.4	0.8-1.4	
Triglycerides (TG) mmol/L	33	1.3 [1.0-2.0]	2.0 [1.2-3.1]	0.21
Range		0.7-2.4	0.8-5.1	
Systolic BP (SBP)	30	126.0 [108.0-139.3]	141.0 [125.0-144.5]	0.70
Range		104.0-150.0	107.0-150.0	
Diastolic BP (DBP)	30	84.0 [70.0-87.0]	87.0 [74.0-91.5]	0.25
Range		64.0-90.0	68.0-94.0	
Average metabolic score (out of 4)	33	1.5 (1.4)	3.3 (1.0)	**0.001**
Range		0.0-4.0	1.0-4.0	

Data given as n (%) or median [IQR] and range (minimum-maximum).

[a] P value for comparison no GHR vs. with GHR groups using chi-squared test for categorical variables Mann-Whitney U test for continuous variables. [b] To convert nmol/L to ng/mL divide by 0.131.

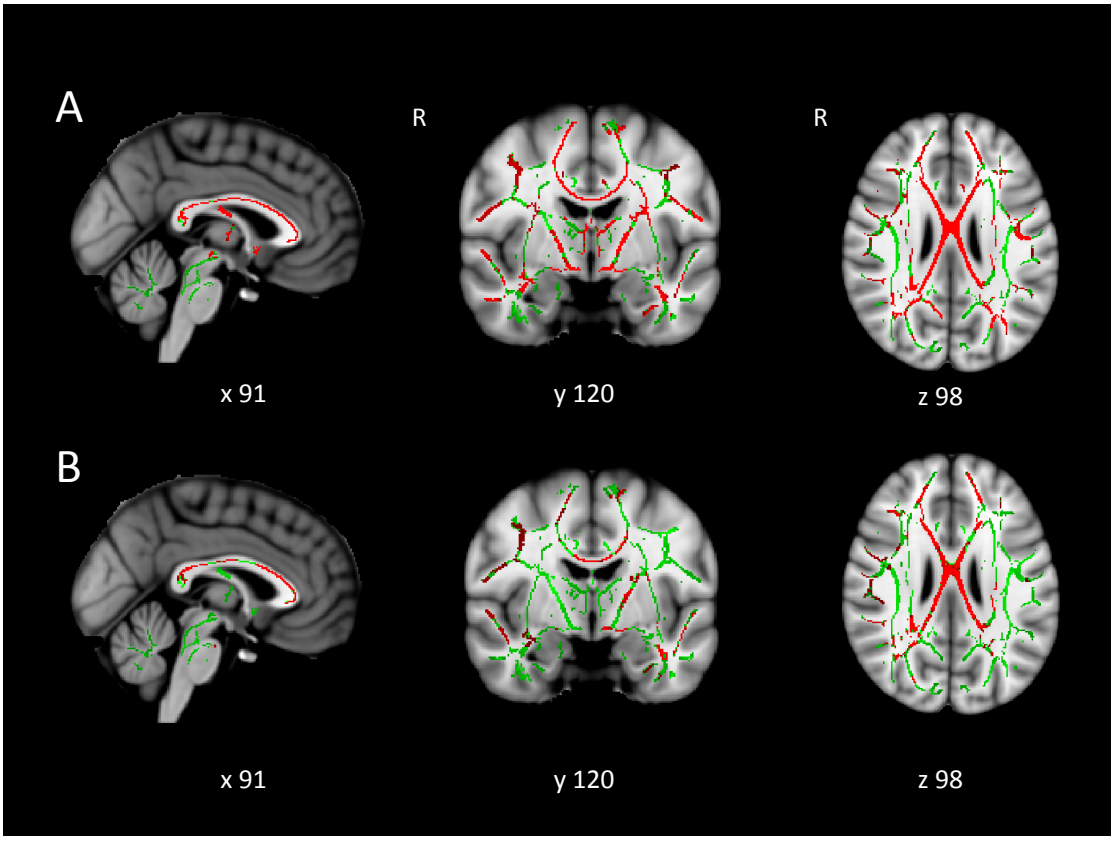
Table 5-3 Endocrine, metabolic, bone and QoL before and after GHR

	n	Before GHR	After GHR	P before vs. after GHR [a]
Absolute IGF-I	10	21.9 ± 4.1	37.1 ± 13.3	**0.008**
Range		15.9-31.7	23.0-68.1	
Age-adjusted IGF-I ratio	10	1.1 ± 0.2	1.7 ± 0.6	**0.016**
Range		0.7-1.3	0.9-2.8	
Dose of GH (mg)	10	0.2 ± 0.0	0.3 ± 0.2	**0.049**
Range		0.2-0.2	0.1-0.8	
BMI (kg/m^2)	10	29.4 ± 3.1	30.0 ± 3.6	0.38
Range		24.9-33.7	25.9-35.8	
Fasting blood glucose (mmol/L)	10	6.3 ± 3.9	5.5 ± 1.5	0.41
Range		3.2-17.0	4.0 -9.1	
HbA1c (mmol/mol	10	37.9 ± 7.4	39.0 ± 8.0	0.36
Range		29.0-53.0	26.0-52.0	
Fasting insulin (ug/L)	10	13.1 ± 11.4	12.0 ± 11.8	0.43
Range		2.5-44.3	1.0-43.0	
Fasting C-peptide (ug/L)	10	911.0 ± 667.6	915.0 ± 483.7	0.97
Range		814.5-2552.0	256.0-2115.0	
HOMA-IR	8	1.9 ± 1.6	1.9 ± 1.8	0.62
Range		1.0-5.9	0.7-6.0	
SHBG (mmol/L)	10	27.3 ± 12.5	29.0 ± 11.6	0.31
Range		11.0-48.0	10.0-42.0	
Total Cholesterol (mmol/L)	10	4.9 ± 1.0	5.2 ± 0.9	0.08
Range		3.2-6.2	3.4-6.1	
Low density lipoprotein (mmol/L)	10	2.8 ± 0.9	3.1 ± 0.9	0.10
Range		1.3-4.0	1.5-4.2	
High density lipoprotein (mmol/L)	10	1.1 ± 0.2	1.1 ± 0.2	0.18
Range		0.8-1.4	0.8-1.5	
Triglycerides (mmol/L)	10	2.2 ± 1.3	2.3 ± 0.8	0.83
Range		0.8-5.1	1.0-4.2	
Systolic BP (mmHg)	10	133.5 ± 14.0	128.2 ± 17.6	0.27
Range		107.0-150.0	107.0-155.0	
Diastolic BP (mmHg)	10	81.0 ± 12.1	79.0 ± 11.1	0.43
Range		58.0-94.0	60.0-95.0	
Metabolic score (out of 4)	10	3.0 ± 1.4	2.2 ± 0.9	0.07
Range		0.0-4.0	1.0-4.0	
AGHDA (out of 25)	10	18.4 ± 5.0	12.0 ± 8.7	**0.004**
Range		10.0-25.0	1.0-25.0	
BDI (out of 63)	10	33.4 ± 16.1	20.7 ± 18.4	**0.001**
Range		15.0-60.0	3.0-56.0	
Epworth (out of 24)	8	11.0 ± 8.1	10.5 ± 6.8	0.47
Range		0.0-20.0	0.0-18.0	
T-score Hip	9	-0.03 ± 1.2		
Range		-1.2 - +2.5		
T-score Spine	9	0.08 ± 1.2		
Range		-1.5 - +2.5		

Data given as mean +SD and range (minimum-maximum).

[a] P value for comparison before GHR vs. after GHR groups using paired Student's t-test (two-tailed)

Figure 5-2 White matter tract fractional anisotropy in patients following TBI



A) *Lower white mater tract fractional anisotropy in patients after TBI. Voxels displaying lower fractional anisotropy (FA) in patients with TBI (n=33) compared to healthy controls (n=35) displayed in red (TFCE P<0.05 corrected and correcting for age and gender), overlaid on combined whole group FA skeleton (green), displayed on standard MNI125 1mm T1 MRI brain scan. Co-ordinates given in Montreal Neurological Institute (MNI) standard space. R indicates right.*

B) *White matter tract FA improvement between scans. Regions displaying higher fractional anisotropy (FA) in TBI patients at follow-up scan (in red) compared to baseline scan overlaid on group FA skeleton (green) using TFCE P<0.05 corrected and correcting for age, time since injury, gender, TBI severity and time between scans. Results overlaid on standard MNI125 1mm T1 MRI brain scan. Co-ordinates given in Montreal Neurological Institute (MNI) standard space. R indicates right.*

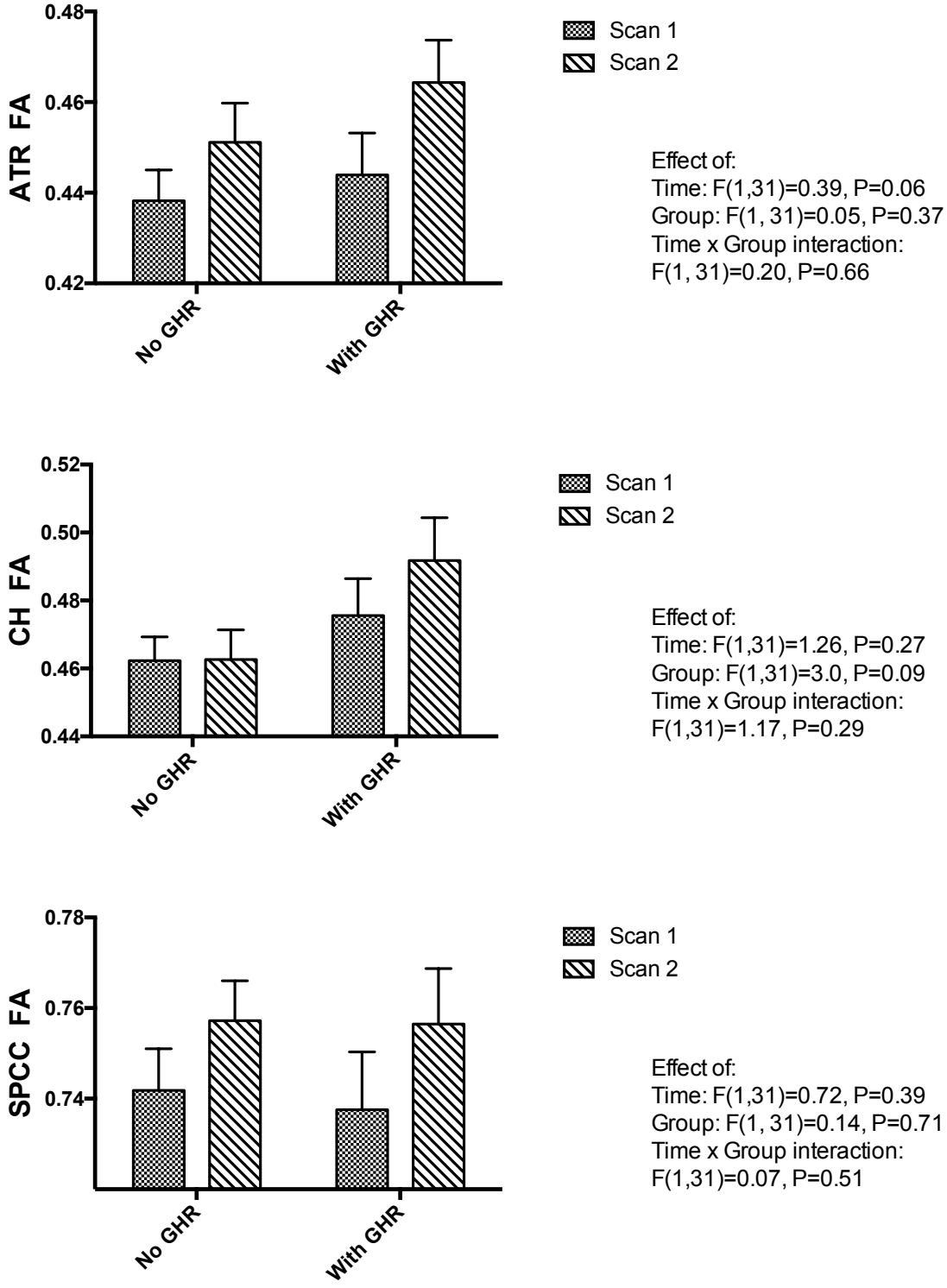
5.3.4 Effect of GHD and GHR on white matter FA

i) No cross-sectional influence of GHD on FA as baseline: Within the TBI group, there was no significant effect of GHD on FA at baseline in whole brain analysis, with or without the inclusion of co-variates (age, gender, TBI severity, time since TBI).

ii) Using the subtraction and interaction TBSS design, there was no significant effect of GHR group or GHR*time. This remained non-significant when including the co-variates: age, gender, TB severity, time since TBI and time between scans.

There was no effect of GHR on the increase in FA over time in either the anterior thalamic radiculus (ATR), cingulum hippocampus (CH) or the splenium of the corpus callosum (SPCC) (GHR group x time interaction P=0.29-0.66) when including all co-variates and or including none. **Figure 5-3**.

Figure 5-3 White matter tract FA in regions of interest in patients after TBI at baseline and recovery over time by GHR status



No difference in improvement in FA in ATR, CH and SPCC between scan 1 (dotted bar) and scan 2 (striped bar) in the group that received growth hormone replacement (GHR) and those that did not. (P results using a repeated measures ANOVA).

5.3.5 Effect of GHR group on cognitive variables and QoL

5.3.5.1 Cognitive variables

Complete cognitive data was available in all patients who received GHR (n=10) and 20/23 who did not receive GHR and who were not GH deficient.

There was a significant effect of time on the Colour Word Stroop Inhibition/Switching task, median time 61.0 s at scan 1 vs. 52.0 s at scan 2, P=0.006 (table). Lower time score on this task relates to better performance. There were trends towards greater performance scores over time in the domains of WTAR, WASI matric reasoning, People test, and Stroop (colour naming) (P=0.054-0.091). **(Table 5-4).**

There were no significant GHR x time interactions in any of the cognitive variables tested when including no co-variates and when correcting for all co-variates (time since injury, time between scans, gender, age at first scan ad TBI severity) (P >0.108). **(Table** 5-4**).**

5.3.5.2 Quality of life variables

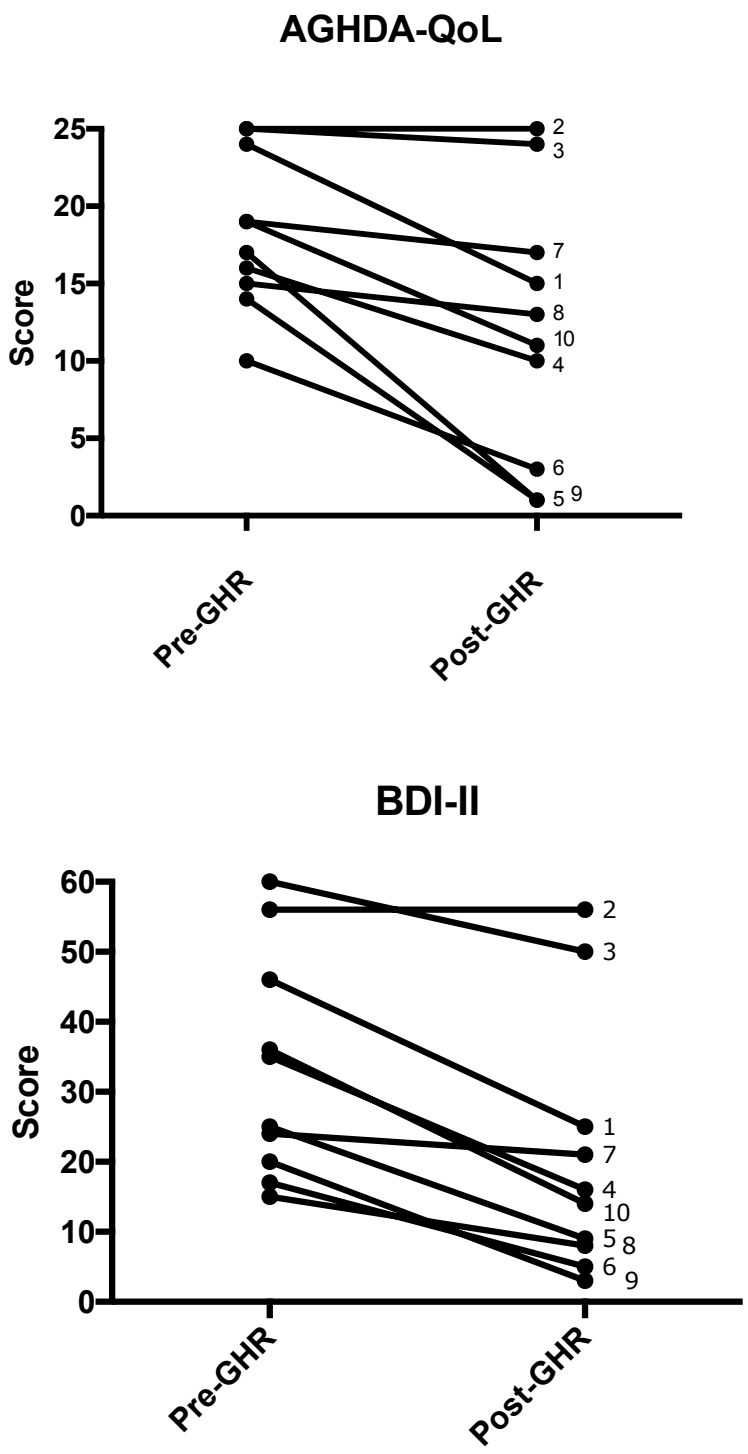
There was a significant decrease in the AGHDA-QoL score following GHR treatment indicating greater improvement in QoL (t=-3.8, df =9, P=0.004, two-tailed). A similar significant reduction in the BDI-II score was aloes observed indicating fewer symptoms of depression following treatment (t=-5.2, df = 9, P=0.001, two-tailed). These results are displayed in **Figure 5-4**. There was no significant change in the Epworth sleepiness scale following treatment. These data are displayed in **Table 5-3**.

Table 5-4 Cognitive outcome measures in patients after TBI at baseline and recovery over time by GHR status

Cognitive domain	*Cognitive variable*	n (no GHR)	n (with GHR)	First Visit	Second visit	Effect of Time[a]	Effect of GH group [a]	Time*Group Interaction[a]
Pre-morbid intelligence: reading ability	**WTAR raw score**	20	10	41.0 [36.0-46.0]	43.0 [34.5-47.8]	0.091	0.939	0.943
Intellectual ability	**WASI matrix reasoning (non-verbal)**	20	10	29.0 [24.0-30.0]	29.0 [26.3-31.0]	0.054	0.317	0.741
Memory: associative memory	**People test (total score)**	20	10	24.0 [20.0-28.0]	27.5 [20.3-31.0]	0.067	0.922	0.218
Memory: immediate recall	**Logical memory I Total**	20	10	41.0 [36.0-49.5]	45.0 [34.5-51.0]	0.552	0.107	0.108
	Logical memory II Total	20	10	26.0 [20.5-34.0]	26.5 [22.3-36.0]	0.666	0.635	0.629
Memory: delayed recall	**Logical memory retention**	20	10	86.7 [78.4-96.1]	90.8 [83.4-100.0]	0.667	0.632	0.630
Processing speed: visual search/complex	**Trail Making Test Trail A (s)**	20	10	24.0 [18.0-31.0]	20.5 [16.3-23.8]	0.188	0.224	0.261
	Trail Making Test Trail B (s)	20	10	47.0 [35.0-67.0]	44.0 [31.0-60.0]	0.275	0.170	0.803
Processing speed: naming/reading	**Stroop Colour Naming (s)**	20	10	31.0 [27.5-40.5]	30.5 [26.5-35.0]	0.068	0.382	0.135
	Stroop Word Reading (s)	20	10	22.0 [18.5-26.0]	22.0 [19.0-24.8]	0.837	0.326	0.254
Executive function: alternating-switch cost	**Trail Making Test Trail B minus A (s)**	20	10	23.0 [15.5-37.0]	19.0 [14.3-35.5]	0.846	0.296	0.466
Cognitive flexibility	**Colour Word Stroop Inhibition/switching (s)**	20	10	61.0 [51.0-74.0]	52.0 [46.0-57.0]	**0.006**	0.712	0.871
Word generation fluency	**DKEFS Letter Fluency F+A+S total**	20	10	37.0 [31.5-46.5]	41.0 [33.0-49.0]	0.810	0.626	0.532

[a] = correction for all covariates (time since injury, time between scans, gender, age at first scan, TBI severity)
*Data displayed as median [IQR] range. Group, time and group*time analysis performed using a repeated measures ANOVA.*
Note: Matrix reasoning, People test, Logical memory: higher score equals better performance; Trail-making, Stroop, letter fluency: lower score equals better cognition
Abbreviations: DKEFS, Delis-Kaplan executive function system; (s), seconds; SF-36; WASI, Wechsler Adult Intelligence Scale; WTAR, Wechsler Test of Adult Reading.

Figure 5-4 AGHDA-QoL and BDI-II scores pre- and post- GHR



Significant reduction in AGHDA-QoL score (t=-3.8, df =9, P=0.004, two tailed) and BDI-II score (t=-5.2, df = 9, P=0.001, two-tailed), indicating improvement in QoL and symptoms of depression following GHR. (AGHDA score out of 25, BDI-II out of 63). Line label 1-10 equates to patient no. see **Table 5-1**.

5.3.6 Effect of GHD and GHR on markers of metabolism

5.3.6.1 Effect of GHD on metabolism

There were significantly higher levels of fasting insulin in patients with GHD compared to those without (median values 10.0 vs. 6.0 ug/L P=0.02). Insulin resistance as measured by the HOMA-IR was significantly higher in those with GHD compared to those without (median value 1.4 vs. 0.9 P=0.002). Total metabolic score (One point for each of raised fasting blood glucose (FBG), HDL, LDL and BP) was significantly higher in patients with GHD vs. those without 3.3 vs. 1.5 (P=0.001), although on the individual measures of FBG, lipids parameters and BP there were no significant differences between groups. BMI was higher in those with GHD (median 28.8 (IQR) 27.5-33.1) than those without (median 24.9 (IQR) 21.6-29.5), although this did not reach significance at P=0.12. The same was true for glycosylated haemoglobin (HbA1c) which was higher in the GHD group (median 38.0 IQR 32.0-43.5) vs. those with no GHD (median 32.5 IQR 30.3-35.2) but was significantly different between the two groups. There was no difference in SHBG (another marker of insulin resistance) between those with and without GHD (P=0.97). Data are displayed in **Table 5-2**.

Bone densitometry (DEXA) was performed only at baseline in those with GHD only (**Table 5-3**). Data were available in 9/10 patients. No patient had evidence of osteoporosis (T score < -2.5). Three out of 10 patients had osteopenia. One patient had osteopenia of the hip (T score -1.5) and the spine (T score-1.1), another patient had osteopenia of the spine only (T score -1.2) and another of the hip only (T score -1.3). Median T score at the hip was -0.05 (IQR) -1.1-0.7 and at the spine -0.1 (IQR) -1.0-0.8.

5.3.6.2 Effect of GHR on metabolism

All patients started GH treatment at a dose of 0.2 mg but this had significantly increased at the end of the study period to a mean dose of 0.3 mg (range 0.1-0.8) to ensure patients were kept in the upper third of the age-related IGF-I reference range. Absolute IGF-I was

significantly higher at the end of the study period (t=3.4, df=9, P=0.08, two-tailed) as was the age-adjusted IGF-I ratio (t=2.9, df =9, P=0.016, two-tailed).

There was a trend towards a lower metabolic score following treatment from mean score 3.0 (± 1.4) to 2.2 (± 0.9) t=0.93, df=9, P=0.07, two-tailed. However there were no other significant differences in BMI, SHBG, HOMA-IR, HbA1c, lipids and blood pressure following treatment with GHR in the study period.

5.4 Discussion

The aim of this chapter was to build on work in the previous chapter which describes an effect of baseline serum IGF-I on WM recovery and memory following TBI. IGF-I is the main downstream mediator of pituitary-derived GH and is therefore considered a proxy measurement of GH reserve. However the gold standard for assessing GH reserve is a diagnosis of GHD via dynamic pituitary function testing, irrespective of the level of IGF-I. This chapter examined the effects of GH replacement (GHR) on WM, cognitive and QoL measures and metabolism in 10 patients who were diagnosed with GHD and underwent GH replacement for a median duration of 14 months.

The patients with and without GHR were fairly well matched in terms of age at first scan, severity, gender, and mode of injury. We did include patients who had sustained a bTBI which is not surprising as bTBI appears to be a particular risk factor for GHD (Baxter et al., 2013) but there was no significant difference in the number of soldiers in each group.

There was no significant difference in the time since injury but there was an outlier in the GHR group who sustained his injury 24 years ago. Given that there were relatively few patients on GHR in the multidisciplinary TBI clinic who were also happy to take part in the study, we decided on balance to include this patient. There was a significantly greater time period between the two scans in patients on GHR compared to those who were not. There could be a number of reasons for this including the desire of the physician to ensure appropriate dose titration and accommodating any interruptions to treatment prior to returning for the second study scan. GHR interruptions occurred in two patients and it was difficult to estimate exactly how much time was spent off GHR, although was not thought to account for more than 2 months in both cases.

Four of the patients with GHD had post-traumatic epilepsy for which they were treated with anti-epileptic agents including phenytoin, carbamazepine and levetiracetem. It is unlikely that these medications or the underlying condition interfered with the diagnosis of GHD. One study found minor abnormalities in stimulated GH in patients who were taking these drugs for epilepsy compared to healthy controls (Franceschi et al., 1984).

However given our stringent protocol for diagnosing GHD in our patients that included a second and sometimes a third dynamic confirmatory test, I think it is unlikely that these medications interfered with the test results sufficiently to alter the final diagnosis. The same is likely to be true for the one patient (patient 7) who had type 2 diabetes. The GST can be an unreliable test in type 2 diabetes (Yuen, 2011), however this patient also had two abnormal GHRH-arginine tests and was therefore very likely to have GHD. We also used BMI and age cut offs for the GHRH-Arginine tests to account for these factors (Colao et al., 2009).

One patient had combined GH/ACTH and gonadotrophin deficiency. That patient was commenced on testosterone replacement at least one year prior to commencing GHR. It is unlikely therefore that testosterone replacement was responsible for improved QoL as is sometimes reported (Moncada, 2006).

Structural brain abnormalities were common in both groups i.e. contusions, superficial siderosis, microbleeds, diffuse axonal injury, and there was no particular difference in the pattern of injury in both group. Overall, no one had grossly abnormal anatomy in this study. One patient with GHD had cystic encephalomalacia which is softening of the brain parenchyma with a cystic component that is usually a late manifestation following a TBI. Of the 4 patients with GHD with normal anatomy, 3 were defined to have a moderate-severe injury based on GCS, LOC and/or PTA duration and 1 had evidence of a mild injury only. Severity of injury was not significantly different between the two groups.

Nine out of 10 patients with GHD had a dedicated pituitary MRI imaging. Two abnormal findings of a hypoplastic stalk and a small volume of pituitary tissue were reported in two individuals. These reports were made by an experienced consultant neuroradiologist. The significant of these findings is uncertain. They could represent previous ischaemic/hypoxic injury resulting from TBI or could be a normal variant.

In terms of WM recovery, we found widespread increases in FA in several WM tracts across the whole brain not only in healthy controls vs. patients but also at scan 2 vs. scan 1. This confirms that patients have microstructural evidence of TBI and this appears to improve following TBI. This result was also reported in the previous chapter. There was

however no effect of GHR on WM recovery. There could be several reasons why this could be false negative result. It is possible that patients were not treated long enough with GHR and at a high enough IGF-I level. It is also possible that this study was underpowered to detect a difference between the two groups and with a larger sample size differences may emerge especially at the cingulum hippocampus. I also had not accounted for lesions in this study, however no patient had distinctly abnormal anatomy and the pattern of injury was similar between the two groups.

The same can be said for the apparent lack of effect of GHR on cognition. The study may have been too small to detect a difference or patients should have been studied at a later time point, especially as there appears to be a strong effect of time alone particularly in the domains of cognitive flexibility. In addition, patient comorbidity may also be an important factor to consider here. At least four patients had significant psychiatric and psychological comorbidity that may have affected their performance on the cognitive tests. One patient had significant psychomotor retardation and was heavily involved with the psychiatric team. Sequentially removing these patients from the cognitive analysis made no difference to the overall results.

However, GHR in this study appeared to significantly improve QoL and reduce symptoms of depression. The QoL improvement is consistent with other studies of GHR outside of TBI and a recent study in patients following TBI (Deijen & van der Veen, 1999; Gardner et al., 2015). I could not find any literature supporting a reduction of depressive symptoms following TBI in those who had received GHR and this could be an important novel finding. Nine out of 10 of our patients had a reduction in their BDI-II score (average point reduction 12.7) and one patient showed no change. This could potentially dramatically improve functioning for these individuals especially as depression is considered one of the most burdensome consequences of TBI (Fann, Hart, & Schomer, 2009). However it is important to point out that 6/10 of these patients were on medication to treat depression following TBI and further investigations are required to determine whether the reduction in depression scores could be attributable to any other intervention or medication dose change.

GHD patients in this study appeared to have a worse metabolic profile at baseline with an increase in insulin resistance (HOMA-IR) and a higher average metabolic score. Formal assessment for metabolic syndrome could not be made as we had not collected waist circumference measurements. BMI was higher in the GHD group with all patients in the overweight or obese categories although BMI was not significantly different between the two groups. Ideally we should have taken a measurement of lean muscle mass and abdominal/visceral adiposity as adipose metabolism impairment is the best recognised metabolic impairment of GHD. However after GHR there was no significant improvement in any of the metabolic variables we measured. There was a trend however towards a lower metabolic score following GHR. Bone health appeared to be lower than a healthy age matched reference group with 3 patients having osteopenia at either the hip/spine or both. It would have been useful to examine the effects of GHR on DEXA imaging at follow-up, however there was no clinical indication to do this.

In conclusion, we found no effect of GHR on WM recovery or cognition following TBI. However we did find a striking improvement in QoL and depressive symptoms following injury. Larger studies examining different lengths of GHR treatment duration are required to determine if GHR has any effect on WM and cognitive recovery from TBI both in GH deficient and GH replete individuals.

6 Neuroinflammation in TBI: A kinetic analysis of the translocator protein (TSPO) positron-emission tomography ligand [^{18}F]GE-180 in the human brain

6.1 Introduction

The translocator protein (TSPO) is a mitochondrial transporter involved in varied intracellular processes, but its expression in the central nervous system (CNS) is relatively low under normal physiological conditions (Cosenza-Nashat et al., 2009). However, activation of microglial cells caused by inflammatory stimuli results in significant upregulation of TSPO expression (M. K. Chen & Guilarte, 2008). TSPO quantification with positron emission tomography (PET) provides a measure of intrinsic neuroinflammation in a variety of CNS diseases. Early PET studies used the isoquinoline [^{11}C]PK-11195 to measure TSPO binding and detected elevations across a range of conditions including multiple sclerosis (Banati et al., 2000), Huntington's disease (Pavese et al., 2006), Alzheimer's disease (Cagnin et al., 2001; Edison et al., 2008), traumatic brain injury (Ramlackhansingh et al., 2011), and ischaemic stroke (Gerhard, Schwarz, Myers, Wise, & Banati, 2005). However, the ligand can be limited by a high non-specific signal, making non-standard approaches to data analysis necessary (Kropholler et al., 2006). In addition, [^{11}C]PK-11195 is a carbon-11 compound, with a half-life of 20.3 minutes, restricting its use to locations with an on-site cyclotron.

A number of second generation TSPO ligands have been developed recently with the promise of improved signal to noise and greater specific binding. [^{18}F]GE-180 is a novel fluorinated radiotracer that binds to the TSPO with high affinity (Wadsworth et al., 2012). Developed from a series of tricyclic indoles, [^{18}F]GE-180 has demonstrated superior specific binding affinity to [^{11}C]PK-11195 in animal models of acute neuroinflammation (Dickens et al., 2014) and stroke (Boutin et al., 2015). The fluorine-18 radiolabelling, with a half-life of 109.8 minutes, also makes [^{18}F]GE-180 more suitable than carbon-11 based compounds for long-distance distribution enabling widespread clinical use.

Other second generation TSPO radiotracers (e.g. [^{11}C]PBR-28, [^{18}F]PBR-06, [^{11}C]-DAA1106, [^{11}C]-DPA713, [^{18}F] FEPPA) show binding affinities influenced by a TSPO polymorphism expressed by individuals which have been classified as high-affinity binders (HABs), mixed affinity binders (MABs) and low affinity binders (LABs) (Owen et al., 2011). Expression of the TSPO Ala147Thr polymorphism results in MAB or LAB depending on whether one or two copies are present (Owen et al., 2012).

In this chapter I present a study in healthy participants using [^{18}F]GE-180 PET imaging. The primary objective here was to study the tracer kinetics and quantification in the healthy human brain. The secondary objective was to investigate whether there were differences in binding between HABs and MABs. Work in this chapter forms the foundation for the next chapter where this tracer is used to quantify TSPO inflammation in patients following TBI and to investigate a link between the metabolic syndrome and central neuroinflammation.

6.2 Methods

6.2.1 Participants

This study was approved by the Westminster Research Ethics Committee, London (13/LO/1596), the Riverside Research Ethics Committee (13/LO/1916), and the Administration of Radioactive Substances Advisory Committee (no. 631/336/30788). Research was conducted in accordance with the Helsinki Declaration. All participants gave written, informed consent.

Ten healthy volunteers (7 males), mean age 41 ± 9, 28–56 years, mean weight 81.8 ± 13 kg, were included in the study. A screening assessment was carried out that included full medical and drug history, blood pressure, height, weight, Allen's test for patency of the ulnar anastomosis, and the Structured Clinical Interview for DSM disorders (SCID). Blood samples were taken for analysis of full blood count, renal profile, clotting screen and TSPO genotyping. Exclusion criteria included pregnancy, a history of prior or current psychiatric or neurological disease, abuse of alcohol or drugs or contraindication to arterial line placement.

6.2.2 TSPO Genotyping

DNA was extracted using the Qiagen QIAmp DNA blood mini kit. TSPO genotyping of the c.439A>G (p.Thr147A1a) (SNP rs6971) was performed using a TaqMan Allelic Discrimination assay. Low affinity binders (n=1) were excluded from the imaging component of the study. During the study design it was felt that on ethical and economical grounds we should not expose LABs to this novel tracer and instead focus on MABs and HABs. Of the 10 participants eligible for imaging, 5 were high-affinity binders and 5 were mixed affinity binders.

6.2.3 Synthesis of [^{18}F]GE-180

[^{18}F] fluorine was produced by the ^{18}O(p,n)^{18}F nuclear reaction on a GE PETtrace 8 cyclotron (The Grove Centre, Amersham, UK). All radiochemistry was performed on the GE FASTlab synthesizer with single use cassettes. Average synthesis time was 43 mins, radiochemical yield 43% and purity was greater than 95% (Wickstrom et al., 2014). The radiotracer was manufactured by GE Healthcare (The Grove Centre, Amersham, UK), transported to Hammersmith Hospital, London, and used within 12 hours of manufacture.

6.2.4 Positron Emission Tomography (PET) Scanning and Image Reconstruction

All participants were scanned at the Clinical Imaging Facility, Imperial College London, Hammersmith Hospital. Prior to PET scanning, an arterial cannula was inserted under local anaesthetic (2% lidocaine) into the radial artery to allow arterial blood sampling. An antecubital venous cannula was inserted for radiotracer administration.

PET studies were performed on a Biograph 6 (6 slice CT) scanner after administration of 182 ± 3.1 MBq via intravenous bolus injection given over ten seconds followed by a 10 mL saline flush. A low dose CT scan preceded the PET acquisition to allow correction for tissue attenuation. Emission data were then acquired over 90 minutes in list mode and reconstructed as 24 temporal frames (6x15, 3x60, 5x120, 5x300, 5x600 seconds) using filtered back projection (matrix size 168x168, zoom 2.6, 5mm Gaussian filter, pixel size 1.56x1.56, slice thickness 3mm) with and without attenuation correction. Standard corrections for scatter, decay and random counts were applied.

6.2.5 Whole Blood, Plasma Activity and Parent Fraction of [^{18}F]GE-180

Arterial blood activity was measured every second for the first 15 minutes of the 90 minute scan using an automated blood sampling system (ABSS Allog, Mariefred, Sweden) connected to the participant via a 1.5 m x 1.0 mm diameter polytetrafluoroethylene

(PTFE) line (blood withdrawal rate 2.5 ml/min). In addition, manual blood samples (10mL) were collected from the radial artery at 0, 5, 10, 15, 30, 50, 70, 90 mins to assay whole blood and plasma activity. Plasma was obtained by centrifugation over 3 minutes at 1800 g. Activity of whole blood and plasma was measured in a Well Counter (CAPRAC -®T/Well Counter) over 10-60 seconds. The first 15 minutes of continuous whole blood activity was combined with the discrete samples to generate the whole blood activity curve for use in data modelling. The continuous plasma-over-blood ratio was estimated using a constant model and a total plasma activity curve was obtained by correcting the whole blood curve for this partition between plasma and blood.

The parent fraction of [^{18}F]GE-180 was measured by high-performance liquid chromatography (HPLC) (Agilent 1100 and 1260 series) of discrete plasma samples (3.5 mL). The parent fraction was fitted to a single exponential plus a constant model. The total plasma activity was multiplied by this parent fraction and then smoothed post peak by fitting to a tri-exponential function to generate an arterial parent plasma input function. A delay correction of up to +30 seconds was applied to the input function to prior to fitting the kinetic modelling stage. This was performed to account for delay in the 1.5 m tube and delay between the radial artery and the brain.

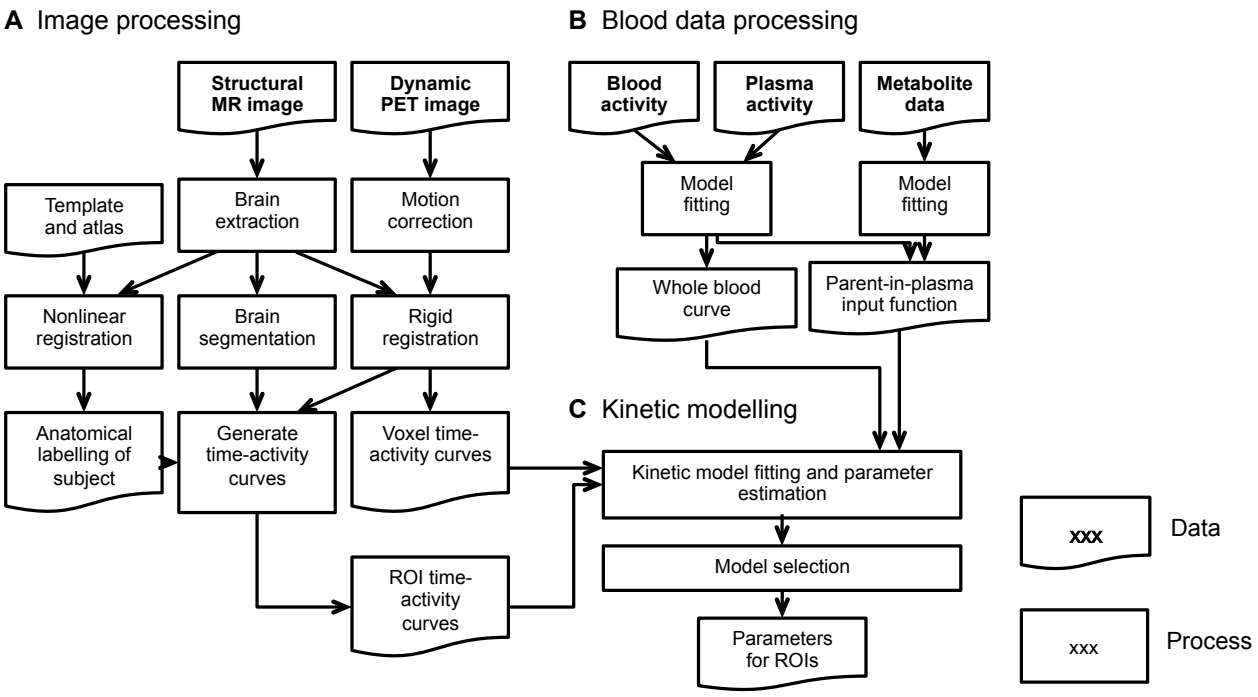
6.2.6 Magnetic Resonance Imaging (MRI)

To provide additional anatomical information to aid analysis of the PET data, each subject had a structural T1-weighted MRI scan on a Siemens Verio 3T scanner (matrix size 256x256, 1x1x1 mm voxel size, TR 9.63 ms, TE 4.74 ms, flip angle 9 degrees).

6.2.7 Data Analysis

A high level overview of the data analysis is provided in **Figure 6-1**. We used the PET data analysis and kinetic modelling toolkit, MIAKAT™ (www.miakat.org), which incorporates software from SPM5 (Wellcome Trust Centre for Neuroimaging) and FSL (FMRIB, University of Oxford) (Searle et al., 2014).

Figure 6-1 Overview of PET data analysis



We used the MIAKAT™ analysis toolkit for image processing (A), blood data processing (B) and kinetic modelling (C) of PET data.

The brain was initially extracted from the T1-weighted MR image. The CIC neuroanatomical atlas was non-linearly registered to the individual's extracted brain in order to generate a personalised set of anatomically parcellated regions (Tziortzi et al., 2011). Frame-by-frame motion correction of the dynamic (non attenuated-corrected) PET data was performed using mutual information co-registration of the individual frames to a reference frame. An average motion-corrected PET image was generated and used for co-registration with the T1-weighted MR image. Finally, regional tissue time activity curves (TACs) were generated for each region of interest (ROI) defined from the CIC atlas that had been transformed into each individual's image space.

Datasets were analysed with one- and two-tissue-compartment models and the Logan graphical method, using the metabolite-corrected plasma input function as previously described (Fujita et al., 2008) with a fixed 5% blood volume correction.

The primary outcome measures were the radioligand delivery rate constant ($K_{1;}$ mL cm $^{-3}$ min $^{-1}$), total distribution volume ($V_{T:}$ mL cm $^{-3}$), and standardised uptake values (SUV). The

SUV ratio and distribution volume ratios (DVR) were estimated using cortical grey matter as a pseudo reference region, since previous studies reported this area as having one of the lowest levels of TSPO binding (Owen et al., 2014)

6.2.8 Time Stability Analysis

To investigate the stability of V_T over different scan durations, a time stability analysis was performed by analyzing data for total time windows that ranged between 40-90 minutes in 10 minute increments.

6.2.9 Statistical Analysis

To determine the most appropriate compartmental model the Akaike information criterion (AIC) was used for model selection (Akaike, 1974), where lower AIC was indicative of a more parsimonious model. To compare characteristics between genetic groups (HAB/MAB), Fisher's exact test (gender) and the Mann Whitney U test (age weight, injected dose) were used. To evaluate differences between genetic groups in time activity curves for blood data, a repeated measures analysis of variance (ANOVA) was used, with time as the within-subjects factor and genotype as the between-subjects factor. A repeated measures ANOVA was also used to compare outcome measures across multiple ROIs, where ROI was used as the within-subjects factor.

6.3 Results

6.3.1 Patient characteristics and drug safety

Injection of [18F]GE-180 caused no pharmacological effects based on patient reports, blood pressure, pulse, respiration rate and oxygen saturation after radioligand administration. There were no significant differences in gender, age or weight between HABs and MABs. We looked for correlations between age and our principal outcome measure. We found no significant correlations between age and VT in both HABs and MABs in any of the ROIs studied (HABS: Spearman's rho = -0.3-0.7 p=0.188-0.873, MABs: Spearman's rho 0.1-0.8, p=0.104-0.94). There was also no significant relationship between age and outcome measures when added as a covariate in the repeated measures ANCOVAs performed. Demographic data are displayed here in **Table 6-1**.

Table 6-1 Patient characteristics of the ten healthy volunteers enrolled in study

Subject	Genotype	Age (yrs.)	Gender	Weight (kg)
1	HAB	47.9	M	97.9
2	HAB	35.5	M	72.1
3	HAB	34.4	M	94.6
4	HAB	48.1	F	69.7
5	HAB	28.4	M	96.6
6	MAB	49.2	M	87.5
7	MAB	39.8	F	70.3
8	MAB	31.0	F	74.2
9	MAB	56.2	M	65.4
10	MAB	39.7	M	90.4

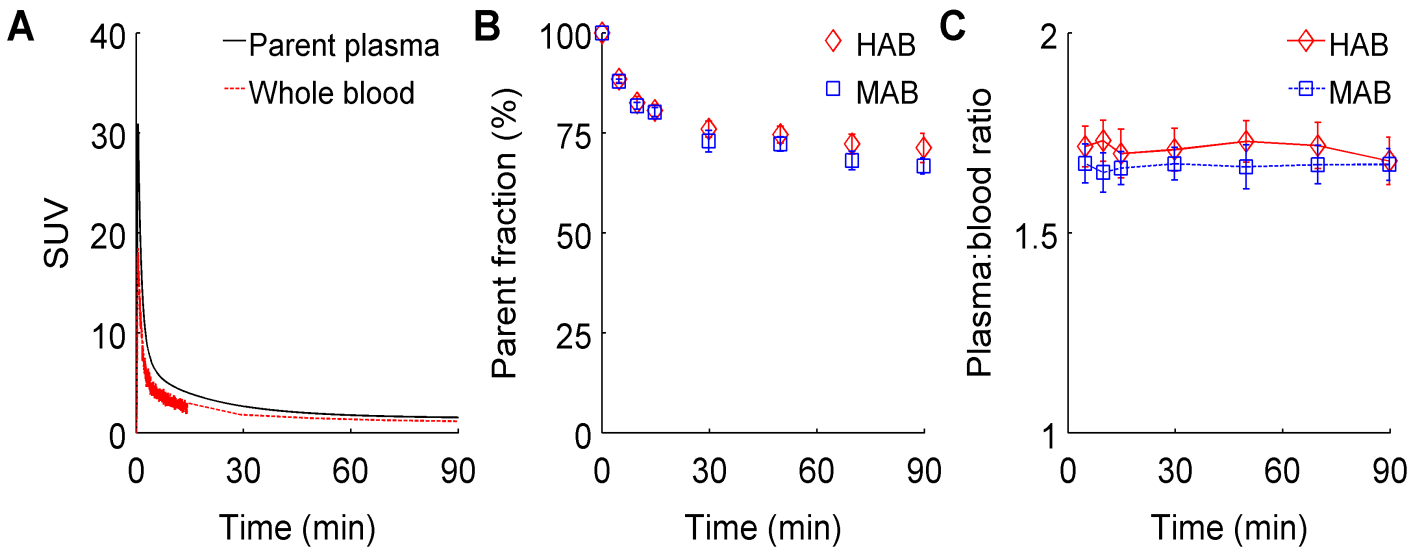
HAB=high affinity TSPO binders and MAB=mixed affinity TSPO binders

6.3.2 Plasma Data

In plasma, the concentration of [^{18}F]GE-180 peaked at about 45 seconds and was followed by a rapid decrease (**Figure 6-2**). The fraction of unchanged [^{18}F]GE-180 over time is shown in **Figure 6-2B**. The parent compound accounted for 65.0-81.7% (min-max across participants) of the total concentration in plasma at 30 minutes, and 57.3-78.3% at 90 minutes. Three polar radioactive metabolites were identified over the course of the 90 minute scanning window (<10% of parent compound). The plasma to blood ratio

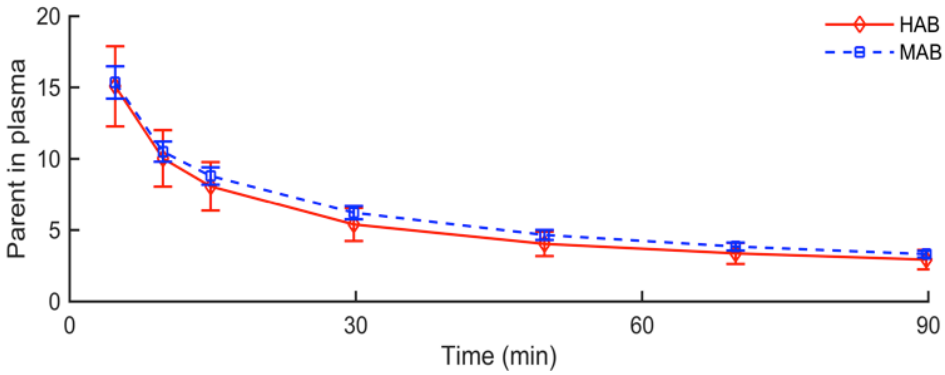
remained constant at ≈1.69 **(Figure 6-2C)** across individuals. There were no significant differences in profiles of plasma over blood There were no significant differences in profiles of plasma over blood (F(1,48)=0.407, p=0.541), parent fraction (F(1,48)= 0.871, p=0.378) or parent in plasma between genetic groups (F(1,48)=0.130, p=0.728). There was no interaction of genotype with time for any of these profiles (p>0.204). The parent in plasma profile for HABs and MABs is shown in **Figure 6-3**.

Figure 6-2 [18^F]GE-180 Blood data: whole blood, plasma, parent fraction and plasma:blood ratio



(A) Whole blood (red) and parent in plasma (black) curves for one subject. (B) The fraction of unchanged parent compound over time, for high affinity binders (HAB, red) and medium affinity binders (MAB, blue). (C) Plasma to blood ratio over time, for HAB and MAB groups. Values plotted for B and C are mean +/- standard error of the mean.

Figure 6-3 [^{18F}]GE-180 Blood data: parent in plasma (HABs vs. MABs)

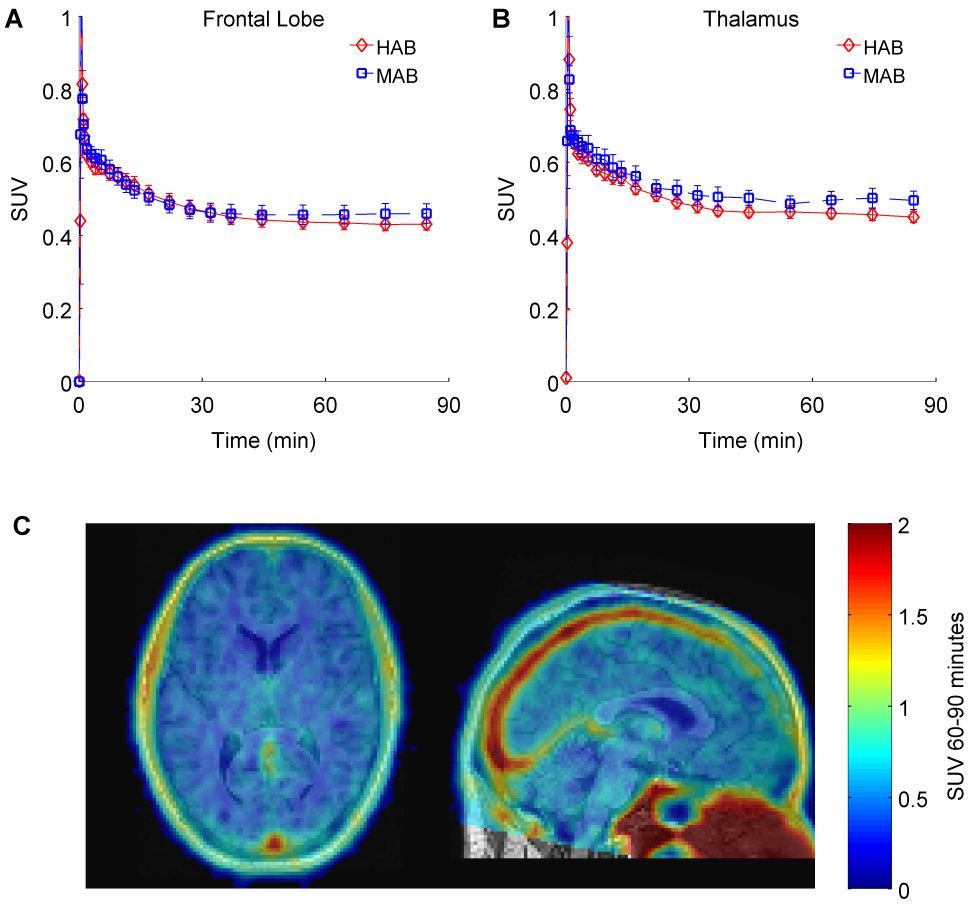


Parent in plasma for groups HABs and MABs over time

6.3.3 Tissue Data

The concentration of the ligand in the brain peaked at around one minute followed by rapid washout for all participants. Group-averaged tissue TACs for frontal grey matter and thalamus are shown in **Figure 6-4A and B**. A 60-90 minute SUV image for a representative MAB subject are shown in **Figure 6-4C.** Overall, there was low uptake of the tracer in brain with images being dominated by signal from blood vessels. There were no significant differences in SUV curves between genetic groups ($F(1,48)=1.396$, $p=0.271$). There was no interaction of genotype with time ($P=0.684$).

Figure 6-4 Example of [18F]GE-180 imaging



Time activity curves (TACs) are shown for high affinity binders (HAB) and medium affinity binders (MAB), in frontal grey matter (A) and thalamus (B). Standardised uptake values (SUV) are plotted as mean +/- standard error of the mean. (C) SUV image calculated from the 60-90 minute PET frames, superimposed on the co-registered T1-weighted MR, for a representative MAB subject.

6.3.4 Kinetic Analysis

The results of the kinetic modeling are shown in **Figure 6-5.** The two-tissue-compartment (2TC) model was superior to the one-tissue-compartment model (1TC), as judged by a lower AIC, in all ROIs except the striatum. Example model fits for 1TC and 2TC are shown in **Figure 6-5**A for a representative MAB subject. The first 10 minutes of **Figure 6-5A** and **C** are shown in more details in **Figure 6-6**.

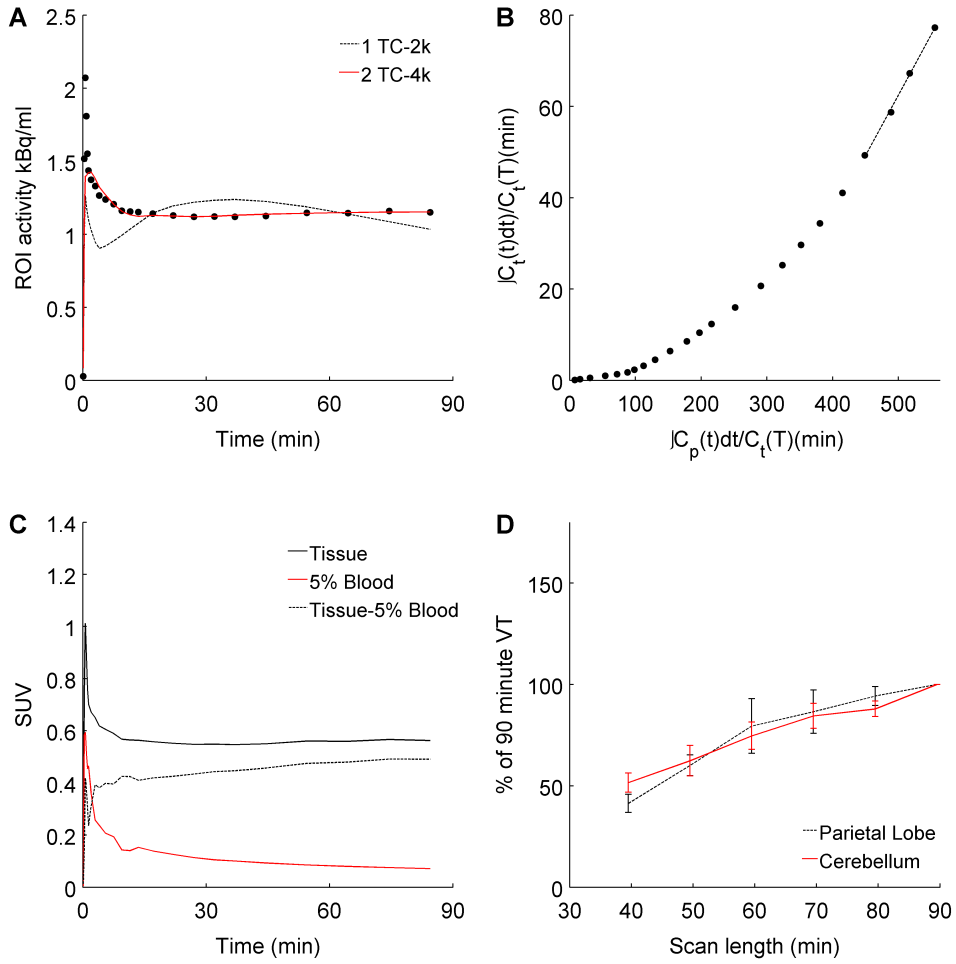
The 2TC generally showed a good fit to the data excepting an initial small mismatch in the blood volume peak, which should not impact on V_T estimates. When we included the 2TC-fit model, it did not outperform the 2TC-fix based on the AIC. Blood volume estimates ranged from 6.3-10.5% (mean 8.4%) across all ROIs. Therefore the 2TC-fix was selected as the model to use for further analysis and gave average rate constants (across all regions and participants) of K_1=0.013 mL cm^{-3} min^{-1}, k_2=0.229 min^{-1}, k_3=0.035 min^{-1}, and k_4=0.010 min^{-1}, resulting in V_T=0.311 mL cm^{-3} **Table 6-2**.

The delivery rate constant, K_1, was low across all ROIs in all participants, indicating low extraction across the blood-brain barrier. This is consistent with the low tissue uptake observed in the images and the predominance of the vasculature structures. There was no significant effect of genetic group on any of the four rate constants. The Logan graphical method was also used to estimate the V_T in each ROI **Table 6-2**. A representative plot is shown in Figure 6.4B. Pooled V_T estimates from the 2TC were positively correlated with V_T Logan (Pearson's r=0.630 p<0.0001, regression equation V_T Logan = 0.3 * V_T-2TC + 0.19). Tissue TAC minus whole blood radioactivity demonstrates that approximately 20% of the activity in a typical ROI comes from blood (**Figure 6-5B**).

6.3.5 Time Stability Analysis and Outcome Measures

The time stability analysis of the 2TC model demonstrated an increasing V_T for each successive time window analysed (shown for two ROIs in **Figure 6-5D.** A comparison of six outcome measures for a number of ROIs is shown in **Figure 6-7**. For all six outcome measures, no significant effect of genetic group was found (p>0.186), nor any interaction between genetic group and ROI (p=0.468).

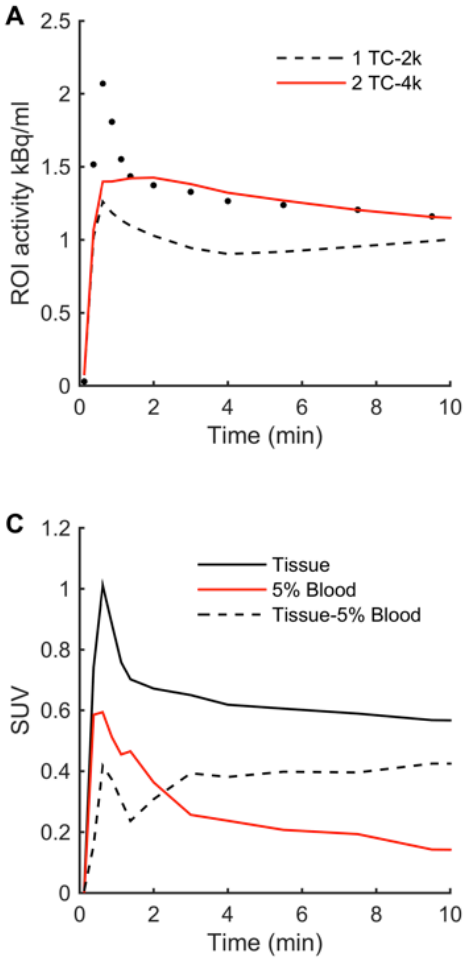
Figure 6-5 Kinetic modelling and time stability analysis



(A)

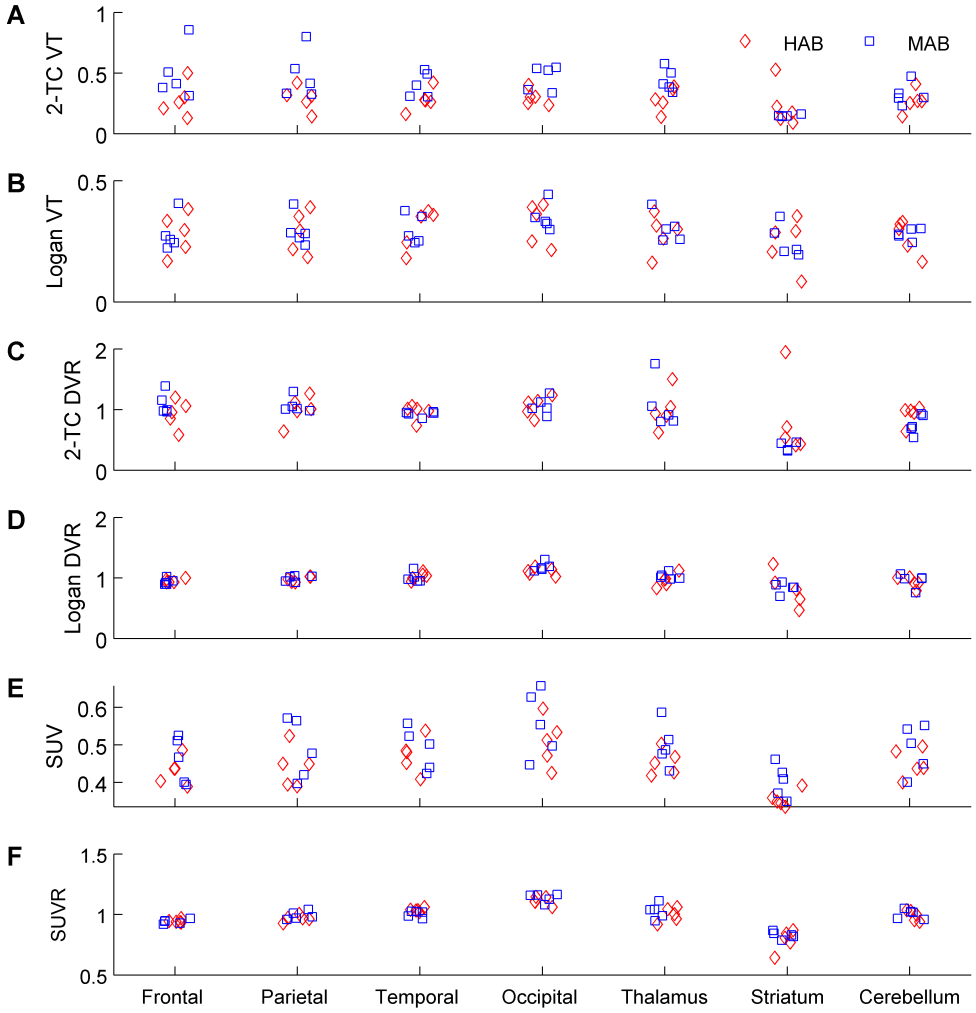
Model fits are shown for one-tissue compartmental-model (reversible 1TC) black dashed line and two-tissue compartmental-model (reversible 2TC), red solid line) against a time activity curve (TAC) (black dots) for the Parietal Lobe. (B) Logan plot (black dashed line for most linear portion of curve) for the same time activity curve as (A) (black dots). (C) Parietal Lobe TAC (solid black line), 5% whole blood activity curve (red solid) and difference of the two curves (black dashed) highlights the significant contribution of blood signal to the TAC. (D) Estimates of two-tissue compartmental-model V_T calculated for 40-90 minute scan windows in 10 minute increments are plotted as the absolute percentage difference compared to the final 90 minute V_T, for parietal lobe (A) and cerebellum (B) grey matter regions of interest. Values plotted are mean percentage +/- standard error of the mean.

Figure 6-6 ***First 10 minutes of Figure 6.5A and 6.5C in more detail***



(A) Model fits are shown for one-tissue compartmental-model (reversible 1TC) black dashed line and two-tissue compartmental-model (reversible 2TC), red solid line) against a time activity curve (TAC) (black dots) for the Parietal Lobe. (C) Parietal Lobe TAC (solid black line), 5% whole blood activity curve (red solid) and difference of the two curves (black dashed) highlights the significant contribution of blood signal to the TAC.

Figure 6-7 Group outcome measures (VT/Logan VT/DVR/Logan DVR/SUV/SUVR)



Six outcome measures are plotted for seven regions of interest, coloured separately for high affinity binders (HAB, red) and medium affinity binders (MAB, blue). (A) Two-tissue-compartmental model (2 TC) volume of distribution (V_T). (B) V_T estimated using Logan plot. (C) Distribution volume ratio (DVR) from 2TC using cortical grey matter as a reference region. (D) DVR estimated using Logan plot and cortical grey matter as a reference region. (E) 60-90 minute standardised uptake values (SUV) (F) 60-90 minute SUV ratios (SUVR) using cortical grey matter as a reference region.

Table 6-2 Parameter estimates and model fits

Model	Region	K_1 (mL/min)	k_2 (/min)	k_3 (/min)	k_4 (/min)	V_T (mL/cm^3)	DVR	AIC wins
1-TC	Frontal Lobe	0.00472(0.0041-0.006)	0.0271(0.023-0.028)			0.171(0.15-0.22)	0.939(0.94-0.95)	2/10
	Parietal Lobe	0.00513(0.004-0.0061)	0.027(0.024-0.028)			0.19(0.16-0.22)	0.969(0.96-1)	1/10
	Temporal Lobe	0.00514(0.0048-0.006)	0.0266(0.025-0.028)			0.182(0.17-0.24)	1.04(1-1)	0/10
	Occipital Lobe	0.00621(0.0057-0.0074)	0.03(0.025-0.032)			0.214(0.19-0.25)	1.12(1.1-1.2)	0/10
	Thalamus	0.00555(0.0046-0.0058)	0.0261(0.024-0.028)			0.182(0.17-0.24)	1.03(0.96-1.1)	0/10
	Striatum	0.00358(0.003-0.0046)	0.0241(0.021-0.025)			0.155(0.14-0.2)	0.826(0.78-0.89)	8/10
	Cerebellum	0.00656(0.0054-0.0076)	0.0339(0.034-0.034)			0.178(0.16-0.22)	0.968(0.91-1)	0/10
2-TC	Frontal Lobe	0.0102(0.0089-0.013)	0.195(0.15-1.6)	0.0301(0.024-0.15)	0.00653(0.004-0.017)	0.346(0.26-0.5)	0.984(0.96-1.2)	8/10
	Parietal Lobe	0.0116(0.011-0.014)	0.192(0.15-0.25)	0.0334(0.025-0.039)	0.00873(0.0045-0.014)	0.33(0.31-0.42)	1.01(0.98-1.1)	9/10
	Temporal Lobe	0.0143(0.012-0.032)	0.217(0.16-1.3)	0.0348(0.03-0.15)	0.00927(0.0063-0.016)	0.306(0.27-0.42)	0.958(0.93-1)	10/10
	Occipital Lobe	0.0238(0.019-0.031)	0.385(0.2-0.62)	0.0395(0.0036-0.054)	0.0101(0.0062-0.014)	0.35(0.3-0.52)	1.07(0.97-1.1)	10/10
	Thalamus	0.0118(0.011-0.014)	0.16(0.14-0.2)	0.0315(0.0026-0.042)	0.00881(0.0062-0.012)	0.376(0.28-0.41)	0.922(0.81-1.1)	10/10
	Striatum	0.00413(0.0033-0.0088)	1.14(0.66-1.7)	4.89(0.62-9.1)	0.14(0.027-0.21)	0.155(0.14-0.22)	0.451(0.41-0.71)	2/10
	Cerebellum	0.0224(0.016-1.3)	0.319(0.23-78)	0.0331(0.0029-0.091)	0.0113(0.0084-0.018)	0.281(0.25-0.33)	0.916(0.69-0.98)	10/10
Logan	Frontal Lobe					0.265(0.23-0.33)	0.931(0.92-0.97)	
	Parietal Lobe					0.284(0.23-0.35)	0.996(0.93-1)	
	Temporal Lobe					0.312(0.24-0.36)	1.01(0.95-1.1)	
	Occipital Lobe					0.34(0.3-0.39)	1.14(1.1-1.2)	
	Thalamus					0.3(0.26-0.31)	0.991(0.98-1)	
	Striatum					0.25(0.21-0.29)	0.844(0.7-0.92)	
	Cerebellum					0.29(0.24-0.3)	0.99(0.91-1)	

Parameter estimates and model fits are shown for the one tissue compartment (1-TC), two tissue compartment (2-TC) model and Logan graphical method. Data are presented as median (interquartile range, IQR). For 1-TC versus 2-TC, the right-most column shows the proportion of the ten participants for which the model was the more parsimonious (defined as having the lower akaike information criterion, AIC).

6.4 Discussion

In this chapter we have described the characterization and quantification of the novel TSPO tracer [^{18}F]GE-180 for the first time in the normal healthy human brain. We generated arterial parent plasma and whole blood input functions and fitted brain TACs to one- and two-tissue compartmental kinetic models and Logan graphical analysis to generate outcome measures across regions and individuals. The following key outcome measures were generated from the analysis: delivery (K_1), volume of distribution (V_T), standardised uptake values (SUV), distribution volume ratios (DVR) and SUV with cortical grey matter as a pseudo reference region (SUVR). In addition, we investigated whether the TSPO Ala147Thr polymorphism in the TSPO binding site influenced these outcome measures (Q. Guo et al., 2013; Owen et al., 2011).

There was consistent and stable metabolism of [^{18}F]GE-180 parent compound across all individuals. There was no difference in blood or plasma activity between the two genotypes and there were only moderate levels of detectable metabolites in all individuals with 70% of the intact parent tracer remaining at 90 min. SUV images across all individuals demonstrated low uptake of the tracer in brain tissue with significant tracer activity apparent in the blood compartments of the brain. The low brain uptake could reduce the signal to noise ratio for this tracer and might mean that variation in the activity within the blood across the groups could bias results. In addition, the low uptake might make the tracer more susceptible to showing increased uptake when there is BBB breakdown.

Analysis of the tracer compartmental modelling showed that the reversible two tissue compartment model provided the best overall fit in the majority of cases. There was a small discrepancy in the model fit at the initial sharp peak of the curve i.e. <5 mins of data acquisition. It is possible that this may be due to increased dispersion of [^{18}F]GE-180 in the vascular bed, although this is difficult to quantify precisely. In addition, the 1.5m line that was used from the radial artery to the arterial blood detection machine may have impacted the model fit. However, this discrepancy should not impact in a significant way on the estimation of V_T as this is based on the integral of the impulse response function (i.e. integral/area under curve of plasma input function) (Gunn, Gunn, & Cunningham,

2001). K1 could be affected by dispersion but would still remain small after any correction and therefore the interpretation of low brain delivery of this tracer is still valid.

Using the 2TC model, the initial rate constant K_1 was consistently low suggesting a low extraction fraction and delivery into brain tissue. Theoretically this could be due to a number of reasons: (1) low lipophilicity - however pre-clinical work has suggested that this tracer is relatively lipophilic (logD at pH 7.4 is 2.95), making this unlikely; (2) [^{18}F]GE-180 could be a substrate for xenobiotic pumps at the blood brain barrier such as the three major ABC transporters, p-glycoprotein-ABCB1 and ABCC1, ABCG2 as can be seen with other tracers with low BBB penetration;(Toth et al., 2014) (3) the low K_1 could be due to increased plasma protein binding although the relationship here is complex and high plasma protein binding does not always lead to low brain penetration. Most molecules to a greater or lesser extent bind to human serum albumin and some tracers also bind to alfa1-acid glycoprotein (Lockhart et al., 2003). However in the case of [^{18}F]GE-180, the binding affinity to these or other plasma proteins may be considerable. A limitation of this study is that the protein binding of [^{18}F]GE-180 was not measured. However in vitro work suggests that in humans the plasma free fraction is approximately 3%.

The median volume of distribution of [^{18}F]GE-180 using the 2TC model across all subjects and brain regions ranged from 0.16-0.38 mL cm^{-3}. There was little variability across brain regions. V_T estimates were lower than those observed for some other second generation TSPO tracers (e.g. [^{11}C]-PBR-28: 4.1 ± 1.6 mL cm^{-3} in grey matter (Park et al., 2015) and [^{11}C]PK-11195: 0.72-1.06 mL cm^{-3}) (Jucaite et al., 2012).

Our time stability analysis demonstrated that V_T did not reach a stable estimate during our 90 minute scan, continually increasing over the 90 minute scanning window. This might lead to an underestimation of V_T. A scan time of 90 minutes was originally selected based on pre-clinical studies and consideration of what would be acceptable for individuals. However, our results suggest that a longer scanning duration might give a more 'stable' V_T estimate. This on-going increase in V_T could have been caused by the accumulation of radiometabolites in brain. However, only modest levels of metabolites were detected in the blood and earlier pre-clinical work demonstrated very low penetration of any metabolite into the brain with 94% parent at 60 minutes (Chau et al., 2015). Metabolites

have not formally been identified but all those observed in this study have been more polar than the parent. It is believed that the 2 main routes of metabolism are O-demethylation and hydroxylation of the aliphatic ring (Chau et al., 2015). Other metabolites could be a combination of the 2 processes or hydroxylation at different sites. The question of accumulation of metabolites in the brain is most relevant when brain uptake is high, which is not the case here. It is also worth noting that these time stability results are consistent with other TSPO tracers which also do not achieve a stable estimate within a 2-hour scanning window e.g. [^{11}C]PBR28 (Fujita et al., 2008)

We did not find evidence of an effect of TSPO genotype on any of our outcome measures. We observed no difference in K_1, V_T, DVR, SUV or SUVR between MABs and HABs. This was an unexpected finding as in vitro work with cold GE-180 displacing [^{3}H]PK11195 has shown a binding affinity of 15:1 between HABs and LABs (D Owen, personal communication). Although we did not acquire PET scans on LABs we would still have expected to see an effect of HABs vs. MABs on our outcome measures. Our expectation was that MABs would show an intermediate binding between HABs i.e. around 50% of the HAB binding (Owen et al., 2012). The fact that we did not observe this difference in vivo may be a consequence of the low uptake seen with this tracer. Genotype effects may emerge in other groups, for example older individuals or diseased individuals where we may expect microglial TSPO expression to be higher. We did consider the affect that increasing age of our participants could have on TSPO binding as has been shown previously (Schuitemaker et al., 2012) however we did not find any correlations between age and VT in all ROIs.

In summary, we report the first PET studies of [^{18}F]GE-180 in humans. Administration of the tracer was used safely and the scan was tolerated well by all participants. A reversible 2TC model fitted the data well and determined that the tracer has a low first pass extraction (~1%) and low V_T estimates in healthy volunteers. There was no observable dependency on the rs6971 polymorphism as compared to other 2nd generation TSPO PET tracers. A low first pass extraction combined with a tissue signal with a relatively large blood component suggests similarities to [^{11}C]PK-11195 in vivo. However more human work with [^{18}F]GE-180 would be informative including studies in patients with neuroinflammatory conditions, subjects of varying ages and a competition study to more

clearly delineate specific binding and in disease states to assess signal in the presence of upregulated TSPO.

7 Analysis of neuroinflammation in TBI using the novel PET radioligand [^{18}F]GE-180 and its relationship with the metabolic syndrome

7.1 Introduction

In the previous chapter I presented the kinetic analysis of this novel TSPO radioligand in 10 healthy human brains. The aim of the last study was to determine which kinetic model best fitted the data and this was determined to be the 2TC compartmental model with a 5% fixed blood volume. This was important to establish as this was one of the first studies in the world to use [^{18}F]GE-180 and its kinetics were not known. We also investigated whether the tracer binding was dependent on the rs6971 TSPO polymorphism which has been seen with other tracers e.g. [^{11}C]PBR28. We examined 5 high affinity binders (HABs) and 5 mixed-affinity binders (MABs) but found no significant difference in outcome measures.

One of the major limitations of this tracer was low tracer uptake. Approximately 1% of the tracer was extracted into the brain circulation, resulting in very low V_T values ranging from 0.1-0.4 mL/cm^3 across multiple regions. This suggests that [^{18}F]GE-180 could be a substrate for pumps in the BBB in the human, but not in rats. This already is a serious limitation to the use of this tracer to quantify TSPO neuroinflammation as we had originally hoped. However, the work of this chapter is to present the [^{18}F]GE-180 data in TBI patients, again by HAB and MAB status and to examine for an influence of the metabolic syndrome in these patients.

TSPO neuroinflammation has been demonstrated in TBI patients using the tracer [^{3}H]PK-11195 (Ramlackhansingh et al., 2011). Tracer binding was apparent even at very long times since injury and the greatest signal was found in subcortical areas such as the thalamus i.e. not at the original site of injury. The aim of this study was to evaluate [^{18}F]GE-180 uptake in TBI patients in the subacute phase and at two time points designed to be 6 months. My second aim was to evaluate if there was a difference in signal

between HABs and MABs. My final aim was to establish whether a TSPO signal is related to the presence of the metabolic syndrome (MetS). For more introductory material for this chapter please to refer to sections **1.5** Neuroinflammation following TBI on page 48 and section **1.6** The Metabolic Syndrome (MetS) on page 58.

7.2 Methods

7.2.1 Participants

This study was approved by the Westminster Research Ethics Committee, London (13/LO/1596) and the Administration of Radioactive Substances Advisory Committee (no. 631/336/30788). All participants gave written, informed consent.

Eighteen patients with TBI were initially recruited into the study. Sixteen of these patients were recruited from the multidisciplinary TBI clinic at St Mary's hospital and the remaining 2 were recruited from Dr Greenwood's private TBI clinic. All patients attended for a screening assessment at the Imperial College Clinical Research Facility (ICCRF). Patients underwent a full clinical interview and examination including a Structured Clinical Interview for DSM disorders (SCID), BP, ECG and Allen's test (to check for patency of the ulnar anastomosis). Patients also had a fasting metabolic assessment (see section **2.5** Methods for assessing insulin resistance (IR) and the metabolic syndrome on page 77 and section **2.1.5** Recruitment for Chapter 7 on page 69) to investigate for presence of MetS. To summarise, all patients had height, weight, % body fat, waist circumference measurements in addition to BP and fasting lipid assessment to diagnose MetS. Fasting blood samples were also taken for analysis of full blood count, renal profile, clotting screen and TSPO genotyping. Exclusion and inclusion criteria following the screening visit are outlined in **2.1.5** Recruitment for Chapter 7 on page 69.

Of the 18 patients who attended the screening visit, 12 patients were included in the study. Two patients were excluded because they had two alleles for the rs6971 TSPO polymorphism making them low-affinity binders (LABs). Two were excluded due to taking a drug that could have an effect on the brain (1, minocycline, 1, internet purchased steroids) and two were excluded due to language barriers that could have affected informed consent and also affected the cognitive assessment.

Of the 12 patients that were included, 8 were high-affinity binders (HABs). Details on the assay used for TSPO genotyping can be found in **6.2.2** TSPO Genotyping on page 180. Five of these patients had confirmed MetS following metabolic assessment.

Of the 12 TBI patient recruited into the study, 10 attended for the follow-up study visit designed to be 6 months later. One patient was lost to follow-up and the other was not able to attend on several other occasions due to intercurrent illness.

Eleven healthy controls were also recruited into the study. Ten of these controls were the same healthy participants used in **Chapter 6** Neuroinflammation in TBI: A kinetic analysis of the translocator protein (TSPO) positron-emission tomography ligand [^{18}F]GE-180 in the human brain**.** Another single control (male, MAB, age 42 yr.) was recruited following the previous chapter work and included in this study. No healthy control had evidence of MetS. Healthy controls were not invited for a follow-up PET visit at 6 months.

7.2.2 PET acquisition

Following the screening visit and final recruitment into the study, patients were invited back for a PET and MRI scanning visit. For details on synthesis of [18F]GE-180 and PET acquisition please refer to sections **6.2.3 Synthesis of [^{18}F]GE-180** on page 181 and **2.7 PET scanning visit** on page 80. To summarise patients had an arterial line inserted for continuous and discrete arterial blood sampling and were injected with ~ 185 Mbq of [18F]GE-180 via a venous cannula followed by a 10 mL saline flush prior to 90 minute dynamic PET acquisition. Prior to injection, a low dose CT Head was performed to allow attenuation correction.

Continuous blood monitoring was performed throughout and discrete samples taken at specific intervals. Plasma and whole blood radioactivity measurements were taken as well as estimates of parent compound using HPLC and metabolites. For details on acquisition of blood data please refer to section **6.2.5 Whole Blood, Plasma Activity and Parent Fraction of [^{18}F]GE-180** on page 181. Blood sampling and recording of data

was performed by the CIF biochemist Gokul Kolipaka. A senior PET radiographer was in attendance with the study investigator throughout the duration of the scan.

On termination of the scan, patients were asked to rest for thirty minutes prior to vital signs being recorded and brief consult with the study physician. Arterial and venous lines were removed with a compression bandage applied to the radial artery site. Patients were encouraged to urinate to maximise excretion of [18F]GE-180 prior to leaving the building. Advice leaflet and emergency contact number was given regarding the arterial line site and precautions advised re. minimising radioactive exposure to others. Patients were sent home by taxi and called by the study physician the following day to address any concerns.

PET data were reconstructed as 23 temporal frames using filtered back projection with and without attenuation correction. Standard correcting for scatter, decay and randoms were applied.

7.2.3 MRI acquisition and cognitive assessment

In all cases, PET acquisition was preceded by a standard T1 MPRAGE brain MRI scan as well as other MRI sequences, data from which is not presented here. For more details on MRI acquisition please refer to **6.2.6 Magnetic Resonance Imaging (MRI)** on page 182. If MRI was not acquired prior to PET scanning, patients were brought back for an MRI scan within two weeks of the PET scan. A full cognitive (both paper and ipad) and QoL assessment were performed prior to scanning as well as the choice reaction time task (CRT) and sustained attention to response task (SART).

7.2.4 PET data analysis

Imaging and blood data were all decay corrected to time of injection prior to any further processing. I used MIAKAT^{TM} v.2, a PET data and analysis and kinetic modelling toolkit, that was downloaded from (www.miakat.org). In the kinetic modelling stage, the 2TC model was applied as this appeared to be the most parsimonious model in healthy controls (see previous chapter). When applicable, cortical grey matter was selected as a pseudoreference region as TSPO is reported to be relatively lower in this region and has

been used in previous studies (Tziortzi et al., 2011). Please refer to sections **2.14** PET analysis pipeline on page 91 and **6.2.7** Data Analysis on page 182.

The primary outcome measures were the radioligand delivery rate constant ($K_{1;}$ mL cm $^{-3}$ min $^{-1}$), total distribution volume ($V_{T:}$ mL cm $^{-3}$), and standardised uptake values (SUV), with and without a reference region for each of the anatomical regions described in the previous chapter.

Comparative analyses were i) HABs vs. MABs in TBI patients at baseline ii) TBI vs. healthy controls (HABs and MABs separately) iii) Baseline vs. follow-up in TBI only (iv) Effect of MetS as an additional within subject factor for the above analyses.

7.2.5 Statistical analyses

To compare characteristics between genetic groups (HAB/MAB), Fisher's exact test (gender) and the Mann Whitney U test (age weight, injected dose) were used. To evaluate differences between genetic groups for blood data, tissue data and outcome measures, a repeated measures analysis of variance (ANOVA) was used, with time as the within-subjects factor and genotype as the between-subject factor. A repeated measures ANOVA was used to compare outcome measures across multiple ROIs by binding status and by the presence of MetS

Only 6 patients had complete baseline and follow-up imaging and for these subjects, blood data and outcome measures for these subjects are plotted individually.

7.3 Results

7.3.1 Patient characteristics

Twelve patients were included in the study (10 male, 11 moderate-severe severity, median time since injury 9.1 months, 8 HABs, median weight 79.0 kg, median injected dose 183.0 MBq). Five out of 12 patients had confirmed MetS. Ten patients attended for their follow-up visit and for these patients the median time between scans was 6.0 months. Data are displayed in **Table 7-2** and **Table 7-3**.

Of the 12 patients, 6 (50%) sustained their injury as a result of an RTA, 5 (42%) via a fall and 1 (8%) from an assault. All patients had reported abnormalities on acute imaging (CT or MRI) and 9/12 had on-going evidence of structural lesions on the baseline research MRI. These abnormalities included superficial siderosis, contusions, microbleeds and gliosis. Details of the injury and imaging findings are displayed in **Table 7-4**.

Almost all patients had cognitive and/or psychological symptoms following TBI and at the time of assessment. 4 (33.3%) patients had stopped work completely as a result of their TBI. In terms of additional medical problems, 3/12 patients had a pituitary abnormality confirmed following their TBI (1 SIADH, 1 ACTH deficiency, 1 combined GH and gonadotrophin deficiency) and were on appropriate treatment at the time of assessment. Data regarding symptoms, other medical problems, medication and occupational status are displayed in **Table 7-5**. Results from the baseline metabolic assessment are displayed in **Table 7-6** and values in red contributed to the final diagnosis of MetS which is based on waist circumference, fasting blood glucose, BP, high-density lipoprotein, triglycerides and medications for raised BP and dyslipidaemia. Please refer to **2.5.1** Criteria for diagnosing the metabolic syndrome in this study **on page 77.**

In terms of patient characteristics by TSPO genotype, there were no significant differences in age, gender, severity, structural lesions, time since injury, presence of MetS, weight and injected dose of tracer between HABs and MABs (P 0.15-0.78). **Figure 7-2**

.

Eleven healthy volunteers were also included in the study for comparison (8 male, 5 HABs, median age 39.8 yrs., median weight 87.5 kg and median injected dose 181.3 MBq). There were no significant differences in age, gender, TSPO status, weight or injected dose of tracer between TBI patients and healthy controls (see **Table 7-1**)

7.3.2 Drug safety and adverse events

The tracer was well tolerated and there were no serious adverse events or drug reactions. One patient developed some cardiac symptoms one week after the first scan but he was fully investigated and no cause found. Another patient fell and fractured her radius between baseline and follow-up scan. Both of these were filed in the notes as adverse events and a copy kept in trial master file. There were no reported complications from the arterial line insertion.

Table 7-1 Baseline characteristics: healthy controls vs. TBI patients

	Controls	TBI	P Controls vs TBI
n	11	12	
Age	39.8 [34.4-48.1]	46.0 [38.9-56.4]	0.41
Range	28.4-56.2	35.0-65.7	
Males (n %)	8 (72.7%)	10 (83.3%)	0.91
HABs (n %)	5 (45.5%)	8 (66.7%)	0.55
Weight (kg)	87.5 [70.3-94.6]	79.0 [69.3-86.5]	0.68
Range	65.4-97.9	66.8-100.6	
Injected dose (MBq)	181.3 [180.0-184.3]	183.0 [181.0-185.0]	0.41
Range	176.8-1888.0	178.3-188.6	

Data displayed as median [IQR] or n%. Tests of statistical significance: Mann Whitney U test for continuous variables and Yates' test for categorical variables.

Table 7-2 Baseline characteristics in TBI patients: HABs vs. MABs

	HABs	MABs	P HABs vs MABs
n	8	4	
Age	41.4 [37.9-52.6]	52.6 [45.0-63.6]	0.15
Range	35.0-59.7	44.0-65.7	
Males (n %)	7 (87.5%)	3 (75%)	0.78
Moderate-to-severe severity (n %)	7 (87.5%)	4 (100%)	0.71
Any structural lesion (n%)	6 (75%)	3 (75%)	0.48
Time since injury	7.1 [4.5-11.2]	15.6 [9.6-25.6]	0.15
Range	2.9-152.0	8.9-67.7	
MetS (n %)	2 (25%)	3 (75%)	0.30
Weight	75.3 [69.3-80.5]	90.3 [72.1-98.5]	0.15
Range	66.8-81.0	66.8-100.6	
Injected dose	183.3 [181.1-184.9]	182.7 [179.3-186.5]	0.57
Range	179.6-188.6	178.3-187.7	

Data displayed as median [IQR] or n%. Tests of statistical significance: Mann Whitney U test for continuous variables and Yates' test for categorical variables.

Table 7-3 Baseline characteristics of 12 TBI patients enrolled in study

Participant no.	Gender	Age at PET scan	Ethnicity	Highest qualification	Injury Severity (Mayo)	Time since injury (m)	Time between scans (m)	TSPO	Metabolic syndrome (MetS)
1	male	38.6	White caucasian	Doctorate	moderate-severe	9.2	0.51	HAB	Yes
2	male	43.0	White caucasian	A Level	probable	152.0	0.49	HAB	No
3	male	37.7	South asian	NVQ	moderate-severe	11.9	0.50	HAB	Yes
4	male	35.0	White caucasian	Masters	moderate-severe	7.4	0.52	HAB	No
5	male	59.7	White caucasian	MBA	moderate-severe	4.4	0.50	HAB	No
6	female	57.2	White caucasian	None	moderate-severe	27.6	0.51	MAB	Yes
7	female	54.0	White caucasian	O level	moderate-severe	4.8	0.54	HAB	No
8	female	48.2	White caucasian	BA	moderate-severe	2.9	0.50	HAB	No
9	male	44.0	White caucasian	Degree	moderate-severe	19.5	0.51	MAB	Yes
10	male	48.0	White caucasian	Degree	moderate-severe	11.7	0.50	MAB	No
11	male	39.8	Chinese	None	moderate-severe	6.8	n/a	HAB	No
12	male	65.7	White caucasian	Degree	moderate-severe	8.9	n/a	MAB	Yes

HAB=high affinity binders, MAB=mixed affinity binders, n/a =not applicable (pt. did not return for follow- up scan)

Table 7-4 Baseline characteristics: Injury details and structural imaging reports

Participant no.	Mode of injury	PTA	GCS	LOC	Other Injuries at TBI	Acute clinical imaging findings	Baseline MRI Report
1	Fall	24-48 hrs	4	1-24 hrs	R 6th nerve palsy	L subdural haemorrhage, contusions frontal lobe, R temporo- and L parieto-occipital lobes	Superficial siderosis
2	RTA: motorcylist	<24 hrs	NK	nil	R tibia fracture	?Bifrontal hyperintensity	Normal
3	RTA: ped vs car	4 days	NK	1-24 hrs	Rib fractures, pneumothorax	Haemorrhagic contusion involving L inferior frontal lobe and inferior operculum	L inferior and opercular contusion
4	Fall	4-6 hrs	NK	1-24 hrs	superficial injuries	Long bilateral occipital fracture, R extradural haematoma, some SAH	normal
5	RTA cyclist	7 days	8	1-24 hrs	superficial injuries	Small white matter bihemispheric haemorrhages	Frontal and temporal microbleeds, superfical siderosis R parietal lobe
6	Fall	1-7 days	NK	nil	Rib fractures	Occipital fracture, R temporal contusion, possible frontal operculum contusion	Gliosis R temporal lobe, microbleeds, contusions R superior middle temporal gyri
7	Fall	24-48 hrs	5	<1 hr	superficial injuries	basal skull fracture, contusion temporal horn, SAH	Contusion L frontal and temporal lobes, shear injury occipital lobe, small scattered foci deep cerebral white matter
8	Fall	24-48 hrs	NK	<1 hr	superficial injuries	R temporal lobe fracture, orbitofrontal contusion	Gliosis R orbit and R temporal lobe
9	RTA: ped vs car	6 days	12	1-24 hrs	superficial injuries	R occipital fracture and haematoma, R frontal contusion	Contusions overlying both orbits, microbleeds R parietal lobe
10	Assault	48 hrs	15	NK	nil	R occipital, R medial maxillary, L zygomatic arch and nasal bone fractures, small SDH	Normal
11	RTA: ped vs car	NK	14	NK	knee dislocation, 4th nerve palsy	Contusion occipital lobe	Parafalcine microbleeds more marked on R than L, medial temporal lobes, right thalamus, DAI thalamus
12	RTA: ped vs car	<24 hrs	NK	<1 hr	superficial injuries	R frontal and subdural blood, small R frontal contusion, SAH R temporal sulci, L occipital fracture	Extensive R cortical damage, small microbleed R parietal lobe and R inferior frontal lobe, superficial siderosis R frontal cortex. Small area of mature gliotic damage L inferior cerebellum ?old infarct

NK=not known, L=left, R=right

Table 7-5 Baseline characteristics: symptoms following TBI, past medical history and medications at first visit

Participant no.	Symptoms following TBI	Other PMH	Medication at time of first research visit	Stopped working after injury
1	Diplopia	Nil	Vitamin D 1000 units	yes
2	Attention, memory , switching, planning, disinhibition, depression, anxiety, social withdrawal, sleep disturbance, microsomia, fatigue,	GHD, testosterone deficiency, osteopenia	GH 0.2 mg sc last 3.5 years, modafinil 20mg, citalopram 20mg od, nebido 1g 12 weeks	no
3	Attention, information processing, fatigue, headaches, sleep disturbance, anosmia, irritability, hearing loss,	GORD	Gabapentin 900mg od, omeprazole 40mg od, Vit D 1000 units	no
4	Attention, fatigue, memory, labile mood, BPPV	Renal colic	nil	yes
5	Slowed thinking, memory, lack of insight, personality change	Nil	Nil	no
6	Memory, sleep disturbance	SIADH, hypertension, dyslipidaemia, Vitamin D deficiency, COPD	demeclocycline 300mg bd, ramipril 5mg od, simvastatin 40mg od, salbutamol 2-3 times pw, vit D 1000 units/day	yes
7	BPPV	nil	nil	no
8	Slowed thinking, impaired attention, memory impairment, headaches, anosmia,	Back pain	Regular neurofen	no
9	Single seizure, memory, attention, anosmia, anxiety, sleep disturbance	ACTH deficiency, TMJ dysfunction, dyspepsia	Hydrocortisone 20mg od	no
10	Absence seizures, irritability, sleep disturbance, memory, executive dysfunction	knee OA	Carbamazepine 200 mg bd, vit d 1000 units, ibuprofen/paracetamol 2-3 times per week	no
11	Possible mood disturbance	nil	nil	yes
12	BPPV, fatigue, anosmia, mood disturbance, sleep disturbance, sexual dysfunction	CKD, hypertension, diabetes	Ramipril 2.5 mg, metformin 500mg bd, gliclazide 20 mg od, fybogel, senna	no

BPPV=Benign paroxysmal positional vertigo, SIADH=syndrome of inappropriate ADH production, GORD=gastro-oesophageal reflux disease, CKD=chronic kidney disease

Table 7-6 Baseline characteristics: metabolic variables in TBI patients

Participant no.	BP systolic	BP Diastolic	Waist circumference (cm)	BMI	Height (cm)	Weight (kg)	%body fat	Insulin (ug/L)	C-peptide (ug/L)	SHBG (mmol)	HBA1c (mmol/mol)	Glucose (mmol/L)	HOMA-IR	Cholesterol (mmol/L)	High density lipoprotein (mmol/L)	Low density lipoprotein (mmol/L)	Triglycerides (mmol/L)	BP meds	Lipid meds	Type 2 diabetes	MetS
1	131	75	94	28.7	168	81.0	24.7	11.8	857	26	38	5.6	1.56	6.5	1.21	4.68	1.34	no	no	no	yes
2	114	67	93	25.8	174	78.2	20.2	7.9	690	13	37	5.2	1.04	5.2	0.94	3.25	2.22	no	no	no	no
3	116	67	95	23.7	185	80.8	23.3	20.4	1731	23	36	5.0	2.59	6.0	0.81	4.17	2.25	no	no	no	yes
4	114	65	76	23.0	177	72.1	14.6	6.0	436	38	34	4.7	0.77	7.4	1.33	5.62	1.00	no	no	no	no
5	109	66	86	25.1	163	66.8	22.4	3.2	269	ND	38	5.1	0.42	7.1	1.34	5.20	1.23	no	no	no	no
6	139	70	100	28.9	152	66.8	42.0	3.2	740	104	44	6.3	0.44	4.7	1.62	2.50	1.27	yes	yes	no	yes
7	122	76	85	24.8	171	72.4	23.0	5.1	502	62	36	4.7	0.65	5.6	1.43	3.67	1.11	no	no	no	no
8	116	64	85	29.6	164	79.7	35.4	6.0	553	155	33	4.7	0.77	5.4	1.47	3.54	0.86	no	no	no	no
9	133	77	112	33.1	167	92.3	33.9	29.8	1464	13	53	8.0	4.13	5.4	0.83	3.41	2.55	no	no	yes	yes
10	133	91	110	32.1	177	100.6	29.2	13.2	1089	27	33	5.4	1.73	5.4	1.05	3.89	1.02	no	no	no	no
11	138	90	90	27.7	157	68.3	21.3	4.0	264	31	42	5.0	0.52	7.9	1.38	5.87	1.42	no	no	no	no
12	158	64	101	30.9	169	88.3	30	5.9	694	49	43	7.3	0.84	6.0	1.96	3.36	1.49	yes	no	yes	yes

Values in red=abnormal values meeting criteria for MetS

7.3.3 Blood data

One patient (Patient 4, HAB, see **Table 7-3**) was excluded from this analysis as it was not possible to place an arterial line. Therefore 11 TBI patients were included in this analysis (7 HAB, 4 MAB).

For the whole group, median parent fraction of unchanged tracer was 0.72 [IQR 0.71-0.77], range 0.61-0.82. There was no difference in parent fraction between HABs and MABs (P=1.0, all time points) and there was no significant difference in weight (P=0.24) or injected dose (P=1.0) between HABs and MABs.

For the whole group, median plasma radioactivity of [18F]GE-180 ranged from 22.1 [IQR 18.7-29.4] range 14.4-51.7 KBq/mL at 5 minutes to median 5.1 [IQR 4.4-8.8] range 2.9-16.0 KBq/mL at the end of the scan. There was no significant difference between HABs and MABs at all time points (P=0.24).

For the whole group, median whole blood radioactivity ranged from 11.5 [IQR 10.2-16.8] range 8.5-28.2 KBq/mL at 5 minutes to median 2.7 [IQR 2.3-4.5] range 1.7-8.7 KBq/mL at the end of the scan. There was no statistically significant difference between HABs and MABs (P=0.24).**Figure 7-1**.

Plasma over blood (POB) ratio is also presented as this was one of final inputs into the arterial input function. Median whole group POB ranged from 1.75-1.85 at all time points and there was no significant difference between HABs and MABs. **Figure 7-2**.

Six patients had arterial blood monitoring at baseline and follow-up. Arterial line insertion was not possible in the 4 other patients at follow-up scan. Data are displayed by individual patients in **Figure 7-3** & **Figure 7-4**.

Figure 7-1 Parent fraction, plasma and whole blood radioactivity of [18F]GE-180 in 11 TBI subjects (Scan 1)

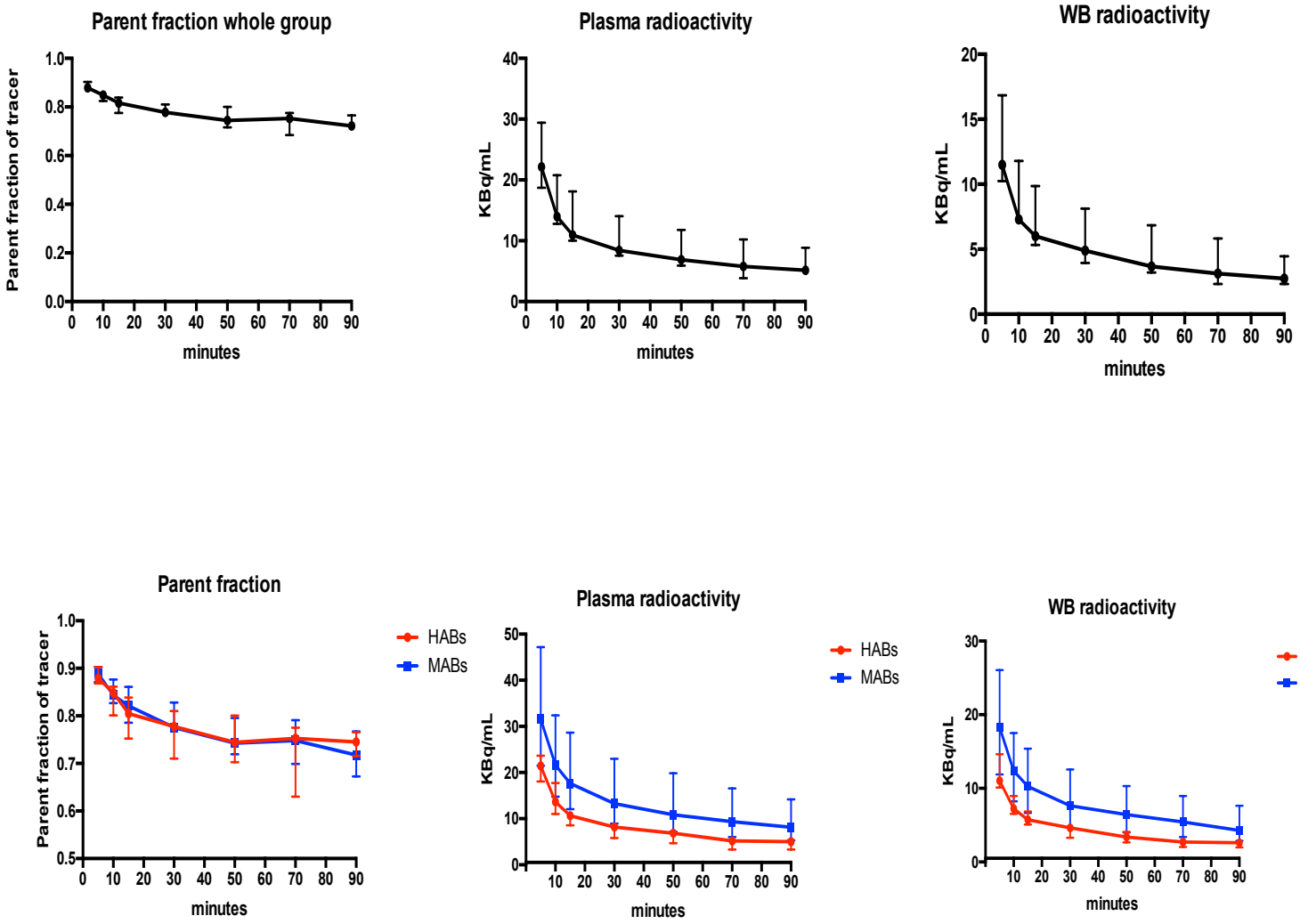
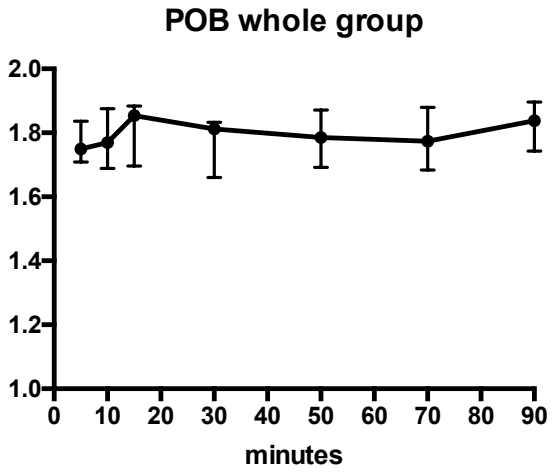


Figure 7-2 Plasma over blood: whole group at baseline and by genotype



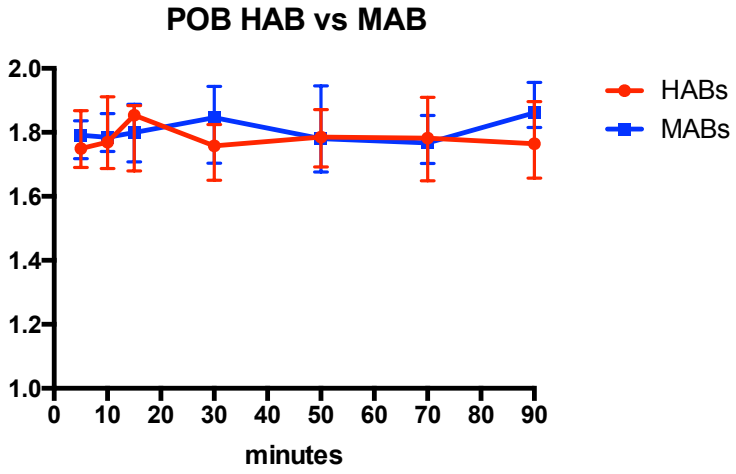


Figure 7-3 Comparison of blood parameters at baseline and follow-up scan (patients 2,3&5)

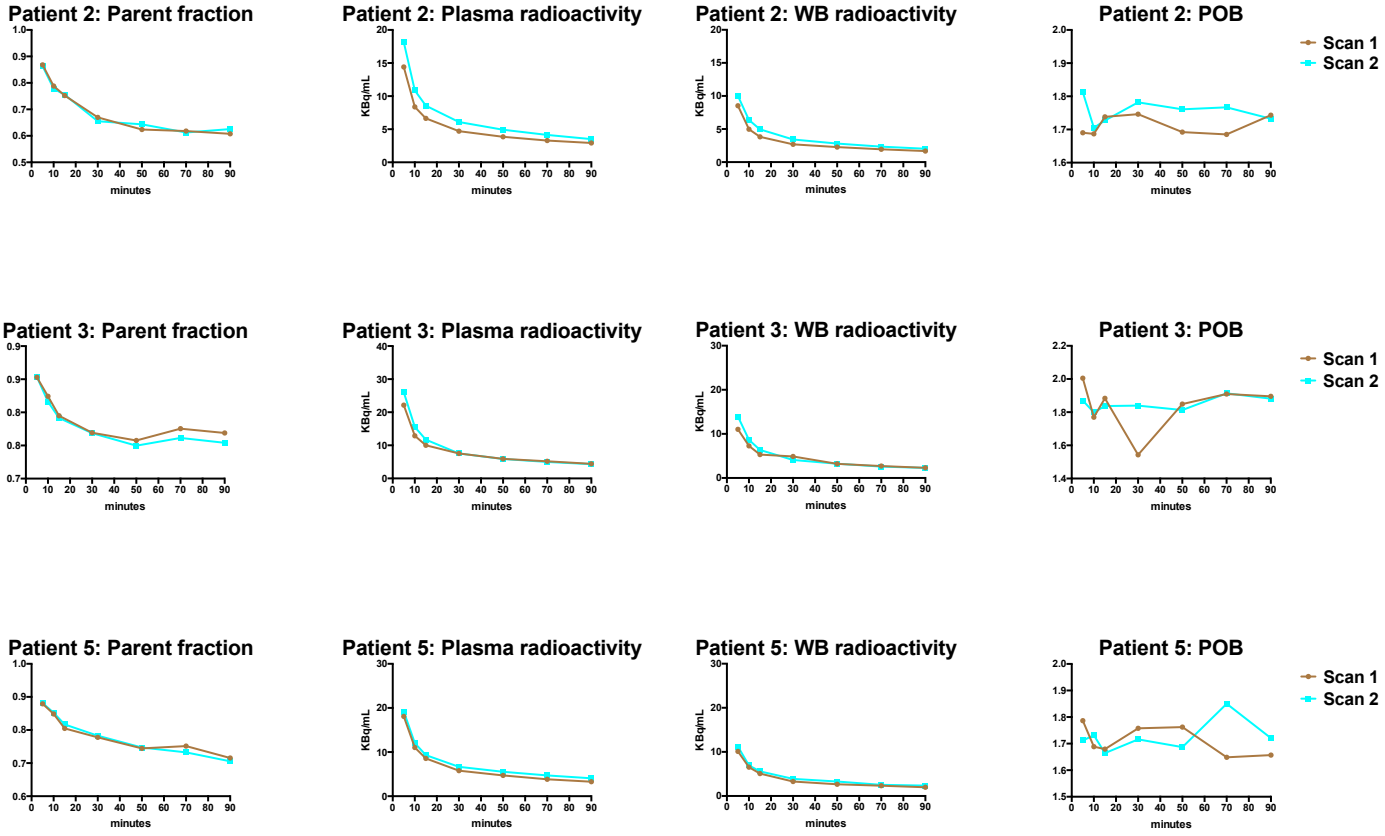
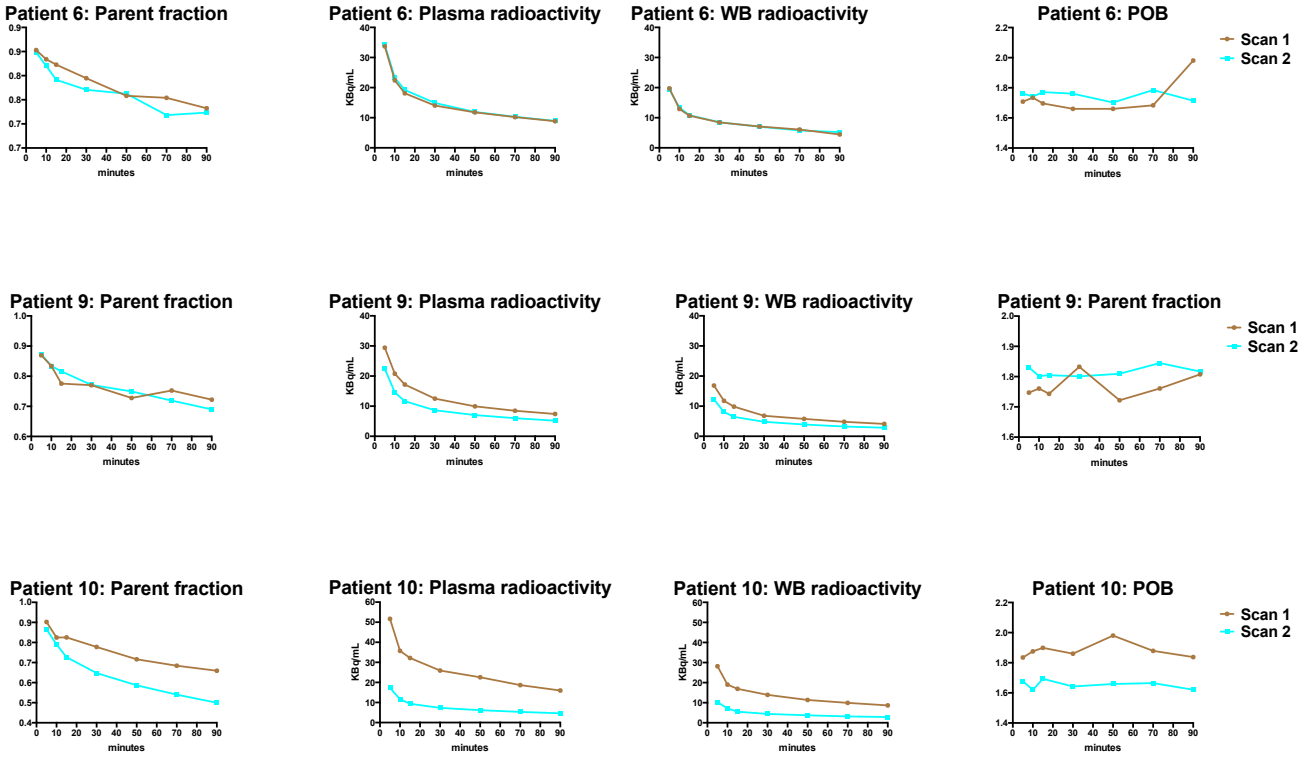


Figure 7-4 Comparison of blood parameters at baseline and follow-up scan (patients 6,9&10)



7.3.4 Tissue Data

SUV images for a representative HAB and MAB subject are shown in **Figure 7-5** and **Figure 7-6**. Tracer uptake appears to dominate in the venous sinuses. Uptake elsewhere in the brain appears to be low and symmetrical. None of the subjects had any specific foci of increased uptake.

Figure 7-5 Representative HAB subject (Patient 1). SUV images superimposed on T1 MRI brain

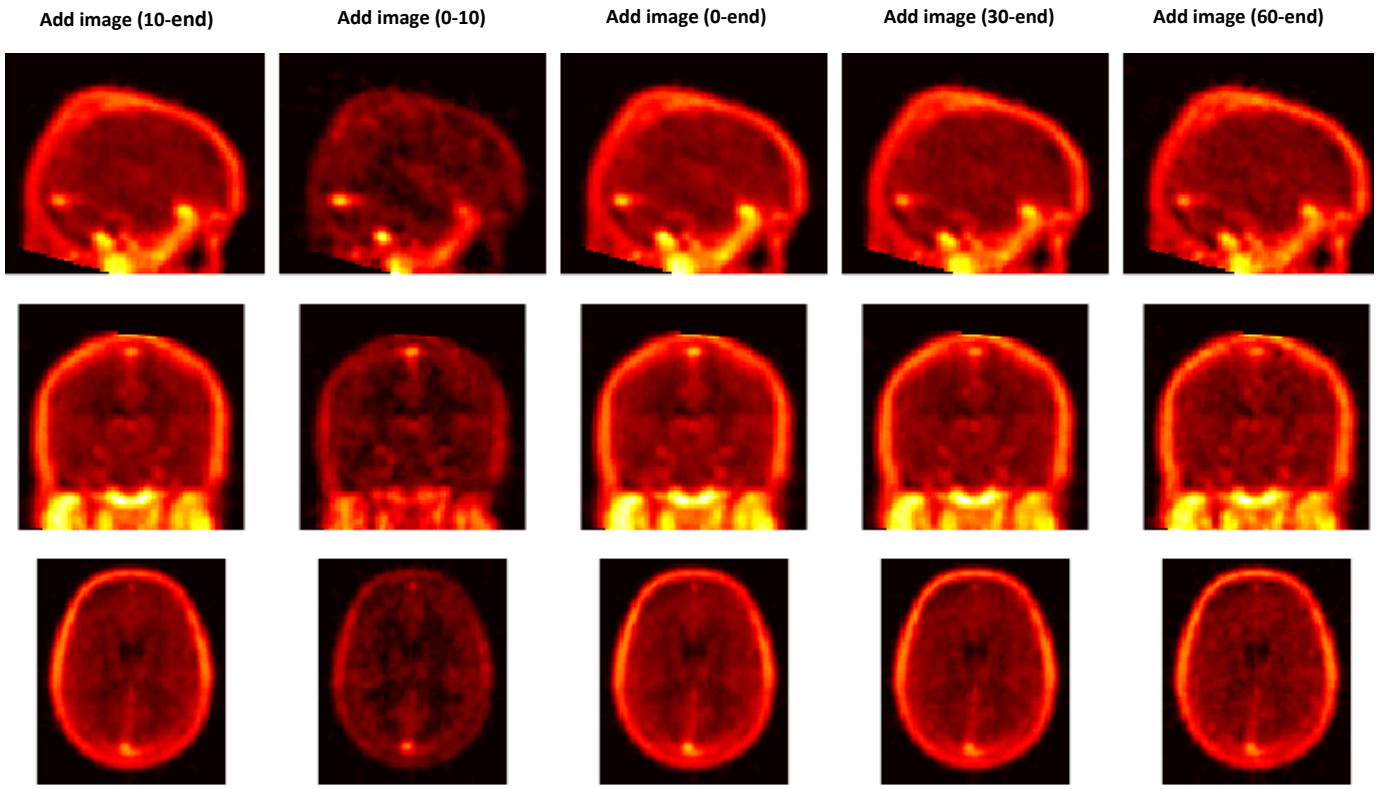
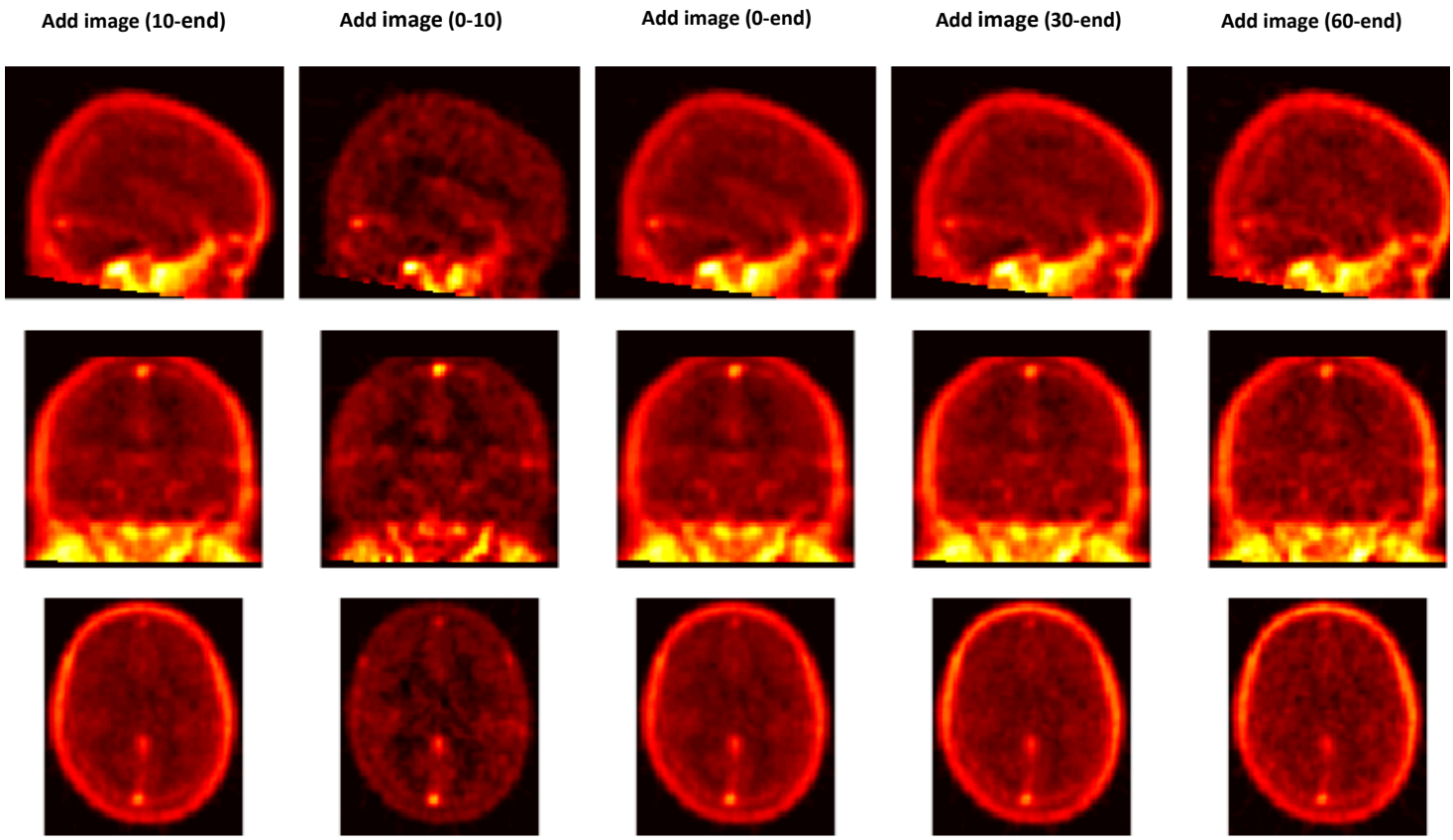


Figure 7-6 Representative MAB subject (Patient 10). SUV images superimposed on T1 MRI brain

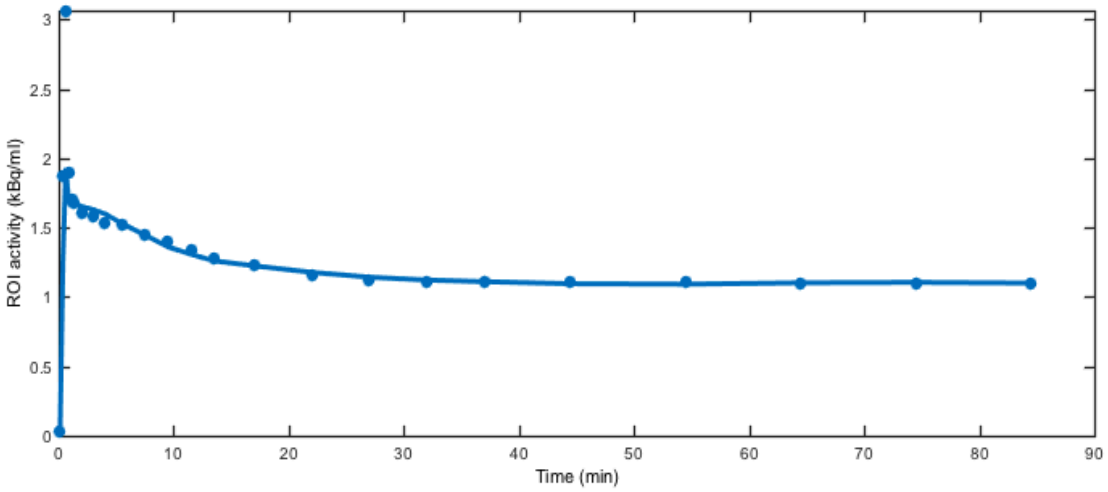


7.3.5 Model Fit

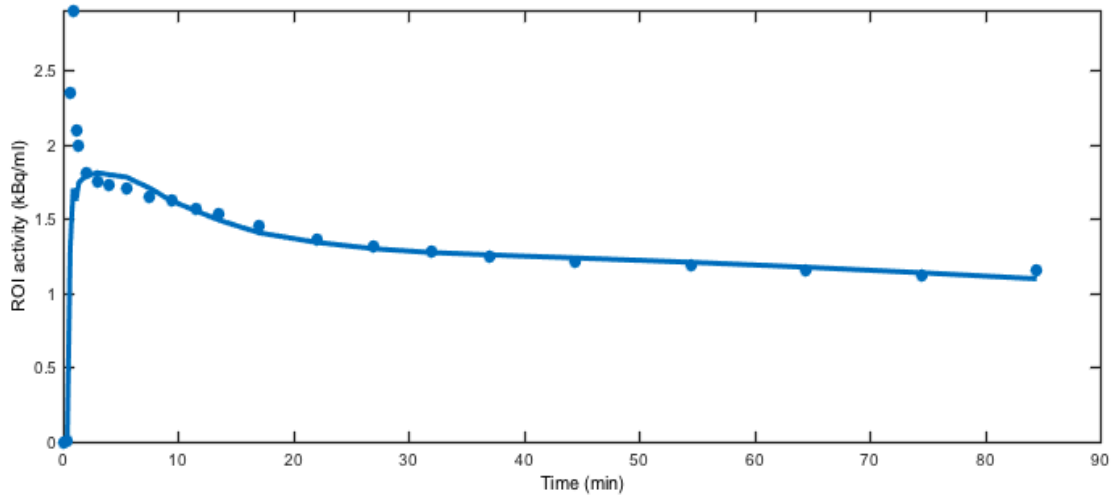
From the previous chapter, the 2TC (5% fit BV) compartmental model was deemed the best fit. For this study, the 2TC (Fit and fix BV) and the 1TC (fit and fix BV) were compared at the kinetic modelling stage. The 2TC (Fix BV) was considered the best fit. There was a slight discrepancy in the model fit at the beginning of the scan as was seen in healthy controls. The 2TC model fit is shown for each patient below. Model fit is applied to the whole brain tissue TAC generated from MIAKAT (**Figure 7-7**).

Figure 7-7 2TC (fix BV) model fit for whole brain tissue TAC for each patient for duration of scan

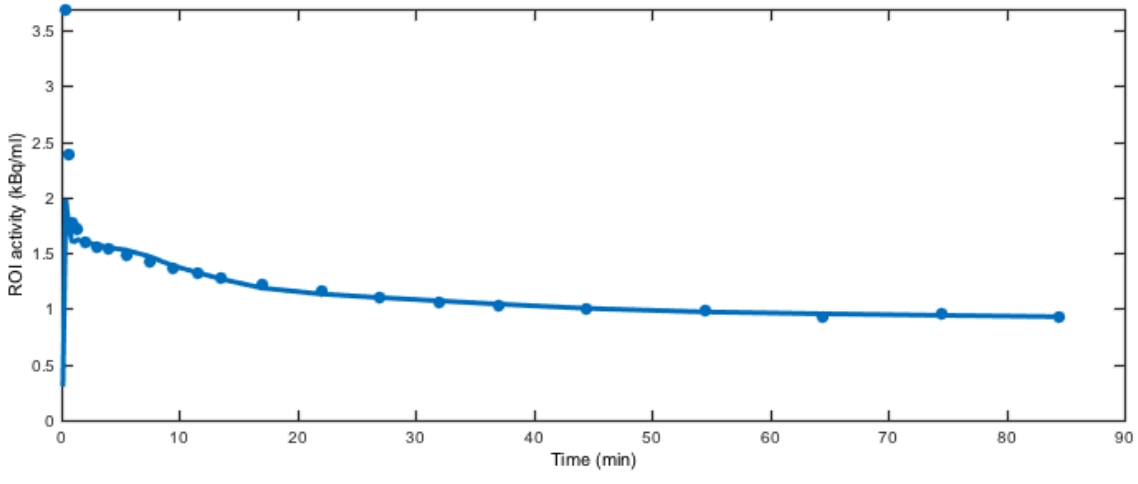
Patient 1



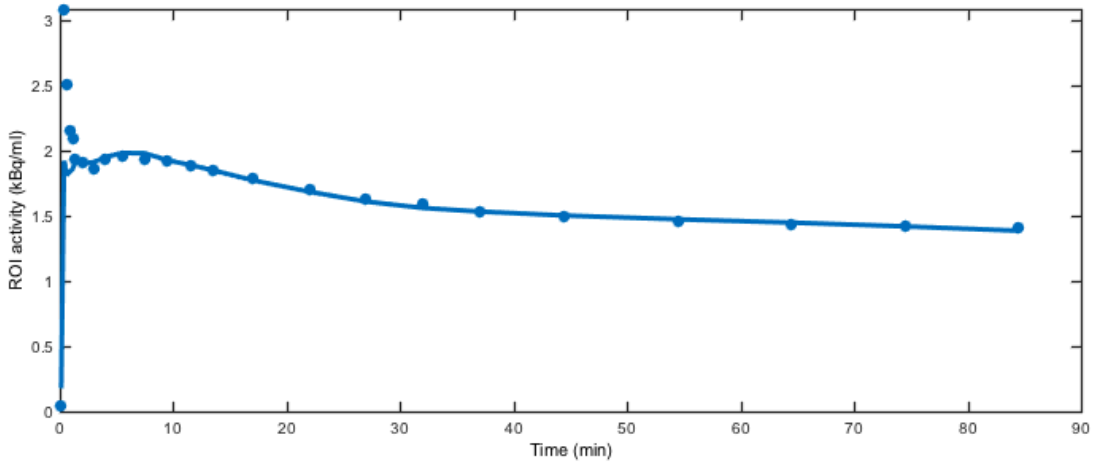
Patient 2



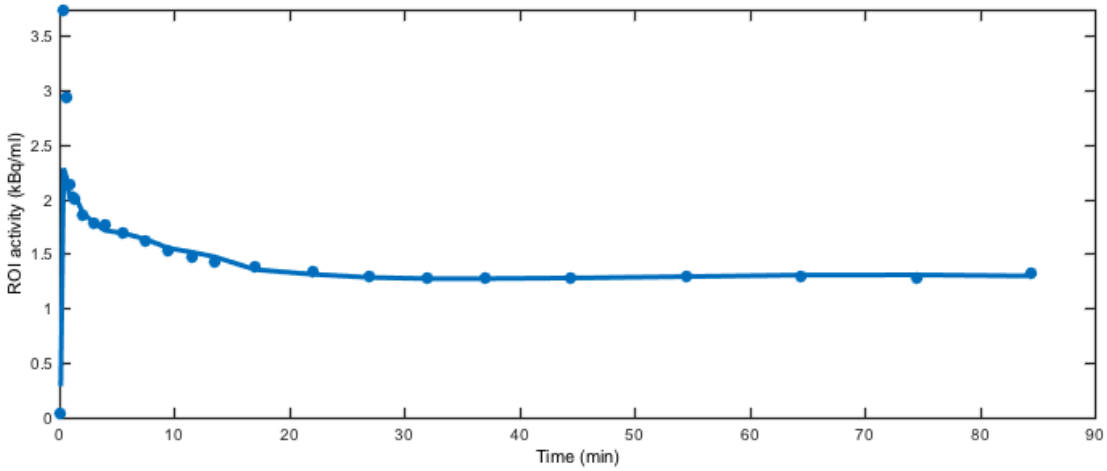
Patient 3



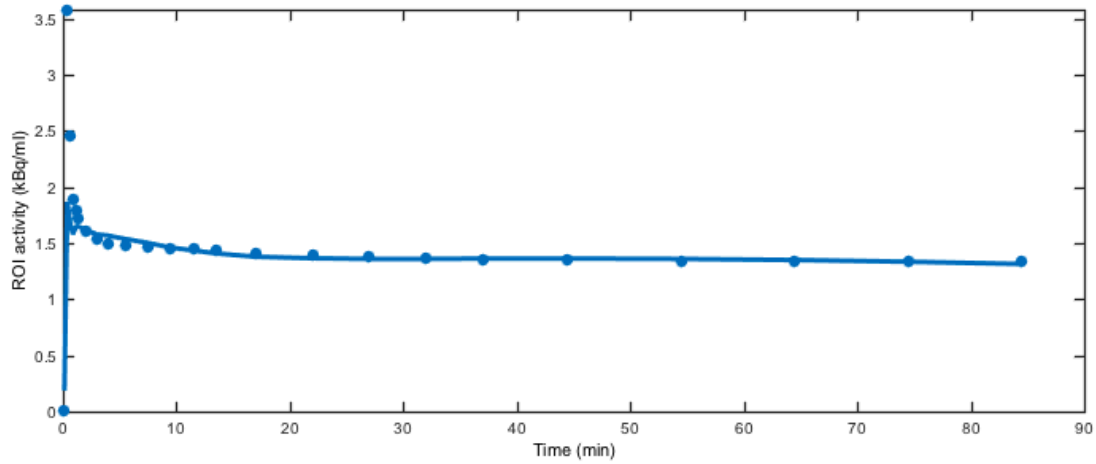
Patient 5



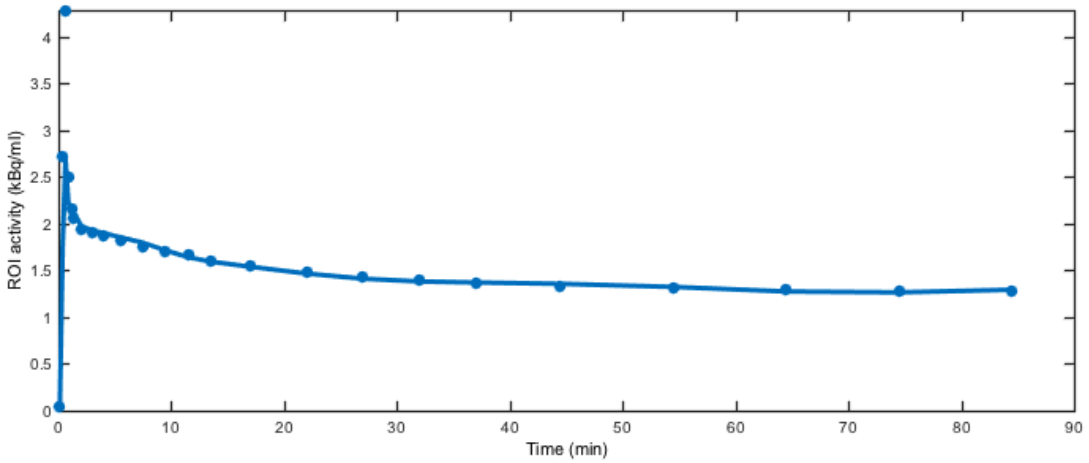
Patient 6



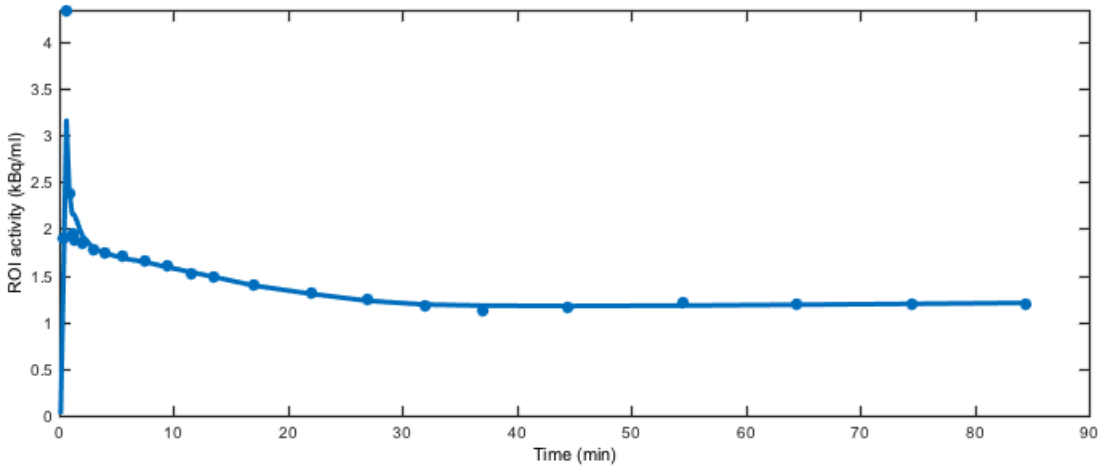
Patient 7



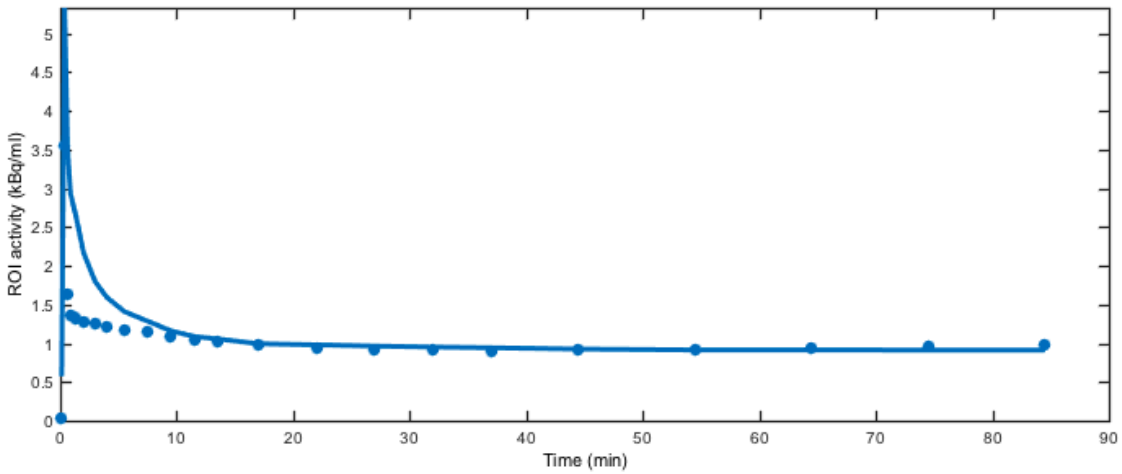
Patient 8



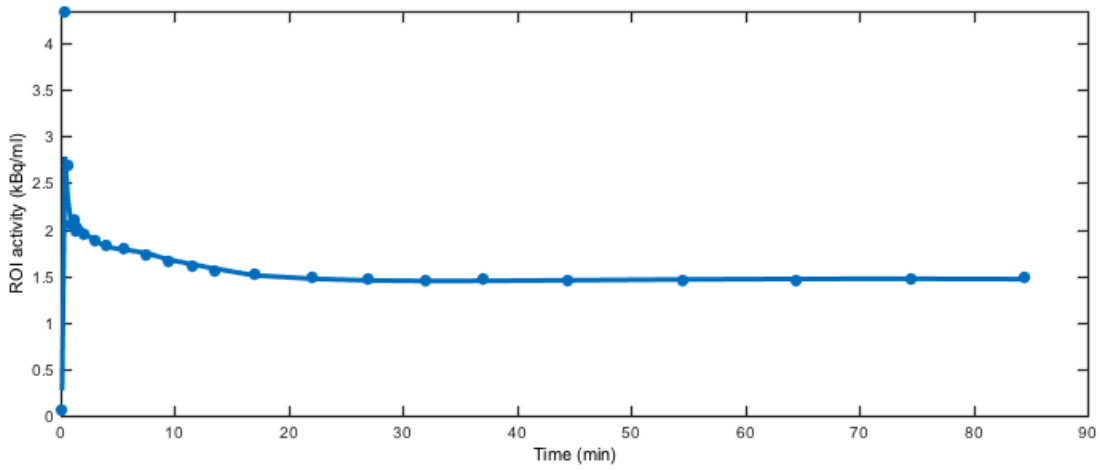
Patient 9



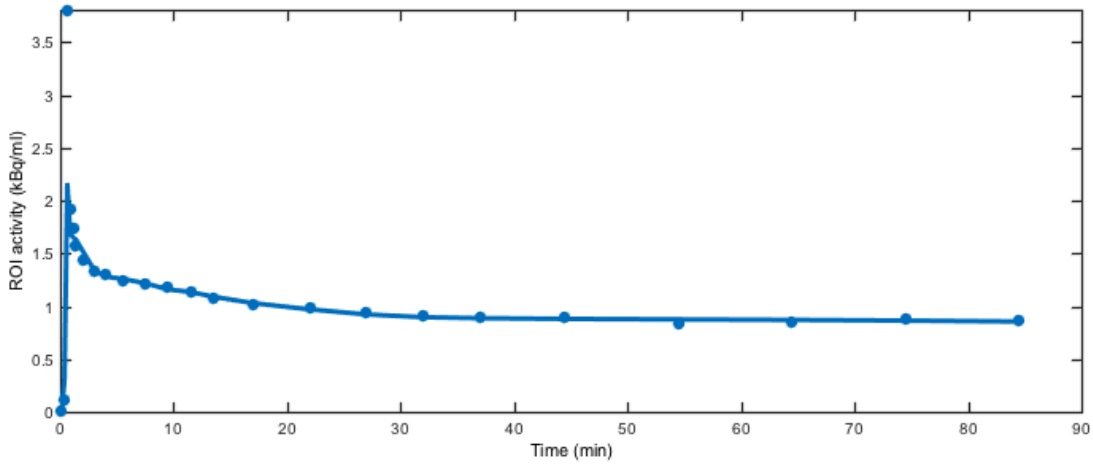
Patient 10



Patient 11



Patient 12



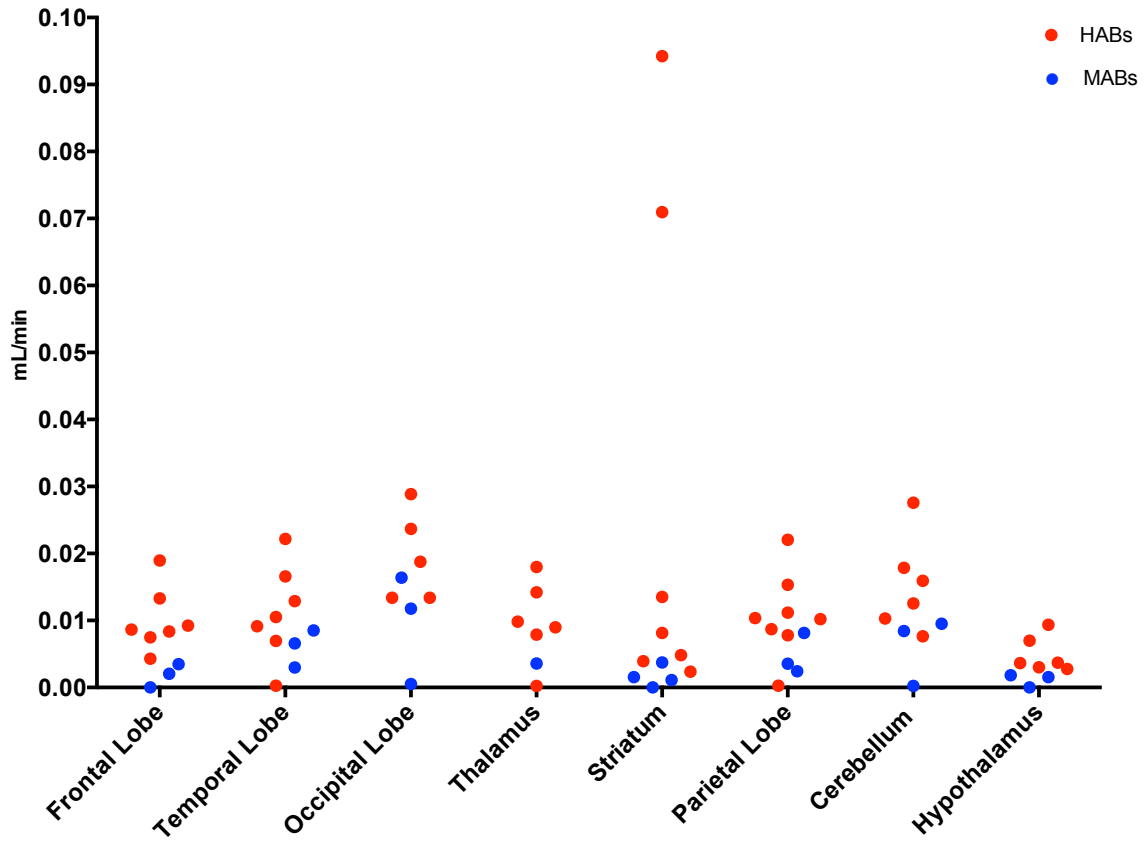
7.3.6 Tracer uptake

Within TBI patients, K1 ranged from 0.008-2.39 ml/min across all regions. There was a trend towards MABs having a lower K1 than HABs in the striatum (P=0.06) but there was no other effect of genotype or metabolic syndrome on K1 across other ROIs **Table** 7-7 and **Figure** 7-8**.** Within HABs only there was no difference in K1 between HABs and MABs **Figure** 7-13**.**

Table 7-7 Table demonstrating 2TC rate constants K1-K4 in TBI patients by genotype

HABs	K1	P HABs vs MABs	K2	P HABs vs MABs	K3	P HABs vs MABs	K4	P HABs vs MABs
n	7		7		7		7	
Frontal Lobe	0.0087 [0.0079-0.0113]	0.55	0.1102 [0.0948-0.1596]	**0.02**	0.0276 [0.0244-0.0329]	**0.02**	0.0094 [0.0053-0.0144]	**0.02**
Parietal Lobe	0.0104 [0.0096-0.0133]	0.06	0.1335 [0.1191-0.1770]	0.24	0.0293 [0.0245-0.0323]	0.24	0.0066 [0.0051-0.0127]	0.24
Temporal Lobe	0.0129 [0.0098-0.0194]	0.06	0.1822 [0.1476-0.2301]	1.00	0.0478 [0.0301-0.0646]	1.00	0.0168 [0.0121-0.0175]	0.55
Occipital Lobe	0.0141 [0.0161-0.4665]	0.55	0.2528 [0.2003-31.7664]	0.24	0.0472 [0.0362-0.0979]	1.00	0.0162 [0.0096-0.0186]	0.55
Thalamus	0.0142 [0.0094-0.0745]	1.00	0.1353 [0.1210-21.6753]	0.24	0.0417 [0.0307-0.1652]	0.24	0.0149 [0.0101-0.0182]	1.00
Striatum	0.0081 0.0044-0.0422	0.06	1.7700 [0.6328-7.1387]	1.00	2.8427 [0.3940-4.8181]	0.24	0.0854 [0.0234-0.1304]	0.24
Cerebellum	0.0179 [0.0142-0.5333]	0.06	0.2220 [0.1647-31.7753]	1.00	0.0302 [0.0267-0.0848]	1.00	0.0142 [0.0078-0.0172]	0.55
Hypothalamus grey	0.0037 [0.0033-0.0082]	0.55	0.7070 [0.6508-1.6766]	0.24	6.0323 [4.6810-7.0465]	1.00	0.1620 [0.1387-0.1689]	1.00
MABs	**K1**		**K2**		**K3**		**K4**	
n	4		4		4		4	
Frontal Lobe	0.0028 [0.0010-0.0792]		7.1624 [1.0090-14.0856]		5.9343 [2.8968-6.6064]		0.1436 [0.0663-0.7196]	
Parietal Lobe	0.0030 [0.0013-0.0058]		0.5739 [0.1708-1.4901]		6.4566 [2.6549-12599.4987]		0.0853 [0.0214-0.1374]	
Temporal Lobe	0.0048 [0.0016-0.0076]		0.7848 [0.1473-7.3992]		3.5199 [0.0304-15304.2525]		0.0080 [0.0036-0.0770]	
Occipital Lobe	0.0237 [0.0061-0.6691]		5.4155 [0.2756-36.2639]		0.0627 [0.0393-533.3176]		0.0121 [0.0010-0.1682]	
Thalamus	0.0845 [0.0019-0.4232]		12.9516 [3.7036-49.3591]		3.4038 [0.2325-12728.5189]		0.0206 [0.0093-0.0599]	
Striatum	0.0013 [0.0006-0.0026]		1.7550 [1.030-2.2570]		7.3367 [2.7557-25.5438]		0.2158 [0.1613-0.7595]	
Cerebellum	0.0080 [0.0039-0.0090]		0.2414 [0.1707-7.1481]		0.0288 [0.0239-4349.6958]		0.0056 [0.0020-0.6344]	
Hypothalamus grey	0.0017 [0.0008-2.3905]		6.1179 [1.3386-222.3930]		6.9653 [1.6717-21.1234]		0.1756 [0.0829-0.9847]	

Figure 7-8 Rate constant K1 in TBI subjects by genotype



7.3.7 Outcome measures

SUV

Standardised uptake values (SUV) were calculated from the 60-90 minute add image. SUV values ranged from 0.3-0.8 and there was no difference between HABs and MABs or an interaction of binding status across ROIs. SUVR was calculated using CGM and again there was no difference in SUVR between regions, between HABs and MABs nor was there an interaction of binding on Vt. There was no main effect of metabolic syndrome for either outcome measure and no group*ROI interaction. **Figure 7-9**.

Volume of distribution (VT)

Median VT ranged from 0.12-0.33 mL/cm^3 across all regions. There was no difference in VT across regions (P=0.20), no main effect of binding status (p=0.20) or interaction of binding status on VT (0.20). There was no main effect of metabolic syndrome on VT (P=0.48) or a group*ROI VT interaction (P=0.47). **Figure 7-10**.

DVR and SRTM

There was no difference in DVR or SRTM across regions, no main effect of binding status or interaction of binding status on either outcome measure. There was no main effect of metabolic syndrome on either outcome measure or a group*ROI interaction. **Figure 7-11**.

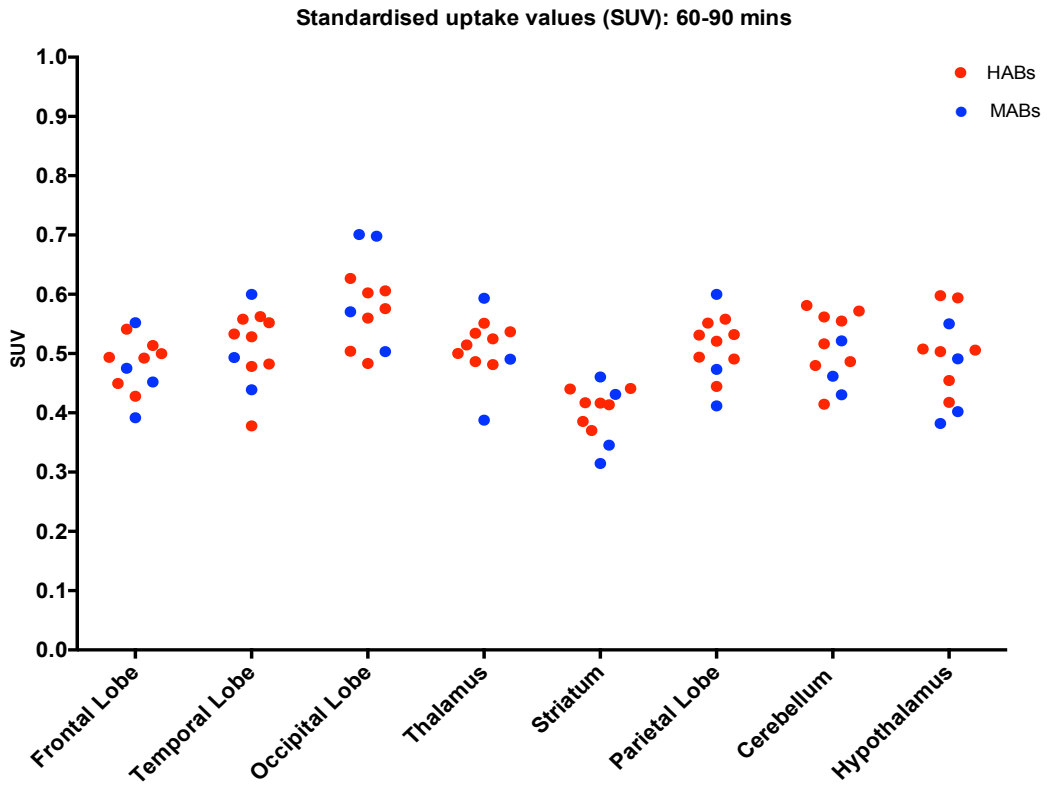
Longitudinal analyses

Individuals VT plots are shown in **Figure 7-12**. There was no significant difference between VT at follow up compared to baseline scan across all regions.

Comparison of VT in Patients vs. controls

For this analysis 11 controls and 11 patients were analysed. There was no difference in K1 between ROI, no main effect of binding status (P=0.45) and no interaction of binding status on K1 (P=0.42). For VT, there was no difference in VT between region (P=0.33), no main effect of binding status (P=0.33) and no genotype group*VT interaction (P=0.33). **Figure 7-13**.

Figure 7-9 SUV and SUVR (from 60-90 min summed image) in TBI patients by genotype



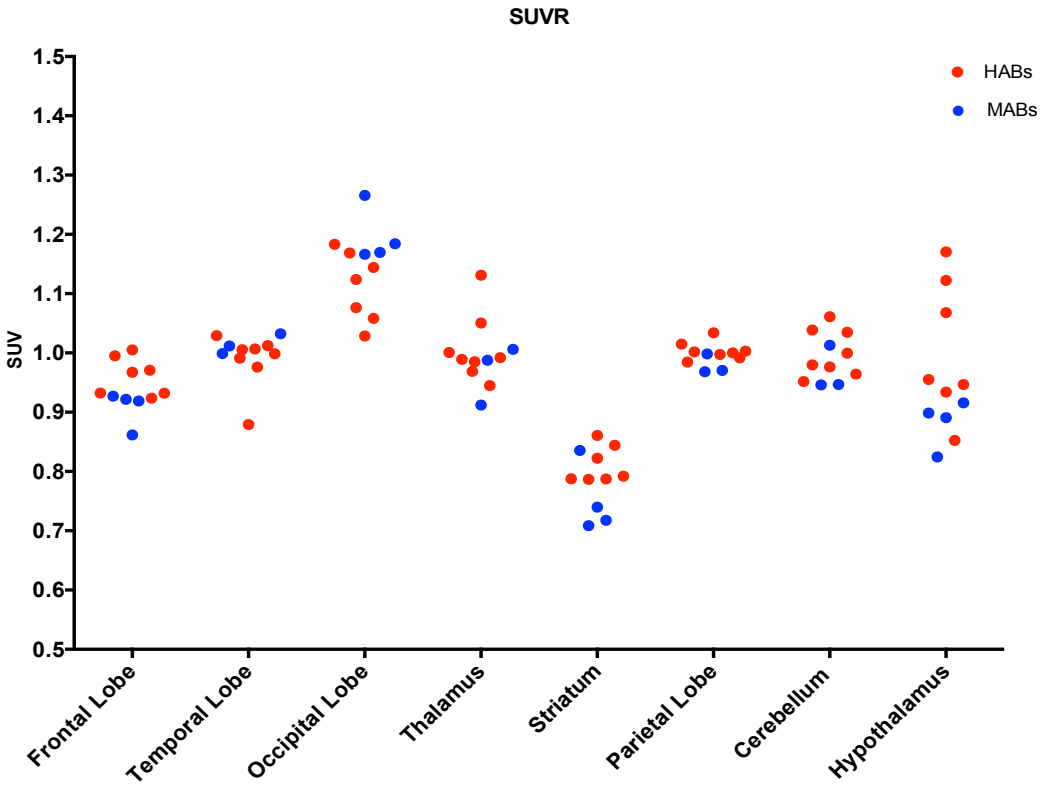


Figure 7-10 Volume of distribution in TBI patients by genotype

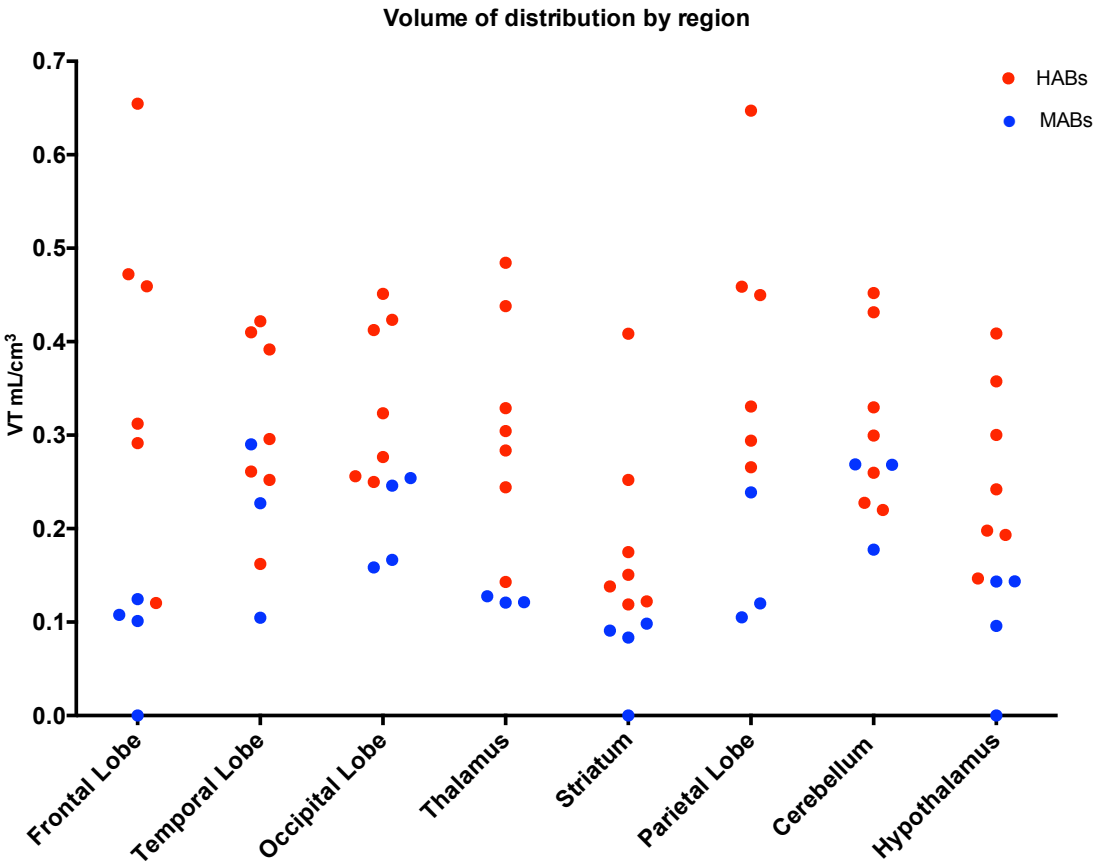

Volume of distribution by region
HABs
MABs
VT mL/cm3
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Frontal Lobe
Temporal Lobe
Occipital Lobe
Thalamus
Striatum
Parietal Lobe
Cerebellum
Hypothalamus

Figure 7-11 Distributed volume ratio (DVR) and simplified reference tissue model (SRTM) in TBI patients by genotype

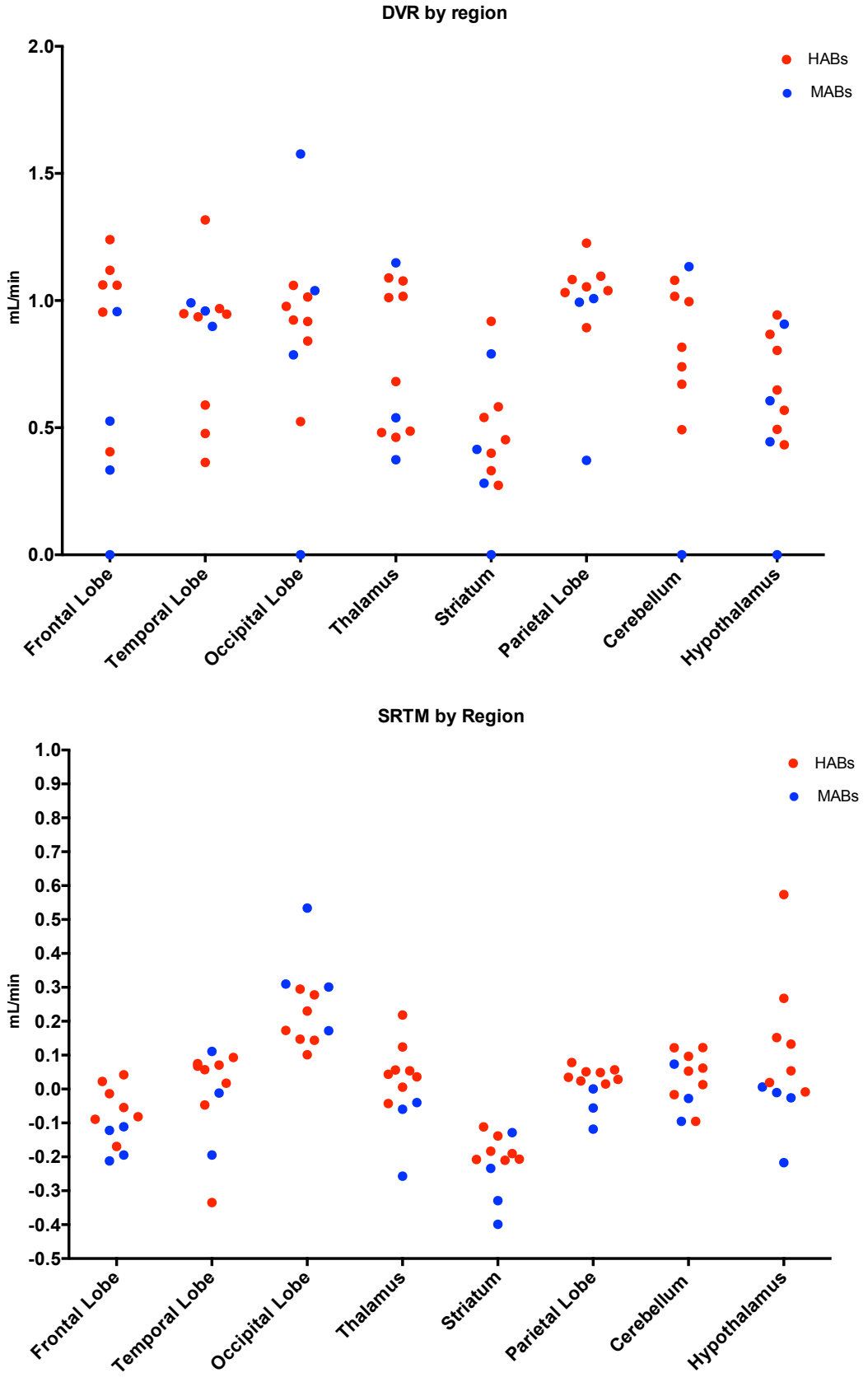


Figure 7-12 Comparison of VT between scan 1 and scan 2 (patients 2,3,5,6,9,10)

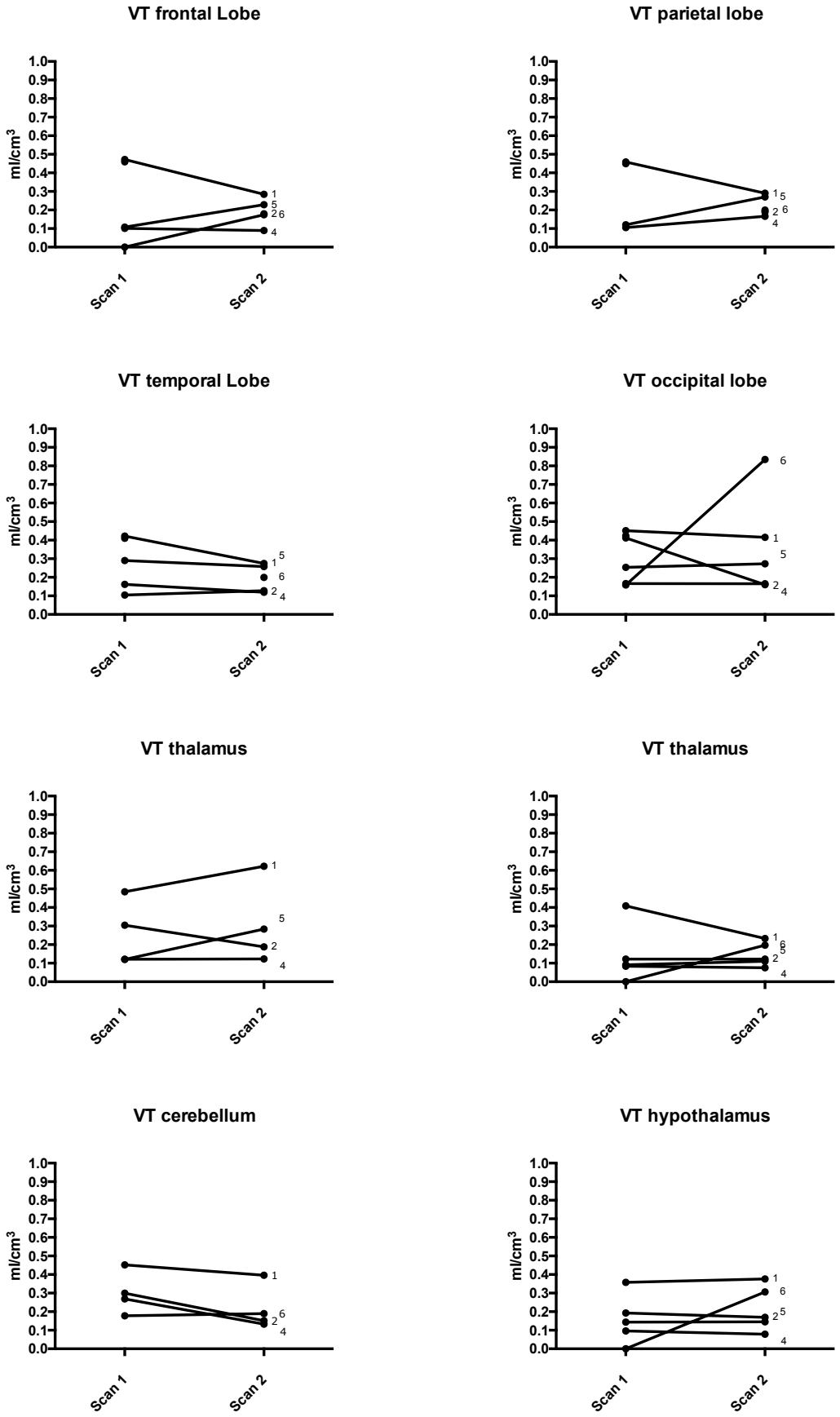
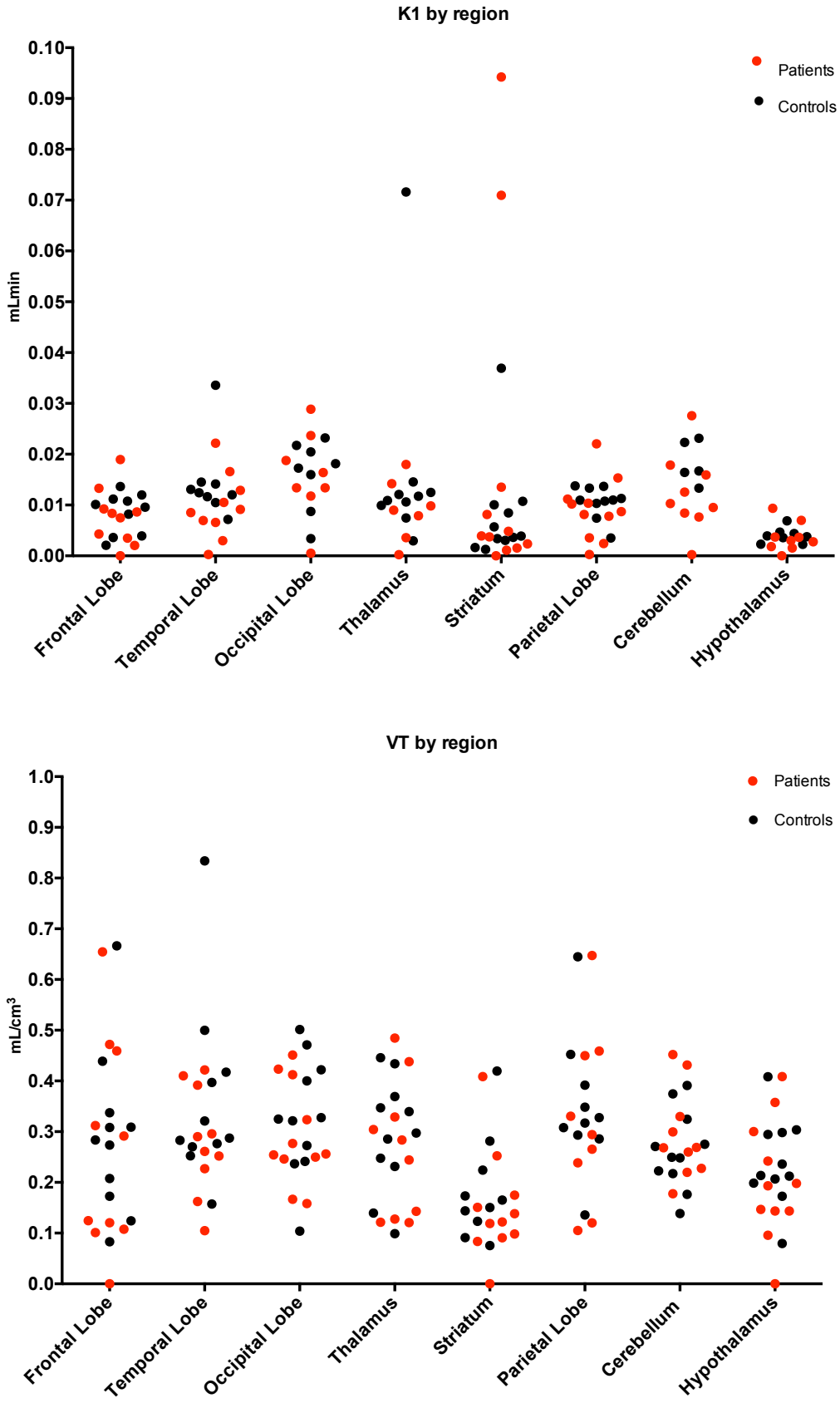


Figure 7-13 Comparison of VT and K1 in Patients vs. Controls



8 Discussion

This preliminary work describes the behaviour of the novel tracer [^{18}F]-GE-180 in 11 patients following a TBI. The work here follows on from the previous chapter where the kinetic behaviour of the tracer was described in 10 healthy volunteers. The main aims of this chapter were to investigate whether the 2TC compartmental model provided the best fit to the data, whether the TSPO polymorphism affected outcome measures and whether patients had higher TSPO binding compared to controls. The secondary aims were to determine whether the metabolic syndrome influenced outcome measures and whether binding measures changed over time.

The 2TC model did provide the best fit to the data as was found in healthy volunteers. Again there was a minor discrepancy in model fit at the start of the scan and this is likely due to effects of dispersion of the tracer. However early discrepancies are unlikely to have an effect of outcome measures which are generally speaking calculated from the later portion of the curve or is an integral of the area under the curve.

In the blood, [^{18}F]-GE-180 appears to have favourable kinetics in that the parent fraction of tracer ranges from 0.9-0.7 throughout the duration of the scan and the plasma over blood ratio of approximately 1.8 suggests good availability of plasma bound tracer. However we did not measure plasma proteins so cannot be certain of the availability of bound vs. unbound tracer. There were up to 4 identifiable metabolites as was seen in healthy subjects, however I cannot report on their polarity as this was not tested. There was no difference between HABs and MABs in blood kinetics. In the individuals who have two scans 6 months apart (6 patients) there was noticeable variability between the plasma over blood ratios at both time points. This could be due to a number of factors including patient factors but also factors relating to the tracer i.e. tracer mass, purity etc. Given the variability, this suggests that blood data should be sought at every time point within an individual.

Late SUV images (i.e. 60-90 mins) demonstrate generalised low uptake of tracer and a high blood signal volume as was seen in healthy volunteers. The time stability analysis in

the previous study suggested that a 90 minute scanning window may not be sufficient to derive reliable outcome measures at 90 mins, and therefore this should be born in mind when considering tissue data and outcome measures at 90 mins.

The rate constants K1-K4 were derived from the 2TC model. K1, the first rate constant and measure of tracer uptake was low (i.e. <0.01 ml/min) throughout all ROIs irrespective of genotype. K1 was not different between patients and controls. This was surprising given that I anticipated that there may be some BBB breakdown in patients which would increase K1. The majority of patients I selected had had a moderate to severe injury but perhaps their injury was not severe enough or recent enough to predispose to BBB breakdown. It is also not clear from the literature whether TBI can lead to BBB breakdown in the subacute phase.

Volume of distribution was <1 mL/cm^3 throughout all regions. This is an extremely low VT and questions the ability of [^{18}F]-GE-180 to quantify TSPO concentrations in the brain. There was no significant effect of binding status on VT but this was a very small sample of patients (n=11). There was no difference in VT between patients and controls in this study. The likely reason for this is that [^{18}F]-GE-180 is a substrate for a BBB transporter affecting uptake and VT. Another explanation is that TSPO expression in the healthy brain and in these TBI patients is truly low and other diseases that elicit a more powerful TSPO response e.g. active MS, should be studied with this tracer before we draw any further conclusions.

In the subset of patients of had two scans 6 months apart there appeared to be no dynamic change in VT and this is not surprising given the known limitations of the tracer.

My original hypothesis was to study the effect of metabolic syndrome on neuroinflammation following TBI. Whilst the appropriate analyses were performed to investigate this, the now known limitations of this tracer mean that the original hypothesis was premature and may have been better investigated using an alternative TSPO tracer such as [^{12}C]PBR-28. However, other tracers are extremely expensive and this may not be appropriate either.

In conclusion, the 2TC compartmental model best describes the kinetic behaviour of [^{18}F]-GE-180 in vivo. Uptake and distribution of the tracer is low in both healthy subjects and in this small study of TBI patients. There appeared be no effect of the TSPO polymorphism of or the metabolic syndrome on outcome measures. The most likely explanation for low uptake and distribution is due to [^{18}F]-GE-180 being a substrate for a BBB transporter. Therefore the tracer may still be suited to diseases with significant BBB breakdown.

9 Main discussion

9.1 Results summary

TBI is major public health problem and one which is often under-represented amongst the medical community. Cognitive and psychological disability following TBI leads to occupational loss, relationship breakdown and huge impairment in quality of life for these individuals. Given the relative lack of targeted treatments for the deficits following TBI, one management approach would be to identify the predictors of good recovery and adjust modifiable risk factors leading to a poorer prognosis.

Pituitary dysfunction following TBI has attracted a lot of attention in the last decade especially due to the wide prevalence (at least reported initially) and the prospect of offering hormonal replacement as treatment options. GHD, the most commonly reported abnormality (likely due to somatotroph cells being located on the outside of the pituitary gland) can be treated with GH injections. This treatment option not only has the advantage of treating the underlying condition but also potentially has a positive effect on cognition and quality of life as is seen outside of TBI.

Several studies have reported a prevalence of pituitary dysfunction to be in the region of 10% and this is consistent with our own (unpublished) experience in the multidisciplinary TBI clinic at St Mary's Hospital. However, in the third chapter of this thesis, we have identified as special group of patients who have a particularly high prevalence of pituitary dysfunction. Soldiers who have been exposed to an IED blast explosion during warfare appear to have a much higher prevalence of pituitary dysfunction than those individuals who have been exposed to non-blast TBI as a result of assaults or road traffic accidents for example. In a group of 19 soldiers, 6 had evidence of pituitary dysfunction compared to 1/39 with nbTBI.

Focussing specifically on the GH/IGF-1 axis as this is the most commonly affected pituitary abnormality following TBI, I report some interesting results in chapters 4 and 5. Baseline serum IGF-I appears to predict white matter recovery in the splenium of the corpus callosum and higher levels of IGF-I at baseline lead to a greater improvement in memory

function over time. In patients who received GH replacement to treat GHD over a period of approximately one year, there was no observable effect on white matter recovery and cognition but there was a highly significant improvement in depression scores and quality of life following treatment.

The second half of this thesis addresses neuroinflammation following TBI. The original hypothesis was that the metabolic syndrome which fuels a low grade systemic inflammatory environment may modulate inflammation in the CNS. Microglia, the resident macrophage in the CNS express TSPO when in the activated state. TSPO expression by microglia is exploited by many PET tracers designed to bind to the TSPO receptor and is therefore considered to be a reliable marker of neuroinflammation.

In chapter 6, I used the novel fluorinated tracer [^{18}F]GE-180 to measure TSPO inflammation in the healthy brain. This study was performed with two aims in mind, to evaluate the kinetics of this new tracer, which were not known prior to this study and to determine whether there was a difference in binding between individuals who expressed a genetic polymorphism for TSPO. The 2TC compartmental model provided the best fit for the data. Overall uptake of the tracer was very low with an extraction rate of ~1% which was also reflected in a low volume of distribution throughout many brain regions. There was no significant difference, in this study, in outcome measures between high-affinity and mixed affinity binders.

Despite the limitations of this tracer, in the final chapter I evaluate [^{18}F]GE-180 in TBI patients using the 2TC model. First pass extraction remained low and there was no difference in outcomes measures between HABs and MABs. There was no effect of the metabolic syndrome on outcome measures in this small study.

9.2 Main findings

9.2.1 Prevalence of pituitary dysfunction is significantly higher in blast TBI compared to non-blast TBI

The prevalence of pituitary dysfunction is significantly higher in bTBI than nbTBI (31.6% vs. 2.6%, P=0.004). There was a trend toward greater PTA (P=0.10) and higher AIS head injury scores (P=0.006) in soldiers who did vs. those who did not have pituitary dysfunction. There was a significantly higher prevalence of skull/facial fractures in those with pituitary dysfunction (P=0.02) and this could be an important predictor of the condition however half the soldiers who had pituitary dysfunction had no evidence of facial/skull fractures. On cross-sectional analysis there was significantly lower FA in the body, genu and splenium of the corpus callosum in soldiers with and without pituitary dysfunction. In addition soldiers with the condition fared worse in multiple cognitive domains and trended towards worse QoL than those who did not have the condition.

This novel finding which has now been published highlights a important group of patients who have potentially treatable conditions. This study was not able to answer about why bTBI appears to pre-dispose to pituitary dysfunction more than nbTBI so we can only speculate. The blast wave may predispose to increased acceleration-deceleration forces which could precipitate shear injury in the region of the hypothalamus-pituitary gland. Vascular damage could lead to hypoxic/ischaemic damage and BBB breakdown which could also lead to pituitary autoimmunity.

Given the high prevalence of the condition our recommendation was to fully investigate all people with moderate to severe bTBI and to comprehensively screen for the condition in those with lower severity categories of bTBI. Following this finding we disseminated this information to our colleagues at the Defence Medical Rehabilitation Centre (DMRC) at Headley Court and the multidisciplinary TBI clinic at St Mary's Hospital continue to receive referrals via this route.

9.2.2 FA is greater in the SPCC and logical memory scores are higher at follow-up in those patients with a higher IGF-I at baseline

In this longitudinal DTI study of 39 TBI patients, FA was greater at follow-up in the SPCC in those who had a baseline IGF-I level above the median of the age-adjusted reference range (IGF-I group x time interaction $F(1,37)=4.62$ $P=0.038$). Logical memory score at visit 2 was also significantly higher in these individuals (IGF-I group x time interaction $F(1,26)=4.38$ $P=0.046$). This result appears to be consistent with studies in stoke where a higher IGF-I at baseline leads to better neurological and functional outcomes at follow-up. Mechanistically this seems plausible as well given that IGF-I is a trophic factor involved in the turnover of oligodendrocytes.

Although IGF-I above and below the median of the age-adjusted reference range is a fairly crude categorisation and one which has no real clinical meaning, we felt it was a good surrogate for examining the effect of GHD as at the time of carrying out the study only 4 patients had confirmed GHD. In a sub-analysis of GHD=4, the presence of GHD appeared to have no influence on FA or cognition.

This chapter also highlighted two other important findings unrelated to endocrine variables. I reported that TBI patients have widespread reductions in FA throughout the whole brain compared to healthy controls. I also report that over time (i.e. Between the two study visits), FA does increase throughout many regions of the brain. This is important as demonstrating that recovery from TBI on a microstructural level is a dynamic process and one that is reflected in cognitive/psychological recovery and one that may be amenable to manipulation. Increasing IGF-I may be one potential factor that could improve white matter recovery in key areas of the brain which are commonly affected in TBI.

9.2.3 GH replacement appears to have no effect on FA and cognition following TBI but does significantly improve QoL and reduce symptoms of depression

This small study of 10 patients diagnosed with GHD following TBI who were then treated with GH replacement for a median duration of 14 months were compared to group of 23 TBI patients who had no evidence of GHD and who did not receive and GH. There appeared to be no influence of group on FA over time in three key regions including the SPCC and no effect either on cognitive variable including logical memory. This was surprising as given IGF-I group appears to affect FA in the SPCC and memory we were expecting that raising GH/IGF-I levels with exogenous treatment would influence these factors. However a group of 10 patients may be too small a sample to expect to see a significant effect of GH replacement. Encouragingly, from visually looking at the data, it did appear that the group who received GH replacement appeared to be going in the direction of FA improvement rather than reduction. Increasing the sample size and/or performing scans at later time points e.g. after 2-3 years on GH replacement, may be the basis for future studies and may be warranted before we conclude that GH replacement has no beneficial effects on cognition or white matter recovery following TBI.

However there was a significant improvement in QoL as measured by AGHDA-QoL following GHR ($t=-3.8$, $df=9$, $P=0.004$, two-tailed) and an even more impressive reduction of depressive symptoms (mean point reduction 12.7) following GHR ($t=-5.2$, $df=9$, $P=0.001$, two-tailed). This probably justifies the use of GHR treatment in patients with GHD following TBI and could have far-reaching effects on occupational status, preserving relationships and increasing motivation to engage with rehabilitation services.

In keeping with what is seen outside of TBI, patients with GHD had greater insulin resistance and worse metabolic score compared to those who did not. There was a trend towards improvement in metabolic score following GHR but insulin resistance did not appear to improve with GHR. This again may be due to a too short a study period but it may be that any metabolic amelioration with GHR may be outweighed by a potentially more sedentary environment in the initial recovery period following TBI.

9.2.4 The kinetics of [^{18}F]GE-180 are best described by the 2TC model and there is no effect of the TSPO Ala147Thr polymorphism on distribution volume outcome measures in the healthy human brain

This chapter presents one of the first studies using the novel TSPO ligand [18F]GE-180 in the healthy human brain. This study has now been published in the European Journal of Nuclear Medicine and Molecular Imaging (Feeney et al., 2016). This purpose of the study was to evaluate the kinetics of this tracer in the healthy human brain prior to its evaluation in TBI.

Several compartmental models were applied to the data and the 2TC model with a 5% fixed blood volume was the most parsimonious. There was a slight discrepancy in model fit at the start of the scan and this may have been due to effects of dispersion. There was a very low first pass extraction of the tracer in the region of 1% and this is likely to be due to [18F]GE-180 being a substrate for an as yet unknown BBB transporter. Within the brain the blood component of tracer distribution was particularly high at around 20%. Volume of distribution was <1 throughout all brain regions. These characteristics of the tracer were disappointing findings and could not have been predicted from pre-clinical work where [18F]GE-180 appeared to have greater specific binding and less non-specific binding compared to PK[11195].

There was no significant effect of the TSPO polymorphism (between HABs and MABs, not LABs) on distribution volume measures in this study. However, given that VT was very low, it would be difficult to say with certainty that there is no effect of TSPO genotype as in disease states or where there is greater uptake of the tracer due to BBB breakdown, differences between genotype may emerge.

Another important finding was the time stability analysis that showed that VT was still rising throughout the 90 minute duration of the scan and too short a scan duration may also be contributing factor to low distribution volumes.

9.2.5 There is no difference in the volume of distribution of [^{18}F]-GE-180 in TBI patients compared to controls

In this preliminary small study of TBI patients, [^{18}F]-GE-180 uptake and volume of distribution was no different between patients and controls and there was no effect of genotype or of metabolic syndrome on the outcome measures studied.

The 2TC model provided the best model fit to the data as was seen in healthy volunteers and there were favourable blood kinetics.

More work in needed in a larger TBI sample and in other diseases known to cause significant BBB breakdown to further evaluate this tracer.

9.3 Limitations

There are several limitations to the interpretation of the data presented in this thesis. Firstly, the study in Chapter 3, Pituitary Dysfunction after Blast Traumatic Brain Injury (bTBI) was by its design a retrospective case-control study which means that although every effort was made to match the groups as much as possible, there were factors (mainly environmental) that could not be controlled for. For this reason, although we make an interesting observation, it was not made in the context of a randomised controlled trial.

The major limitation to Chapter 4 Association of serum IGF-I with white matter, neuropsychological and cognitive recovery following traumatic brain injury, was that I could not find an association with IGF-I and FA using the whole brain analysis approach and neither was there a correlation between delta FA at the corpus callosum and IGF-I. This potentially undermines the association we found between FA at the SPCC and IGF-I.

For the Chapters 5 & 7, the major limitations relate to the small numbers enrolled in the two studies with the potential for there to be significant type II errors. We hope to address this by adding to these datasets and re-analysing the data prior to publication.

9.4 Conclusion

The aim of this thesis was to investigate the role of hormones and metabolic factors in the recovery from TBI. The findings that pituitary dysfunction (mainly GHD) has a high prevalence in blast-TBI, that a high IGF-I at baseline may enhance recovery following TBI and that GH replacement improves QoL and reduces depression all highlight the importance of endocrine factors following TBI. The work here provides some foundation for future studies investigating the role of hormone replacement on recovery from TBI and other neurological conditions.

I had hoped to also explore the role of the metabolic syndrome in the recovery from TBI and on neuroinflammation *per se*. However the novel TSPO tracer [^{18}F]-GE-180 which had promising pre-clinical results appears to be limited by low uptake and distribution volumes. Instead, this thesis has detailed one of the first kinetic analyses of this tracer in healthy brain and in a preliminary study of TBI patients. This work will be of use to researchers using or planning to use [^{18}F]-GE-180 in their studies.

10 Future Directions

Arising from this thesis, there are several datasets that I would like to expand upon and interrogate further. I would like to work more on the data presented in Chapter 5 (The effect of GH replacement therapy on the recovery from TBI). In particular I would like to analyse repeated QoL measurements in patients taking GH to establish whether the positive effect on QoL is sustained. Ideally I would like to add more people to this study as n=10 may be too small to answer the question about GH replacement on WM recovery. In addition I would like to do additional analyses such as voxel based morphometry especially as data is available to do this. Time permitting, I would like to publish this work, (even if we find no effect on WM recovery) as there is little in the literature that answers questions about GH replacement in adults following TBI.

Regarding the TSPO PET chapters, I hope to continue to work closely with other researchers using this tracer to determine whether there is increased TSPO signal in brain injured patients. Unfortunately I was not able to specifically answer the question about

whether the metabolic syndrome influences the recovery from TBI. However I do think body/brain interactions are an important area of research and ideally I would set about answering this question in different ways e.g. designing a large observational study to look for correlations between markers of insulin resistance and functional outcome from TBI.

More broadly, I hope I am able to apply the skills learnt during my PhD and utilise them during my return to clinical training in Endocrinology and Diabetes with a view to paving new research avenues in the field of clinical neuroendocrinology.

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12 Other Publications

Brief Communication Arising from 'Evidence for human transmission of amyloid-β pathology and cerebral amyloid angiopathy'(Jaunmuktane et al., 2015)

12.1 Seeds of Neuroendocrine Doubt

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CF wrote the article. GS contributed to writing the article. JC, MS, AG and RL revised the manuscript and contributed to its content.

Dear Editors,

The possibility of human-human transmission of Alzheimer's disease (AD) has never been considered until recently. A landmark study, published in September 2015 in *Nature*, reports the probable seeding of β-amyloid (a pathological hallmark of AD) from cadaveric human growth hormone (c-hGH) pituitary preparations who also developed iatrogenic Creutzfeldt-Jakob Disease (iCJD) from the same source (Jaunmuktane et al., 2015). Here, we argue that c-hGH was historically used to treat rare neuroendocrine disease and that these diseases or treatment with GH could result in β-amyloid pathology *per se*. Without a

control group of the same patient cohort who neither received c-hGH nor developed iCJD, we believe that the authors are premature to infer an iatrogenic source of AD.

Cadaveric-hGH was previously used to growth hormone deficiency (GHD) in children. In the UK, GH was extracted from a cadaveric pool of approximately 400,000 pituitary glands and offered as an intramuscular treatment to selected patients. Given scarce resources and laborious extraction, its use was coordinated in the UK by a Medical Research Council working party in 1959 that was superseded by the Health Services Human Growth Hormone Committee in 1977 (Milner et al., 1979). In the UK, 1,908 people were registered as having received c-hGH from 1959 to 1985 when it was withdrawn worldwide after an initial report documenting three deaths from CJD in the US and one in the UK. This number rose to 226 incidences worldwide in 2012 (Stagnaro, 2015). From 1985 onwards c-hGH became obsolete after being superseded by synthetic recombinant forms.

The diverse clinical indications for c-hGH treatment until 1985 are clearly presented in a comprehensive epidemiological review which utilised data from the Health Services Human Growth Hormone Committee records in the UK (Buchanan, Preece, & Milner, 1991). Of the 1908 patients registered as having received c-hGH in the UK, 1004 (52.6%) had 'idiopathic' isolated GHD, 188 (10%) 'idiopathic' panhypopituitarism, 230 (12.1%) craniopharyngioma, 194 (10.2%) other intracranial tumour, 62 (3.3%) other neurological disease e.g. hydrocephalus, post meningitis and 62 (3.3%) genetic short stature and growth delay or both and 44 (2.3%) Turner's Syndrome. The remaining causes included Prader-Willi syndrome, low birth weight, haematological disease, histiocytosis and septo-optic dysplasia. These well-recorded historical data demonstrate the heterogeneity and severity of neuroendocrine disease necessitating c-hGH treatment in the UK prior to 1985.

Supplementary material from the article in question reports the clinical details of all 8 patients and the indication for c-hGH treatment, summarized in Table 1. All patients received c-hGH as children or adolescents and duration of treatment was for 2-12 years. From an etiological perspective, 3/8 subjects had panhypopituitarism (one also with mental retardation and microcephaly), 1/8 craniopharyngioma and 4/8 are reported to have been treated for short stature alone. This is surprising as short stature alone was an

uncommon indication for c-hGH prior to 1985, although trials were starting to emerge for this indication just prior to the withdrawal of c-hGH(Poidvin et al., 2014). Short stature should not be confused with GHD as the former can be constitutional and the latter is a neuroendocrine disease which can lead to short stature. It is therefore likely that the 'short stature' group disguises a more complicated clinical picture.

At the other end of the clinical spectrum, one of the 4 patients reported as having moderate to severe gray matter and vascular β-amyloid pathology had a craniopharyngioma. These unusual pituitary tumours can be large, often require neurosurgery and/or cranial radiation and are prone to recurrence and multiple pituitary hormone deficiencies. Interestingly, the one patient in the sporadic CJD control group (aged 65 yrs. approx.) who also had similar levels of cerebral amyloid angiopathy (CAA) to the iCJD group was labelled as an outlier and it was commented that the subject had a surgical intervention 40 years before death (Jaunmuktane *et al.* Extended Data Figures 2&3).

Jaunmuktane and colleagues' article was preceded by a case series from the same research group of the clinical, imaging, molecular and autopsy findings of the whole iCJD cohort (n=22)(Rudge et al., 2015). One patient had a history of long term cognitive problems and additional MRI findings included one patient with septo-optic dysplasia and partial agenesis of the corpus callosum. It is not clear whether these particular patients were the ones included in the current article, but together with the historical records and the supplementary clinical details provided, highlight our argument that these patients had complex, rare and potentially severe neuroendocrine disease.

With this in mind, an alternative hypothesis contributing to the findings is that these pre-existing and underlying conditions could by themselves lead to β-amyloid pathology and abnormal brain structure. GH is known to have various cognitive effects on the brain and isolated GHD in children can lead to lower IQ, impaired cognition, reduction brain volumes and white matter abnormalities (Nyberg & Hallberg, 2013; Webb et al., 2012). If GHD continued into adulthood (as it usually does) and was untreated then significant cognitive impairments are likely to have persisted (Rinderknecht & Humbel, 1978).

Traumatic brain injury (TBI) we now know is a common cause of GHD in children(Rose & Auble, 2012) and this may not have been widely acknowledged prior to 1985. TBI has been associated with widely distributed Aβ deposition in post-mortem brain tissue (Le Tissier et al., 2012). Other neurological diseases including epilepsy, fragile X syndrome, Down's syndrome and Parkinson's disease have all been linked with significant increased levels of amyloid precursor protein (APP) and β-amyloid protein questioning the specificity of this neuropathology to AD. Aβ deposition may result from common underlying processes such as neuroinflammation, microglial activation and genetic factors(Kokshoorn et al., 2010).

The authors comment on the striking CAA in this patient group and hypothesize that these individuals 'would be at increasing risk of cerebral haemorrhages had they lived longer'. However, large long-term follow up studies of children who received recombinant GH from 1985 onwards (i.e. no risk of human-human transmission) were found to have a 5-7 fold increased risk of cerebrovascular disease and, in particular, subarachnoid haemorrhage, suggesting that this cohort have predisposing factors leading to increased risk of intracranial haemorrhage, irrespective of having received c-hGH(Carel et al., 2012; Poidvin et al., 2014).

In conclusion, the authors present an interesting finding worthy of further investigation, but we feel that caution must be exercised in interpreting the result, especially in the absence of an appropriate control group. We believe it is also important to present a neuroendocrinological perspective and consider plausible alternative hypotheses to offer a balanced view on this high profile communication.

Table 1

Patient	Sex	History	Substantial Aβ	Focal Aβ	Aβ angiopathy
1	M	GH as a child for ~5 years for panhypopituitarism	No	Yes	No
2	F	GH as a child for ~10 years for panhypopituitarism, mental retardation and microcephaly	No	No	No
3	M	GH as a child for ~5 years for short stature	No	Yes	No
4	M	GH as a child for short stature from ~7 for ~12 years	Yes	No	Yes
5	F	Craniopharyngioma aged ~10 years, GH from 11 years for ~8 years	Yes	No	No
6	M	GH for short stature from ~9 years for ~5 years	Yes	No	Yes
7	M	GH as an adolescent for ~3 years for panhypopituitarism	No	No	No
8	M	GH for restricted growth from 16 years for ~2 years	Yes	No	Yes

Summary table of the clinical indications for c-hGH treatment in childhood and incidence of β-amyloid for each patient (adapted from article text and supplementary information)