Neuropathology and cognitive dysfunction after early hypoglycaemia

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In loving memory of my Grandma, Angela Pitts

‘ However long your stay on this small planet lasts, and whatever happens during it, the most important thing is that - from time to time - you feel life’s sweet caress ’

- William Boyd, *Sweet Caress*

Declaration

I, Georgia Pitts, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

Hypoglycaemia is the most common metabolic problem in neonatal medicine, occurring during the first days of life and usually resolving within the same time frame. However, some neonates and infants experience severe and recurrent episodes of hypoglycaemia, the most common aetiologies being congenital hyperinsulinism (CHI) and ketotic hypoglycaemia (KH). Children with CHI are at risk of lasting brain injury, while children with KH are considered to be protected from adverse sequelae owing to the presence of ketone bodies during hypoglycaemia. This thesis investigated the neuropsychological and neuroimaging profiles of these two patient groups in neurologically normal school-aged children. Thirty-one patients with CHI and twenty-one patients with KH participated in the study alongside a cohort of healthy controls. A comprehensive battery of neuropsychological tests revealed specific impairments in attention and motor skills in both patient groups, with additional impairments observed in children with CHI. Automated and manual measurements of subcortical volumes, as well as whole brain analyses (voxel based morphometry and tract based spatial statistics) were conducted. Compared to controls, patients with CHI have reduced volume of subcortical structures, as well as extensive white matter volume loss (accompanied by decreased intracranial volume) and reduced white matter integrity across the entire brain. Patients with KH did not significantly differ from controls on any brain measures, but the only significant difference between patient groups was in thalamic and intracranial volumes. Integrity of subcortical structures and white matter was found to be predictive of scores in memory, motor skills and attention. This study is the first to show the extent of brain abnormality as a result of CHI in neurologically normal children. Furthermore, the finding that both patient groups share a similar cognitive profile refutes the notion that children with KH are protected from adverse sequelae. The implications of these findings are discussed.

Contributions

Unless otherwise stated here, all recruitment, neuropsychological assessment and analysis included in this thesis is my own work.

Recruitment and assessment of many of the patients included in this thesis was conducted as part of the *HBI* study (n=20), conducted by Dr Anitha Kumaran. Dr Jemima Bullock conducted the neuropsychological assessments of all patients recruited to the *HBI* study.

Recruitment and assessment of healthy controls to the STEPSOUT study (n=17) was conducted by Dr Marina Martinos. Data for the CA study (n=9) was collected by Dr Sharon Geva. Recruitment and assessment of the 64 children in the MRC study '*Hypoxia/ischaemia in children: Patterns of neuropathology and associated memory impairment* ' (referred to in chapter five) was conducted by Dr Janine Cooper, Dr Monica Munoz and Dr Sebastian Jentschke.

Chapter Five: All hippocampal volumes were measured by Professor David Gadian.

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Abbreviations

AD – Axial Diffusivity

ANOVA – Analysis of Variance

ANCOVA – Analysis of Covariance

BET – Brain Extraction Tool

BRIEF – Behaviour Rating Inventory of Executive Functions

CHI – Congenital Hyperinsulinism

CI – Confidence Interval

CMS – Children's Memory Scale

CPT – Continuous Performance Test

CSF – Cerebrospinal fluid

CT – Computed Tomography

DTI – Diffusion Tensor Imaging

DWI – Diffusion Weighted Imaging

D-KEFS – Delis-Kaplan Executive Function System

EODM– Early Onset Diabetes Mellitus

FA – Fractional Anisotropy

FSL – Functional MRI of the Brain Software Library (Oxford, UK)

ICV – Intracranial Volume

IQ – Intelligence Quotient

KH – Ketotic Hypoglycaemia

MABC-2 – Movement Assessment Battery for Children

MD – Mean Diffusivity

MRI – Magnetic Resonance Imaging

RD – Radial Diffusivity

RF – Radiofrequency

SDQ – Strengths and Difficulties Questionnaire

TBSS – Tract-Based Spatial Statistics

TEA-Ch – Test of Everyday Attention for Children

VBM – Voxel-Based Morphometry

WASI – Wechsler Abbreviated Scale of Intelligence

WIAT-II – Wechsler Individual Attainment Test

WISC-IV – Wechsler Intelligence Scale for Children

Chapter 1

General Introduction

1 General Introduction

Hypoglycaemia (low blood sugar) is common in the newborn, and usually resolves within the first few postnatal days without any serious medical intervention required. However, some neonates, infants and children experience severe and recurrent episodes of hypoglycaemia and are at risk of lasting brain damage and cognitive dysfunction as a result. The two most common causes of hypoglycaemia after the immediate postnatal period are congenital hyperinsulinism and ketotic hypoglycaemia.

This thesis is concerned with the long-term outcome of children with these diseases. This introductory chapter describes the two phenotypes. This is followed by a review of relevant studies examining cognitive and motor outcome and those studies that have reviewed neuroimaging data. Finally, the goals of this research are presented.

1.1 Background

1.1.1 Glucose and the brain

Glucose is the main source of energy and the main substrate for cerebral oxidative metabolism in the human brain. The brain consumes 20% of glucose-derived energy, making it the biggest consumer of this energy source in the human body (Mergenthaler, Lindauer, Dienel, & Meisel, 2013). The overall cerebral utilisation of glucose varies over the course of development; the total utilisation of glucose is at its greatest during childhood, being twice as great as in the fully developed adult brain,

and is at its lowest during the neonatal period (Chugani & Phelps, 1986; Pascual, Wang, Hinton, & Engelstad, 2007). Glucose metabolism in the brain varies regionally according to postnatal age within the first year of life. In the neonatal period a high utilisation of glucose is observed in the thalamus, hippocampus and sensorimotor cortices, and this evolves to greater glucose utilisation in the basal ganglia, parietal, temporal and primary visual cortices by two to three months of age. Between six and eight months of age, glucose utilisation increases in the frontal cortices, stabilising at around one year. These variations in glucose utilisation reflect the functional and structural maturation of different brain regions over the first year of life (Chugani, 1998), and the utilisation of glucose in infants and children reflects the formation and establishment of neural circuits through division and migration of neurons and precursor cells (Pascual et al., 2007). The immature brain is thought to be particularly sensitive to lack of adequate fuel during these times of critical development (Alkalay, Sarnat, Flores-Sarnat, & Simmons, 2005).

1.1.2 Neonatal hypoglycaemia

The most common metabolic problem in neonatal medicine is hypoglycaemia (Boluyt, van Kempen, & Offringa, 2006). Despite this there is no universally accepted definition of hypoglycaemia. Textbook definitions of hypoglycaemia have been found to vary between blood glucose levels of 1 and 2.5 mmol/L (Koh, Eyre, & Aynsley-Green, 1988), while in clinical practice levels falling anywhere below 3.5 mmol/L are considered to be significantly low and to warrant intervention. Generally, a glucose value of less than 2.8 mmol/L in infants and children is considered to be of clinical significance (Arnoux et

al., 2010), although the neurological response to these levels will vary between individuals (Rozance & Hay, 2006).

In the neonate, hypoglycaemia is a common occurrence, as the newborn has to independently adjust to, and meet the demands of, a new environment. Often this reflects a normal adaptation to extra-uterine life (Cornblath et al., 2000). A multitude of causes exists for the occurrence of hypoglycaemia in the neonate, including those that are due to hormonal abnormalities (e.g. transient hyperinsulinism) brought on by congenital perinatal stress factors; for example in infants who are premature or small for gestational age (Arya, Mohammed, Blankenstein, De Lonlay, & Hussain, 2014). In addition, deficiencies in cortisol or growth hormone, varied defects of carbohydrate breakdown and synthesis and a wide range of metabolic conditions can all cause hypoglycaemia in the neonate.

These episodes of hypoglycaemia usually occur within the first postnatal days of life, and are often transient. However, for some neonates, infants and children, severe and recurrent episodes of hypoglycaemia persist past the neonatal period, often occurring throughout infancy and childhood, especially if the underlying condition is difficult to manage (Hussain, Blankenstein, De Lonlay, & Christesen, 2007). It is these patients who are the subject of this thesis.

1.2 Congenital Hyperinsulinism

1.2.1 Incidence

Congenital hyperinsulinism (CHI) is the most common cause of persistent and severe hypoglycaemia in the neonatal period and throughout childhood (Hussain, 2005; Hussain et al., 2007). The condition is not limited to neonates but can also occur in infants and children (Kapoor et al., 2009). The incidence of CHI is estimated to be around 1/50,000 to 1/27,000 live births in central Europe, making it a very rare disease (Mazor-Aronovitch et al., 2007; Warncke et al., 2016). However, in smaller communities (such as Finland) the occurrence is thought to be greater and it is greater still in communities with a high rate of consanguinity, where CHI may be seen as often as 1/2500 births (Al-nassar, Sakati, Al-ashwal, & Bin-abbas, 2006; Hussain & Aynsley-Green, 2003).

CHI is an extremely heterogeneous disease in terms of its clinical presentation, aetiology and response to medical treatment, but in all cases, is characterised by an inappropriate and unregulated secretion of insulin, resulting in profound hypoglycaemia which is accompanied by a suppression of production of alternative fuels (such as ketone bodies) that can be used for cerebral oxidative metabolism.

1.2.2 Pathophysiology

Under normal conditions, the secretion of insulin is tightly regulated by pancreatic β-cells in order to maintain fasting blood glucose levels in a narrow range, between 3.5-5.5 mmol/L (Nessa, Rahman, & Hussain, 2016). β-cells in the pancreas are very

sensitive to glucose levels in the blood plasma and respond to these levels appropriately via regulated secretion of required amounts of insulin. In CHI, dysregulated and inappropriate secretion of insulin from pancreatic β-cells causes a profound depletion of blood glucose accompanied by an abnormal hormonal response to this depletion. Indeed, glucose insufficiency is not just characterised by the concentration of blood glucose, but depends on factors such as cerebral blood flow, cerebral glucose utilization rate and cerebral uptake of alternative fuels (Rozance & Hay, 2006).

While glucose is the main substrate in the brain, alternative fuels can be available for cerebral energy metabolism and a healthy response to hypoglycaemia is hallmarked by a rise in the presence of these substrates. Lactate is one of the best characterised alternative fuels for the brain (Rozance & Hay, 2006), in addition to ketone bodies (namely β-hydroxybutyrate and acetoacetate). The cerebral uptake and metabolism of ketone bodies has been shown in adults after a period of starvation, but also in children between six weeks and seven years of age after fasting (Persson, Settergren, & Dahlquist, 1972). In patients with CHI, production of these alternative substrates is supressed owing to the dysregulation and continued secretion of insulin, which inhibits lipolysis and ketone body synthesis (Hussain & Aynsley-Green, 2003).

Potassium channels (K_{ATP} channels) in the membrane of the β-cells maintain the electrical potential of the β-cell membrane, and play a crucial role in the regulation of insulin secretion. Disruption to the function of the K_{ATP} channels leads to unregulated entry of calcium into the β-cell, a process that is thought to stimulate insulin secretion.

Unsurprisingly, given its importance in regulating the secretion of insulin, the most common cause of persistent CHI is a functional abnormality of the K_{ATP} channels (Hussain, 2005; Mohamed, Arya, & Hussain, 2012; Nessa et al., 2016). There are, however, other mechanisms of insulin secretion that are not directly related to K_{ATP} channels. For example, mutations in the genes coding for glucokinase and glutamate have been identified as genetic causes of CHI (Hussain, 2005b). To date, there are nine genes that have been identified in congenital hyperinsulinism. These are the ABCC8, KCNJ11, GCK, SCHAD, GLUD1, SLC16A1, HNF1A, HNF4A and UCP2 genes (Nessa et al., 2016). However, in about 50% of cases with CHI there is an unknown genetic cause (Hussain, 2005a).

1.2.3 Clinical presentation

Neonates and infants with CHI can present clinically through myriad symptoms. Severe disease-specific symptoms include loss of consciousness and seizures, while non-specific symptoms include lethargy, poor sucking reflex, irritability and jitteriness (Hussain, 2005a; Mohamed et al., 2012). In CHI, seizures are the most common presenting symptom of hypoglycaemia, typically occurring in 50% of patients (Al-nassar et al., 2006; Arnoux et al., 2011), but some studies report seizures in up to 75% of patient cohorts (Ağladıoğlu et al., 2013). In patients with non-specific symptoms, delays between presentation and diagnosis (and therefore effective treatment) are likely to result in increased propensity for hypoglycaemic encephalopathy, but early presentation with severe symptoms such as seizures is also considered to result in poor outcome (Rozance & Hay, 2006).

1.2.4 Treatment

Treatment for CHI is dependent on aetiology and responsiveness to medication. Patients with medically responsive hypoglycaemia are treated with drugs that either directly target the K_{ATP} channels (Diazoxide), block calcium channels (Nifedipine), or increase glycogenolysis and gluconeogenesis (Glucagon). There are also drugs such as Octreotide that have a broader effect on the β-cells (Hussain, 2005a). Most patients who are medically treated remain drug-dependent for many years, although spontaneous recovery does occur in some cases when response to medical management is good. Another exception to long term drug-dependency is observed in those with the transient neonatal form of CHI; these patients experience hyperinsulinism in the first few months of life which then resolves after treatment with Diazoxide (Arnoux et al., 2010). In the case of medically unresponsive CHI, a partial, or near total, pancreatectomy is usually performed (Figure 1.1). If a focal lesion is identified (through a Positron Emission Tomography [PET] scan), a lesionectomy is performed, removing only the diseased part of the pancreas. In these cases, patients are typically completely cured of the disease and do not experience further episodes of hypoglycaemia. In diffuse disease, which is more difficult to manage, a near total (90-95%) pancreatectomy is performed to control the secretion of insulin. In these cases hypoglycaemia often persists post-surgery, and the patient is likely to develop diabetes at a later age (Hussain & Aynsley-Green, 2003; Hussain, 2005a).

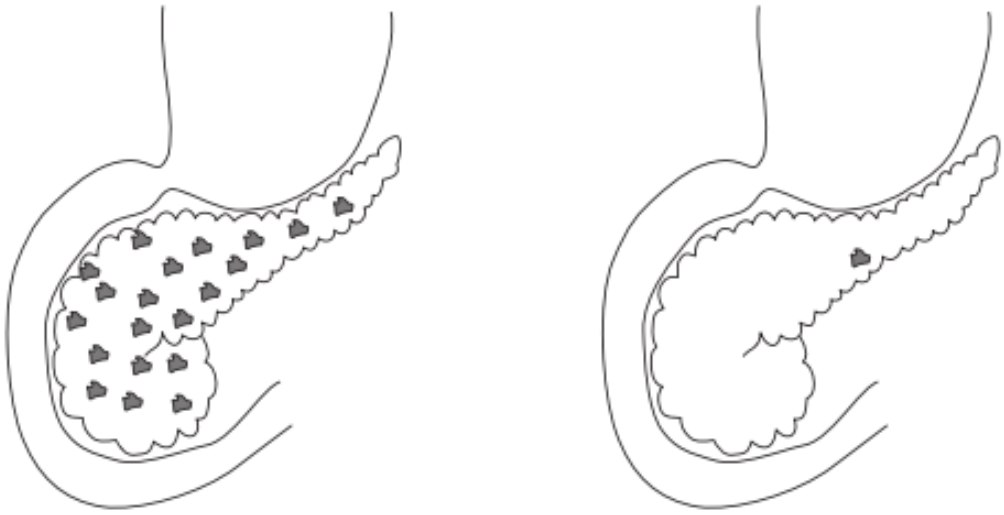

Figure 1.1 Diffuse and focal disease of the pancreas in CHI. Darker blobs represent diseased tissue. In diffuse disease (left) a near total pancreatectomy is required to regulate the secretion of insulin. In focal disease (right), identification and removal of the lesion often results in complete recovery (adapted from Kapoor et al., 2009).

1.3 Ketotic Hypoglycaemia

After CHI, ketotic hypoglycaemia (KH) is the most common cause of hypoglycaemia beyond infancy, and typically first occurs between 18 months and five years of age (Haymond & Pagliara, 1983). Sometimes termed idiopathic hypoglycaemia of childhood, it is a rare condition with an estimated incidence of four per 100,000 (Cohen, Zwiebel, & Jeanmonod, 2015). Ketotic Hypoglycaemia was first described in 1924, but lacked a full characterisation until a study describing characteristics of such patients was published in 1964 (Colle & Ulstrom, 1964; Pollack & Pollack, 1993). Typically, children with KH present with extreme lethargy, protracted vomiting and sometimes seizures (Colle & Ulstrom, 1964). The condition usually resolves spontaneously by nine years of age (Daly, Osterhoudt, & Weinzimer, 2003; Hussain, 2005b).

1.3.1 Pathophysiology

Ketotic Hypoglycaemia has a different pathophysiology to CHI altogether (Figure 2). Although blood glucose levels fall, a healthy response to hypoglycaemia is observed; i.e. a suppression of insulin production and an increase in the production of alternative fuels, namely ketone bodies (Haymond & Pagliara, 1983; Nessa et al., 2012). Children with this disorder are likely to become hypoglycaemic during periods of limited food intake, such as during an intercurrent illness (e.g. an upper respiratory tract infection, gastroenteritis), or after a prolonged period of fasting (Hussain, 2005b; Pollack & Pollack, 1992; Sperling, 2009). This is thought to heighten their vulnerability to hypoglycaemia by limiting the amount of available energy substrate (Daly et al., 2003). However, the exact pathophysiology underlying KH is unclear (Pollack & Pollack, 1993), and it is generally considered a poorly defined diagnosis by exclusion (Hussain, 2005b). It has been proposed that children with KH represent one end of the spectrum of tolerance to fasting (Haymond & Pagliara, 1983; Pollack & Pollack, 1993). Owing to the raised levels of ketone bodies and free fatty acids, which can be used as alternative fuels during episodes of hypoglycaemia in KH (Hawdon, 2015; Rozance & Hay, 2006; Rozenkova, Guemes, Shah, & Hussain, 2015), the risk of hypoglycaemic encephalopathy in this group is thought to be low (Grunt, McGarry, McCollum, & Gould, 1970). However, only a few studies have assessed the neurological outcome of those patients with KH.

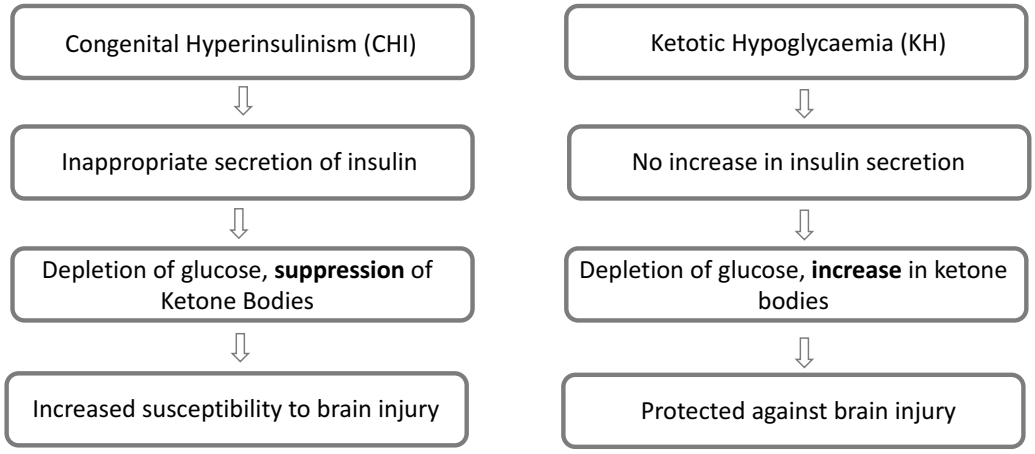

Figure 2. Diagram showing the role of insulin and ketone bodies in patients with CHI and patients with KH

1.4 Outcome: Cognitive and psychomotor functioning

1.4.1 Early, transient episodes of hypoglycaemia: effects on motor and cognitive development

Hypoglycaemia has been shown to have negative and lasting effects on cognition and development, even in patients who experience it for a relatively short time. The incidence of hypoglycaemia is between 10-15% in otherwise healthy babies (Harris, Weston, & Harding, 2012). There have been a number of reports focusing on at-risk infants, who experience transient episodes of hypoglycaemia because of other adversities, such as prematurity or being the infant of a diabetic mother. Reports of developmental delay (in cognitive and motor abilities) are common in this group who experience transient hypoglycaemia.

In a study investigating the relationship between neonatal hypoglycaemia and motor and mental development (measured by the Bayley Scales of Infant Development) at 18

months of age in small for gestational age (SGA) preterm-born infants, those with hypoglycaemia had significantly lower scores than those without hypoglycaemia. In addition, a significant association was observed between developmental scores in both domains and the number of days during which a neonate was hypoglycaemic, with frequent, moderate hypoglycaemia being associated with a greater developmental deficit than more severe but less frequent hypoglycaemia (Lucas, Morley, & Cole, 1988). In a follow-up study, these authors found that there was an association between number of days of hypoglycaemia and reduced motor and arithmetic scores at seven years of age, an indication that this relationship persists past the infancy period (Lucas & Morley, 1999). Duvanel and colleagues (1999) conducted a longitudinal study comparing hypoglycaemic SGA premature children and normoglycaemic SGA premature children. They found that at three-and-a-half years of age, the hypoglycaemic group had significantly lower perceptual and motor skills (although these did not persist at a five-year assessment). Replicating the finding of Lucas et al. (1988), this study found that those who experienced recurrent, mild episodes of neonatal hypoglycaemia had significantly lower scores than those with one documented episode of hypoglycaemia and the difference between these two groups persisted throughout the different time points of assessments, up to five years of age (Duvanel, Fawer, Cotting, Hohlfeld, & Matthieu, 1999).

In a large cohort (n=832) of moderately preterm-born children of pre-school age, neonatal hypoglycaemia was associated with parental reports of developmental delay (Kerstjens, Bocca-Tjeertes, de Winter, Reijneveld, & Bos, 2012). Developmental delay was quantified via a questionnaire measuring development in fine and gross motor

ability, problem solving skills and personal-social functioning. Developmental delay was recorded if the total score (a composite of domain scores) was more than two standard deviations below the mean of the reference population. Neonatal hypoglycaemia was the only co-morbidity found to be significantly associated with later developmental delay and the risk of developmental delay increased as recorded glucose values decreased (Kerstjens et al., 2012).

Adverse effects following transient neonatal hypoglycaemia have been noted even in infants who were born at term. A study of 35 infants who had symptomatic hypoglycaemia in the first week of life, with assessments carried out at a minimum of 18 months after birth, indicated a high proportion of adverse neurodevelopmental outcomes (identified in 26 infants, six of whom had cerebral palsy), with cognitive delay being more common than motor delay (Burns, Rutherford, Boardman, & Cowan, 2008). The authors found that by 18 months one-third of their cohort had developed epilepsy and a further third also had visual abnormalities, including squint, cortical visual impairment and field defect. Motor abnormalities have been reported in other studies investigating this population. In a small sample of eight patients with transient neonatal hyperinsulinism, it was found that all patients (evaluated between two and seven years) exhibited motor clumsiness (Murakami et al., 1999).Therefore both cognitive and motor skills have been shown to be at risk in this population.

Impairments in academic achievements have also been noted in these cohorts. In a large cohort study of 1,395 children who experienced transient hypoglycaemia on the

first day of life, a single episode was significantly related to lower academic proficiency compared to normoglycaemic controls when assessed at ten years of age (Kaiser et al., 2015). While prematurity was a risk factor for adverse outcome, the association existed even when excluding those participants who were very premature, and the association still remained after controlling for a multitude of confounds including gestational age, maternal education and socio-economic status.

In contrast to other studies reviewed in this section, some have found no indication that neonatal hypoglycaemia is associated with developmental adversities. Brand and colleagues (2005) examined infants who experienced transient hypoglycaemia on the first day of life. They assessed 75 children at four years of age using a developmental assessment (the Denver developmental scale), a non-verbal intelligence test (the Snijders-Oomen non-verbal intelligence test) and a parental questionnaire (the Child Behaviour Checklist; CBCL), and compared infants with and without hypoglycaemia. Within each analysis hypoglycaemia was classified according to five separate thresholds. They found no stable difference between the two groups when using these different thresholds. The authors argue that this indicates that transient hypoglycaemia on the first day of life does not carry a risk of adverse neuropsychological sequelae (Brand, Molenaar, Kaaijk, & Wierenga, 2005). This finding was echoed in a study by McKinlay and colleagues (2015), who examined premature infants with hypoglycaemia at a two-year follow up and found no association between the presence of neurosensory impairment, or impairment in executive functions, visual abilities and motor outcome, and hypoglycaemia (McKinlay et al., 2015).

Although some found no association between neonatal hypoglycaemia and poor developmental outcome (Brand et al., 2005; McKinlay et al., 2015), most studies reviewed above point to unfavourable outcome following transient neonatal hypoglycaemia, with some calling for further research into the association between neurological outcome and low blood sugar readings (Marlow, 2013). From these studies, a case can be made for expecting poorer cognitive and motor outcome for hypoglycaemic neonates relative to their normoglycaemic peers. However, in these studies the link between hypoglycaemia and poor outcome cannot be made with confidence, as other factors (such as prematurity) may be the driving force for differences between groups, with children born preterm already predisposed to poorer outcome (McKinlay & Harding, 2015). In addition, the studies reviewed above have focused on transient hypoglycaemia with wide ranging aetiologies, but the outcome may be even poorer when the hypoglycaemia is severe and recurrent, as in CHI.

1.5 Early, severe and recurrent hypoglycaemia: cognitive and motor outcome

Few studies have assessed the cognitive and motor outcome of patients with CHI, and there are fewer still that assess functioning at a later age when deficits in neural systems might become more apparent. Many of these studies do not use standardised assessments that allow judgements of performance relative to normative population means to be made. Standardised assessments also allow reliable comparisons between studies to be drawn, enabling more robust conclusions about outcome. This section

will present a review of the literature regarding the cognitive and motor outcome of patients with CHI and KH.

1.5.1 Early cognitive and motor development in CHI

Studies examining outcome in CHI in late infancy and early childhood do tend to report a high incidence of adverse cognitive outcome and developmental delay, with reports of psychomotor and neurological impairment often reported in about one-quarter to half of patients (Ağladıoğlu et al., 2013; Al-nassar et al., 2006; Harken, Filler, Avruskin, & Crigler, 1971). Table 1.1 gives a brief account of all the studies discussed in the following section.

Developmental delay has been reported frequently in patients with CHI. Ismail and colleagues (2005) reported that 64% of their cohort of 14 patients had developmental delay, with two of these being severely delayed (Ismail & Werther, 2005). Another study found that 18 of 43 patients had developmental delay (Al-nassar et al., 2006). A limitation of both of these studies is that the authors do not state the domains in which delays were present. In a study conducted by Avatapalle and colleagues (2013) that examined outcomes between children with transient-CHI compared to those with persistent-CHI, abnormal neurodevelopment (in speech and language, motor skills and vision) was reported in 39% of children aged between two-and-a-half to five. Mild developmental delay mostly reflected delays in speech and language, and was identified in 12% of participants. Twenty-seven percent of children were classed as

severely impaired, indicating a functional impairment requiring supportive therapy at home and at pre-school. Of these 27%, all showed abnormal development in speech and language and motor skills, while half showed visual impairments (Avatapalle et al., 2013). In both the mild and severe categories, 40-50% of children had a seizure disorder. Importantly, rates of poor outcome did not differ between patients with transient CHI and those with persistent CHI. Examining outcomes of children with CHI up to three years of age, Ludwig and colleagues (2011) also found delayed motor skills and delayed speech development (Ludwig et al., 2011). Taken together, these studies suggest that there is high rate of speech and language and motor delay in patients with CHI.

Retrospective telephone questionnaire-based studies of patients with CHI, which collect historical data about development and school performance, have also found high rates of developmental adversities. In a cohort of 68 patients with CHI, who ranged in age from one to 68 years, developmental delay was recorded in one-third (Steinkrauss et al., 2005). However, this was a retrospective study, and many of the participants would have been adults at the time of interviewing family members about development. This could render the results somewhat unreliable. Another study employing semi-structured interviews found that in 21 patients with CHI, 38% exhibited fine or gross motor problems, while 14% exhibited speech delay. Explicit ages were not stated in this study, but participants were grouped by age categories (e.g. infancy/early childhood, Mazor-Aronovitch et al., 2007).

In contrast to these studies citing high instances of developmental delay and psychomotor impairment, one study found that in children with CHI under one year of age, development of speech and language, fine and gross motor skills, social behaviour and sensory screening was normal in 17 of 18 infants (Kinnala, Rikalainen, Lapinleimu, & Parkkola, 1999). However, at this age range, there is little opportunity to examine various fine aspects of ability. Similarly, another group studied six children aged between one and three years, and found abnormal scores on cognitive, language and motor skills, as measured by the Bayley Scales in only one participant (Levy-Shraga et al., 2013). Again, it could be argued that these patients are too young to draw meaningful conclusions, as subtle abnormalities in functioning might only become apparent at a later age.

Overall, studies examining outcome in infancy and early childhood point toward developmental delay, but there a number of methodological weaknesses that limit the conclusions that can be drawn from them. These include lack of standardised assessments (Avatapalle et al., 2013), failure to state the methods used to assess functioning and the age range of participants (Al-nassar et al., 2006; Mazor-Aronovitch et al., 2007), failure to state the number of participants who were assessed (Ludwig et al., 2011), or being based on parental report. Nevertheless, these studies raise many questions, including whether these suggestions of poor performance at infancy are good predictors of outcome in childhood.

Table 1.1 CHI: outcome in infancy and early childhood

Authors	*Number of participants (n)*	Age of participants (years)	Domains investigated	Tests used	Findings
Harken et al., 1971	3	1-2 years	Neurological function	Neurological examination	Two significantly impaired
Kinnala et al., 1999	18	<1 year	Fine/gross motor, speech, social behaviour, sensory screening, independence	Neurological examination (no standardised assessment)	94% normal functioning
Ismail et al., 2005	14	Not given	Development	Not specified	64% with developmental delay
Steinkrauss et al., 2005	68	1-68	Adaptive behaviour	Questionnaire: SIB-R[1]	33% exhibit developmental delay
Mazor-Aronovitch et al., 2007	21	1-6.5	Hypotonia, fine motor, gross motor, speech	Lab-specific (not standardised)	38% showed fine/gross motor problems
Ludwig et al., 2011	Not given	0-3	Motor and speech	Bayley scales of infant development	% not specified, but trends for motor and speech delay noted
Avatapalle et al., 2013	67	2.5-5	Speech and language, motor skills, vision	Not specified (not formal psychometric assessment)	39% of cohort exhibit either mild or sever abnormal development
Levy-Shagra et al., 2013	6	1-3	Cognitive, language and motor	Bayley scale of infant development	83% normal development

[1] Scales of Independent Behaviour - Revised

1.5.2 Outcome in childhood: cognitive ability and motor skills in CHI

Few studies have assessed the neurological outcome and cognitive performance of older, school-aged, children with a history of CHI. Those that have will now be described. A brief summary of all these studies can be found in Table 1.2.

In one of the first studies to report on outcome in children with CHI, Harken and colleagues (1971) found that out of seven children aged four to 12 years, four had severely impaired function upon neurological examination (Harken et al., 1971). In a large cohort composed of 90 patients with CHI, seven were found to have severe psychomotor impairment (developmental/intelligence quotient [DQ/IQ] <60, major intellectual or motor impairment, attending a special school, and major neurological impairment), and 12 patients were found to have intermediate impairment (DQ<60 / IQ<80, two failures in school or in a special class, two intellectual or motor disorders; Menni et al., 2001). Of note, the authors classed a 'normal' psychomotor profile as having a developmental quotient above 80, and one minor disability. This might be considered to encompass those who, in fact, are impaired. Even when considering this latter group to be normally developing, a high rate of impairment exists in this cohort.

Other studies have also pointed to compromised intelligence quotients and neurological functioning. Neurological abnormalities (defined as brain atrophy, seizures or motor disabilities) or low IQ were reported in ten out of 26 patients with CHI (Cresto, Abdenur, Bergada, & Martino, 1998), with 40% of these children having an IQ of less than 60 (in the

impaired range). In one of the largest cohorts examining outcome after CHI, composed of 114 children (median age 11, mean age 14), an incidence of adverse neurodevelopmental outcome (defined as neurological abnormalities in psychomotor development, and abnormalities in cognitive outcome ascertained via intelligence assessments and degree of schooling) was reported in 44% of cases, with 18% reported as severe. Here, severe neurological impairment was classified as a functional impairment (severe abnormality of tone and movement), but it is not stated how a severe cognitive impairment was classified (Meissner, Wendel, Burgard, Schaetzle, & Mayatepek, 2003).

These studies have examined large numbers of participants and all indications point to significantly compromised neurological functioning. However, much like the studies listed in the previous sections, interpretation is limited because of lack of clarity with regards to methodology, failure to state the age range of participants (Cresto et al., 1998; Meissner et al., 2003), lack of clarity regarding the spectrum of impairment in those who were classed as having adverse neurodevelopment, and failure to state which assessments were used in order to arrive at these conclusions. It is unclear how many participants had only cognitive or neurological impairment, and how many had a combination of the two. While pointing to impairments in general domains gives an idea of overall outcome, it is unclear which neural systems serving specific cognitive functions might be compromised as a consequence of hypoglycaemia.

Standardised parental questionnaires have also been used to assess outcome in older children with CHI; using this method, Steinkrauss and colleagues (2005) found that

one-third of patients who were of school age required special education. This is three times the rate in the general (U.S.) population. In another questionnaire-based study, of 121 patients who were treated surgically for CHI, the proportion of patients obtaining abnormal scores on standardised parental questionnaires regarding abnormal adaptive functioning and day-to-day social and emotional functioning was significantly higher than in the general population. In addition, 18% had documented speech delay, 16% had learning disability, 11% had a physical disability and 10% had a diagnosis of Attention Deficit Hyperactivity Disorder (Lord et al., 2015). Forty-eight percent of this large cohort (n=121, age range 3:50 years) exhibited some sort of behavioural deficit as identified through standardised questionnaires. However, these results should be interpreted with caution, as a high proportion of those assessed had diabetes secondary to pancreatectomy. Thus a causal relationship between low blood sugar and these findings cannot be established. Despite this, the studies reviewed above do indicate a high propensity for adverse cognitive and motor outcome in patients with CHI but it is not clear if this manifests as overall compromised function, or if specific areas of cognition and motor skill are more affected than others.

In one of the few studies to assess specific aspects of cognition and motor functioning with standardised assessments, Rother et al. (2001) investigated outcome in patients with CHI who were school-aged. All eight patients with CHI, who were aged between eight and 15, exhibited attentional impairment, which was significantly more marked than impairment in IQ (which itself was present in half the sample) and impairment in memory scores (visual memory was assessed and found to be significantly impaired relative to intellectual ability in only one patient). This study points to a behavioural

phenotype of impairment in attention in later childhood in children with CHI, as well as providing further evidence for a risk of impaired intellectual function, but it is limited by a small sample size.

A handful of studies have found less severe (or an absence of) adverse effects on later cognition after early episodes of hypoglycaemia (Dacou-Voutetakis, Psychou, & Maniati-Christidis, 1998; Levy-Shraga et al., 2013). Levy-Shagra et al. (2013) found that one of eight patients (aged between three and ten years) performed below the norm in the mental processing scale of the Kauffman assessment battery for children. However, half of the children had significantly lower achievement scores than their 'mental processing' (analogous to IQ) scores. This indicates that subtle cognitive impairment might be present in those with CHI later in life, which in turn might place restrictions on learning. Importantly, parental reports of daily living functioning (including younger children) indicated that 71% of the cohort scored below the normal population. In another study (Dacou-Voutetakis et al., 1998), 13 patients of school age were examined. Outcome was assessed from school progress and social behaviour and no impairment was reported. While academic progress is a good indicator of general functioning, no specific domains of functioning were examined, therefore subtle impairments are likely to be unrecorded.

Table 1.2 CHI: Outcome in later childhood

Authors	*Number of participants* (n)	Age of participants (years)	Domains investigated	Tests used	Findings
Harken et al., 1971	7	4-12	Neurological function	Neurological examination	Four out six severely impaired functioning
Cresto et al., 1998	26	Not given	Intelligence	Briac, Termen, Gesell and WISC[2] test, school performance	10 patients with neurological abnormality and/or low IQ
Dacou-Voutetakis et al., 1998	13	2-20	Academic progress, social behaviour	Review of school record	No impairment detected
Menni et al., 2001	90	1 -15	DQ/IQ, academic achievement, behavioural disorders, neurologic disorders	Not specified	18% with intermediate disability, 8% with a major disability
Rother et al., 2001	8	8-15	Attention, intelligence, Memory, academic achievement, social/emotional functioning	WISC, TOVA[3], WRAML[4], W-J-R[5], CBCL[6]	50% with intellectual deficit. 100% with deficit in attention. 50% with evidence of abnormal emotional/behavioural functioning

[2]Weschler Intelligence Scale for Children
[3] Test of Visual attention
[4] Wide Range Assessment of Memory and Learning
[5] Woodcock-Johnson Revised
[6] Child Behaviour Checklist

Authors	*Number of participants* (n)	Age of participants (years)	Domains investigated	Tests used	Findings
Meissner et al., 2003	114	Range not given	Neurological function and mental outcome	Neurological examination	44% had abnormal neurodevelopment
Steinkrauss et al., 2005	68	1-68	Adaptive behaviour and special education	Questionnaire: SIB-R	34% of patients required special education
Levy -Shagra et al., 2013	8	4-9	Intelligence, achievement, Adaptive behaviour, social/emotional functioning	K-ABC[7] CBCL, Vineland	88% normal
Lord et al., 2015	121	3.5-50.7	Adaptive behaviour, internalising and externalising behaviours, neurobehavioural deficits	Questionnaire: ABAS[8] and CBCL	48% report neurobehavioural problems. Significantly greater proportion of children scoring >2s.d. from the mean compared to normal population

[7] Kaufman Assessment Battery for Children
[8] Adaptive Behaviour Assessment System

1.5.3 Epilepsy secondary to hypoglycaemia

Most studies of outcome after CHI report a relatively high percentage of epilepsy in cohorts, attributed to brain damage caused by hypoglycaemia (Caraballo et al., 2004). Epilepsy is considered to be one of main sequelae of hypoglycaemia, with some arguing that it develops in more than 50% of cases (Fong & Harvey, 2014) and is often related to hypoglycaemic brain damage (Gataullina, Delonlay, Lemaire, & Boddaert, 2014). Typically, epilepsy resulting from hypoglycaemic brain damage is generalised (Kumaran, Kar, Kapoor, & Hussain, 2010). One study found that epilepsy only developed in those who experienced brief hypoglycaemic seizures; in those who did not present with a seizure, epilepsy did not develop (Gataullina et al., 2014). Epilepsy is obviously an important and notable clinical feature of hypoglycaemia. The studies listed above have reported a prevalence of epilepsy in 13-25% of patients (Ağladıoğlu et al., 2013; Avatapalle et al., 2013; Cresto et al., 1998; Lord et al., 2015; Mazor-Aronovitch et al., 2007; Meissner et al., 2003; Menni et al., 2001). Importantly, epilepsy is known to have serious implications for cognitive functioning in a wide range of domains (Berg, 2012). The inclusion of patients with epilepsy in these studies at rates of up to one-quarter of the sample renders it difficult to know what is causing the noted impairments in these participants; the hypoglycaemia itself, the resulting epilepsy, or both.

1.6 Early hypoglycaemia in diabetes: cognition

Children with Early Onset Diabetes Mellitus (EODM) offer a promising parallel population against which to compare children with CHI. Treatment for EODM may result in over-administration of insulin, resulting in hypoglycaemia with a pathophysiology mirroring the mechanism of children with CHI. Many studies report adverse outcome in those who develop diabetes at a young age and attribute this to early episodes of hypoglycaemia (Lin, Northam, Rankins, Werther, & Cameron, 2010; Northam et al., 2001; Warren & Frier, 2005).

Hypoglycaemia is more frequent and harder to control in very young children compared to those with late onset diabetes (Spinks & Mann, 2013). There is a concern that episodes of hypoglycaemia in EODM could result in cognitive dysfunction and many groups have examined the outcome of children with diabetes who have experienced early episodes of hypoglycaemia. Here, early onset diabetes accompanied by episodes of severe hypoglycaemia has been associated with reduced IQ (Ack, Miller, & Weil, 1961; Northam et al., 2001; Rovet, Ehrlich, & Hoppe, 1987), poorer scores on tests of motor skills and visual-motor integration (Hershey, Bhargava, Sadler, White, & Craft, 1999; Rovet & Ehrlich, 1999; Rovet et al., 1987), psychomotor functioning (Bjørgaas, Gimse, Vik, & Sand, 1997) and tests of attention, working memory and processing speed (Bjørgaas et al., 1997; Hannonen, Tupola, Ahonen, & Riikonen, 2003; Northam et al., 2001;Rovet & Ehrlich, 1999). These findings chime well with the results of the (limited) studies that have examined later outcome in CHI.

Significantly lower memory scores have also been found in children with diabetes who experience hypoglycaemic seizures compared to those who do not (Hershey et al., 1999; Kaufman, Epport, Engilman, & Halvorson, 1999), in keeping with the proposed vulnerability of neurons in the hippocampus to an abundance of insulin (Auer, 1986). Rovet and Ehrlich (1999) found that hypoglycaemic seizures in EODM (but not late onset diabetes) resulted in lower scores on assessments of memory.

Hence, evidence suggests that in the context of diabetes, episodes of hypoglycaemia are associated with neurocognitive difficulties, especially in the domains of attention, memory and fine motor skills, but also in general intellectual abilities. However, any comparisons between patients with EODM and CHI are compromised by the fact that in EODM, episodes of hypoglycaemia are unlikely to occur at a very early age (in contrast to patients with CHI) and hypoglycaemia is occurring in an environment where functioning may already be compromised due to existing disease and where cognitive adversities cannot be attributed to a single cause. However, from these studies it is important to note that cognitive functions appear to be compromised in children who experience hypoglycaemia, even past the neonatal period, which suggests extended vulnerability to such insult. The brain undergoes a protracted period of functional development from the neonatal period to adolescence, with increasing metabolic demands (Chugani, 1998); this process of development is likely to render the brain especially sensitive to an environment lacking in glucose at various points throughout development.

1.7 Ketotic hypoglycaemia: early and late cognitive and motor outcome

Owing to the presence of ketone bodies during episodes of hypoglycaemia, children with ketotic hypoglycaemia (KH) are considered protected from the adverse effects of low blood sugar. Accordingly, studies investigating cognitive outcome in this group are sparse. In the first study to investigate cognition in patients with KH, Colle and Ulstrom (1964) reported on the developmental status of eight patients. They found that two patients appeared to have compromised functioning, although it is unclear how cognitive functioning was assessed. From these results, the authors concluded that in order to make a diagnosis of KH, among other medical factors 'the children should be normal or near normal in intelligence'. By this token, a psychomotor impairment would satisfy an exclusion for a diagnosis of KH, which is echoed in the diagnostic criteria of KH outlined by Pershad and colleagues (Pershad, Monroe, & Atchison, 1998).

Another study following a series of 24 children with idiopathic hypoglycaemia (14 of them with KH) found that in children with KH (between the ages of one and 13 years), low IQ (below 90) was found in six, and abnormal EEG recordings (at rest) were observed in five. Thus more than one-third of the sample either had low IQ or abnormal EEG - although the overlap between these two variables was not specified (Kogut, Blaskovics, & Donnell, 1969). Importantly, this is the only published study to employ standardised assessments, and the findings contend the criteria of diagnosis outlined by Colle and Ulstrom (1964) and Pershad et al. (1998).

Further evidence of adversities following KH is found in a study by Grunt et al (1970), who found that out of eight children between the ages of 19-64 months, three showed specific signs of Central Nervous System (CNS) dysfunction, while two showed mild developmental delay. All five cases showed some form of motor delay or impairment, as well as a number of other impairments (in attention, language or number skills). Despite this, all children performed within the average range on tests of intellectual functioning (Grunt et al., 1970). However, in a larger series, Daly et al. (2003) found that only two out of 24 children with KH between the ages of five months and seven years showed signs of developmental delay.

Thus, while perhaps less pronounced than the impairments seen in patients with CHI, there is some evidence to suggest a degree of restriction in functioning in those with KH. This is, however, an under-researched cohort, possibly because of the assumption that ketone bodies are available as an alternative fuel during episodes of hypoglycaemia. There are a limited number of studies that allow predictions about outcome after KH, with a lack of consensus between the results of the studies that are published. In addition, to the best of the author's knowledge, no published studies have examined incidences of neuropathology in KH.

1.8 Neuroimaging studies

Generally, the studies reviewed so far indicate that cognitive and motor impairment are common after early episodes of hypoglycaemia. The next question that arises is what are the brain-based origins of these impairments. Although not well

characterised, the vulnerability of the developing brain to hypoglycaemic insult is widely accepted. The threshold for cell death secondary to hypoglycaemia is not universal and varies amongst different populations of neurons (Auer, 1986) . This section will briefly review mechanisms of damage associated with hypoglycaemia and will then review the findings of imaging studies conducted in neonates and children.

1.8.1 Mechanism of damage

In the maturing brain glucose not only plays the role of the primary fuel. It is also necessary for normal biosynthetic processes to proceed. Therefore, brain damage after hypoglycaemia is not only a result of energy failure, but involves multiple processes, such as increased levels of the excitatory amino acids aspartate and glutamate (Auer, 2004; Vannucci & Vannucci, 2000). This release of excitotoxins is accompanied by large fluxes in calcium, causing leaks and breaks in the cell membrane, leading to necrosis. The increase in calcium is in part mediated by glutamate receptors, in particular those that are gated by N-methyl-D-aspartate (NMDA). Thus, regions that are densely populated with NMDA receptors (such as the hippocampus and thalamus) may be particularly vulnerable to hypoglycaemia (Auer & Siesjö, 1993).

1.8.2 Neonatal imaging studies

There are a number of imaging studies investigating early effects of hypoglycaemia on the brain during the neonatal period. Most of these report a primarily occipital pattern of damage that affects both grey and white matter in the occipital and parietal lobes

(Alkalay, Flores-Sarnat, Sarnat, Moser, & Simmons, 2005; Aslan & Dinc, 1997; Barkovich, Ali, Rowley, & Bass, 1998; Filan, Inder, Cameron, Kean, & Hunt, 2006; Kinnala et al., 1999; Murakami et al., 1999; Spar, Lewine, & Orrison, 1994; Tam, Widjaja, Blaser, Macgregor, & Moore, 2010; Traill, Squier, & Anslow, 1998). Indeed, a study retrospectively examining the cause of occipital lobe injury in a cohort of 21 infants found that perinatal hypoglycaemia was the most common cause (74%) of injury (Wang, Mb, Hou, Ma, & Feng, 2012). Thus, there is an abundance of evidence to suggest that early hypoglycaemic episodes particularly affect posterior regions of the brain. However, Burns et al. (2008) reported a much more varied pattern of brain damage. Using Magnetic Resonance Imaging (MRI) they examined the cortex, white matter and basal ganglia, employing a grading system to highlight extent of damage. Abnormalities in white matter ranged from high signal intensity to infarction, while abnormalities in grey matter were defined as loss of cortical markings or high signal intensity. Only two patients were found to have normal MRI findings; 94% of the cohort of 36 had white matter abnormalities (80% of these were classified as moderate or severe). Contrary to the predominantly posterior pattern of brain damage cited in other studies, they found that 40% of cases showed global involvement, 18% showed posterior changes only, and 36% showed periventricular changes. Forty percent of these showed abnormalities to the basal ganglia and thalamus, although it is not clear how this was defined. Cortical abnormalities in the form of cortical highlighting were identified in more than half of the cases. Clearly, the structures affected by hypoglycaemia are diverse, although specific damage to subcortical structures (the basal ganglia, thalamus and hippocampus) has been noted in other studies (Barkovich et al., 1998; Kinnala et al., 1999).

While the above studies appear to characterise a pattern of injury in association with hypoglycaemia, they have some shortcomings, namely that many of the patient cohorts are made up of patients with diverse aetiologies or the inclusion of patients with comorbid disorders. This includes patients with hypoxia-ischaemia, which is deleterious to the brain, especially in the context of hypoglycaemia (Chalmers et al., 1991). The cases mentioned here were neonates who experienced episodes of hypoglycaemia for a wide range of reasons, with cohorts including those with transient hyperinsulinism, transient neonatal hypoglycaemia and those with CHI. Importantly, none of these studies have focused exclusively on those with CHI, who may manifest a much more compromised imaging profile owing to the lack of alternative fuels available for energy metabolism. The question arises as to whether the changes observed in these cohorts persist past the early years, and if a more severe profile is noted after severe and recurrent hypoglycaemia. Indeed, one study reported a reversal of the damage that was observed in the period immediately after the hypoglycaemic event when scans were repeated at an older age (Kinnala et al., 1999). This could be due to pseudo-normalisation; an apparent return to healthy brain tissue which does not reflect the underlying integrity of the tissue (Tam et al., 2010).

1.8.3 Childhood imaging studies

A limited number of studies have focused on identifying the lasting effects of early hypoglycaemic insult in older children. These studies, detailed below, frequently report white matter damage alongside lesions to the thalamus and basal ganglia.

In a cohort of 50 patients who experienced hypoglycaemia as neonates (including patients with CHI, glycogen storage disease and fatty acid oxygenation defects), lesions were identified in 56% on MRI. The most common type of brain lesions were those affecting the white matter in the parieto-occipital area, followed by lesions to the basal ganglia, thalamus and brainstem. Finally, a group of patients with lesions in temporo-parietal areas (with extension into the hippocampus) were identified (Gataullina et al., 2013). Importantly, the authors conclude here that the site of damage corresponds to the age at which hypoglycaemia occurred. They found that hypoglycaemia occurring before the age of six months resulted in posterior white matter lesions, hypoglycaemia occurring between six and 22 months resulted in basal ganglia lesions, and hypoglycaemia occurring after 22 months resulted in lesions to the parietotemporal cortex. This patient cohort was made up of patients who experienced hypoglycaemia due to hyperinsulinism, fatty acid ß-oxidation defect or glycogen storage disease (Type 1). Therefore, generalisations about the specificity of damage pertaining to hyperinsulinism cannot be made. However, the authors note that all patients with hyperinsulinism who had abnormal MRI scans showed white matter lesions to posterior white matter. The involvement of posterior white matter lesions as well as basal ganglia involvement in some patients is consistent with the neuroimaging profile of infants that has been reviewed above. However, there was a wide age range (one month to five years) of participants included in this study, which makes conclusions about the lasting damage of hypoglycaemia difficult to draw.

Avatapalle et al. (2013) studied a more homogenous subset of patients, examining MRI scans of 15 patients with hyperinsulinism aged between two-and-a-half to five years.

Visual inspection of these scans showed changes to the brain in 73% of cases, in the form of infarcts, high signal intensities, gliosis to parietal and occipital lobes and basal ganglia involvement, as well as white matter atrophy and periventricular leukomalacia. However, only patients with abnormal motor development, severe cognitive dysfunction and seizures were scanned and these included patients with a history of hypoxia-ischaemia. This introduces a level of bias into this cohort, only capturing the most severe end of the spectrum. In general, the inclusion of patients with comorbidities reduces the ability of these studies to draw conclusions about brain injury associated specifically with hypoglycaemia. Kara and colleagues (2007) reported a case study of a six-year-old child with CHI, visually assessing T1 and T2-weighted Magnetic Resonance Imaging (MRI) scans. They reported global atrophy, bilateral hyperintensity in the putamen and atrophy of the cerebellum (Kara, Aydin, Aslan, & Gürer, 2007).

From the studies reviewed in these two sections, it is clear that a significant degree of pathology after hypoglycaemia is evident, with almost all studies citing white matter lesions or atrophy. This is reported even in those who experience hypoglycaemia for a short time, i.e. when hypoglycaemia is not persistent. Table 1.3 gives a short account of the studies reviewed above.

As a marker of underlying neuropathology, studies have found reduced head growth or reduced head circumference in children with a history of early hypoglycaemia. Burns et al. (2008) found that nine of 35 infants had significantly reduced head circumferences compared to the normal population. They conclude that this is

consistent with the high rate of white matter injury seen in their cohort. In their study of hypoglycaemic premature neonates, Duvanel et al. (1999) found significantly reduced head circumference at 12 months, 18 months and five years. Another study examining outcome in children with CHI found reduced head circumference in their cohort which correlated with neurological outcome; children with neurological abnormalities showed poorer head growth (Cresto et al., 1998). Caraballo et al. (2004) found microcephaly present in 40% of their cohort of 15 patients with epilepsy secondary to hypoglycaemia. Head circumference is thought to correlate with brain weight, volume, and the amount of myelin (Duvanel et al., 1999); thus smaller head circumference and slowed head growth is likely to indicate brain pathology.

Table 1.3 Imaging studies. CT = Computed Tomography, MR = Magnetic Resonance

Author	*n*	Age range	Aetiology	Imaging method	Regions affected and % of patients exhibiting damage
Traill et al., 1998	2	<1 week	Transient neonatal hypoglycaemic	CT	Both showed reduced cortical and white matter density in parietal and occipital lobes
Murakami et al., 1999	8	9 months-8 years	Low birth weight, placental dysfunction, delay in oral intake	MR	75% high intensity in periventricular deep white matter in parieto-occipital region. 63% dilation of lateral ventricles 50% focal atrophy in cortex of occipital lobe
Aslan & Dinc, 1997	1	10 months	Not specified	MR	Ventricular dilation of occipital horns
Barkovitch et al., 1998	5	<1 month	SGA[9], CHI	MR	80% damage in parietal and occipital lobes (grey and white matter). 20% frontal lobe and globus pallidus.
Kinnala et al., 1999	18	<1 month	Not specified	MR	33% showed abnormal scans, with 22% either in the parieto-occipital white matter or thalamus. In 16% damage evident at later scanning
Alkalay et al., 2005	1	11 months	Unknown	CT	Atrophy of occipital lobes, dilated lateral ventricles
Filan et al., 2006	4	<1 week	Transient hyperinsulinism in 3 cases, 1 case unknown	MR	100% occipital damage, in 75% of cases damage also evident in corpus callosum and optic radiation
Yeon et al., 2006	2	10 months	Unknown	MR	Atrophy of parieto-occipital region and splenium of corpus callosum

[9] Small for gestational age

Author	*n*	Age range	Aetiology	Type of imaging	Regions affected and % of patients exhibiting damage
Kara et al., 2007	1	6 years	CHI	MR	Atrophy of cerebellum, as well as global atrophy, hyperintensity in putamen
Burns et al., 2008	36	1-42 days	IUGR[10], polythemia, IDM[11], HI, KH, other metabolic abnormalities	MR	94% White matter injury (Majority global or periventricular) 51% cortical abnormality 40% Basal ganglia/thalamic damage
Tam et al., 2010	25	1 week	HIE[12], IUGR, IDM	MR	32% restricted diffusion in occipital lobes (identified through DWI)
Gataullina et al., 2013	50	1 month-5 years	FAOD[13], CHI, GSD[14]	MR	38% white matter lesions, in posterior and frontal regions 10% basal ganglia lesions 10% grey matter damage in temporo-parietal/hippocampal regions
Avatapelle et al., 2013	15	2.5-5 years	CHI	MR	73% showed significant changes in white matter and basal ganglia, specific proportions not given

[10] Intrauterine growth restriction
[11] Infant of diabetic mother
[12] Hypoxic-ischaemic encephalopathy
[13] Fatty acid oxygenation defect
[14] Glycogen storage disease

In summary, reliable reports on the long-term consequences of hypoglycaemia on the developing brain and the cognitive correlates of such insult are lacking. Generally, there has been a dearth of high quality studies in this field; Boluyt and colleagues (2006) noted that out of 18 studies documenting outcome after hypoglycaemia experienced in the first week of life, only two were considered high quality (notably, neither of these included studies focusing on patients with CHI). Cohorts are often made up of diverse patient groups and include cases with comorbid conditions that are known to have adverse effects on the immature brain. It is therefore difficult to discern a pattern of brain pathology that is specific to hypoglycaemia, and to relate this putative pattern to cognitive and behavioural dysfunction. Indeed, few studies have related evidence of brain damage to behavioural function in the early years (Burns et al., 2008) and none have done so during later childhood. A limited number of studies (two published) have used standardised assessments of cognitive and motor functioning in children with CHI, and in those that have, sample sizes are too small to draw meaningful inferences. Furthermore, no published studies have performed quantitative neuroimaging analyses to ascertain and statistically verify a pattern of brain pathology specific to hypoglycaemia. There is a need to examine the effects of hypoglycaemia on school-aged children who are free from comorbidities, and where disruption to neural systems serving specific functions becomes more apparent (Boardman, Wusthoff, & Cowan, 2013).

1.9 Current research

1.9.1 The HBI study

This thesis is a follow-up study of a previous study, the Hypoglycaemia and Brain Injury (*HBI)* study. The HBI study was conducted by Anitha Kumaran (2012) and was a comparative study of neuroimaging and cognitive outcomes of children with CHI and KH. Owing to the protection of ketone bodies, children with KH were used as a healthy control group against to which to compare patients with CHI. Imaging and neuropsychological data from 21 children with CHI (including those with epilepsy) and 14 children with KH were analysed. Standardised age-appropriate neuropsychological tests assessing intelligence, academic attainment, memory, attention and motor skills were administered. It was hypothesised that children with CHI would be considerably more impaired than their KH counterparts across all domains of cognition that were assessed and that the abilities of children with KH would not differ from the standard population means on tests of cognition. Accordingly, it was hypothesised that neuroradiological findings in children with KH would be unremarkable, while abnormalities were expected in patients with CHI. Analysis of behavioural data showed that patients with CHI were impaired on measures of intelligence, academic attainment and memory in relation to standard population means and that they performed significantly below patients with KH in these domains. They also performed significantly below patients with KH on a measure of fine motor skills, although gross motor coordination and balance did not differ between groups. In contrast to the hypotheses, both patient groups were impaired on tests of attention, with no significant differences between patient groups in this domain.

Similarly, visual inspection of MRI scans showed a high incidence of abnormality in both the CHI (38%) and KH (36%) groups. Focal white matter lesions were evident in 14% of patients in both groups, with diffuse lesions present in 33% of those with CHI and 29% of those with KH. Despite the similarity of white matter lesions, A Tract-Based Spatial Statistics (TBSS) analysis (a quantitative neuroimaging analysis sensitive to detecting microstructural white matter changes), demonstrated focal differences in the corpus callosum in patients with CHI relative to those with KH. Hippocampal atrophy was detected in both groups, although more frequently in those with CHI.

This study has suggested that patients with KH are not free from neuropathology and cognitive adversity following hypoglycaemia. They showed a degree of impairment in some aspects of attention, as well as incidences of white matter abnormalities comparable to those seen in patients with CHI. However, aside from performance on tests of attention, patients with KH outperformed patients with CHI on every measure of functioning.

The lack of a typically-developing control group against which to compare both patient groups makes it hard to draw conclusions about imaging abnormalities and this is compounded further by the fact that patients with epilepsy were included in the CHI cohort. The inclusion of patients with epilepsy makes it difficult to attribute conclusions about the diversity of cognitive difficulties and the neuroimaging profile in the CHI group to the effects of hypoglycaemia alone, as epilepsy is known to have a

deleterious effect on many aspects of cognition (Berg, 2012) and is associated with structural changes in the brain (Bernasconi et al., 2004; Cross, 2015).

1.9.2 Aims of this thesis

This thesis aimed first to determine the long-term effects of hypoglycaemia as a result of CHI and KH (in the absence of any other compromising condition) on cognitive and motor outcome later in childhood. The results of this investigation will be presented in Chapter Three. The lasting effects of hypoglycaemia on the brain are determined using quantitative imaging methods, comparing healthy controls, patients with CHI and patients with KH. Chapter Four describes differences in grey and white matter volume as seen on T1-weighted MRI scans, found using a whole brain analysis, while Chapter Five describes the volume of subcortical structures, quantified via manual and automatic segmentation. Chapter Six describes the integrity of white matter tracts throughout the brain, using diffusion scans. Finally, Chapter Seven explores relationships between brain pathology and function, attempting to uncover the contribution of structural abnormalities to cognitive and motor outcome in patients with CHI and KH.

Through comparison of these two patient groups to typically developing children the lasting effects of early and severe hypoglycaemia are discovered. In addition, comparing the neuroimaging and cognitive profiles of those with CHI and those with KH, in the absence of any other neurological condition, allows interpretation regarding the neuroprotective role of ketone bodies during hypoglycaemia. Based on previous studies, it is expected that children with CHI will show significant restrictions in

neuropsychological functioning and will also show damage to white matter and possibly to subcortical structures. The lack of imaging studies in children with KH makes it difficult to hypothesise whether they will show brain pathology, but, in accordance with the HBI study mentioned previously, it is expected that they will show a degree of compromised cognitive functioning, specific to the domain of executive functioning.

Chapter 2

Cohort Descriptives

2 Cohort Descriptives

This chapter provides an overview of participant selection and recruitment. This thesis combines data from previous studies along with data collected specifically for this doctoral research. The data is presented in two cohorts - a 'large cohort' and a 'small cohort'. For patient data, the large cohort is composed of all patient data from previous studies and from the author's doctoral research. The small cohort is composed solely of data gathered by the author for this thesis. This chapter describes in depth the cohorts whose data are reported in the thesis and gives details of the subgroups that contribute to each experimental chapter.

2.1 Recruitment

2.1.1 Patients

Patient data from two studies are reported here. One study was completed before this doctoral research began (Hypoglycaemia and Brain Injury '*HBI',* conducted by Anitha Kumaran), and the other was conducted by the author of this thesis for this doctoral research project (Cognitive Outcome and Neuropathology after Hypoglycaemia '*CNH'*, conducted by the author of this thesis). All patients who took part in the HBI study and who met the inclusion criteria (see Box 2.0; n=30) were invited to participate in the CNH study by returning to Great Ormond Street for a repeat neuropsychological assessment and MRI scan. Invitations were made by post and follow-up telephone calls. Of the eligible patients who were seen as part of the HBI study, four patients with CHI and six patients with KH returned for repeat assessments. Cognitive and behavioural data acquired as part of the CNH study is used

in this thesis. Twelve patients with CHI and eight patients with KH did not return; their original data are included in the behavioural and neuroimaging analyses reported in the following chapters. These patients make up a proportion of what is hereon referred to as the 'large cohort' of patients.

New patients (i.e. those who had not previously taken part in the HBI study) were identified through screening of metabolic outpatient and patient discharge lists. Invitations were sent by post and followed-up with telephone calls. Thirty-seven patients with CHI and 19 patients with KH were identified. Figure 2.1 shows recruitment and assessment pathways for patients, both from the HBI study and the CNH study.

Exclusion criteria
Born before 33 weeks gestation
Known neurological disorder (e.g. epilepsy)
Diabetes (developed after pancreatectomy)
Ongoing medical conditions (e.g. cardiac problems) other than hypoglycaemia
Hypoxia

Box 2.0 Exclusion criteria for patients in the CNH study, also applied to non-returning patients who took part in the HBI study.

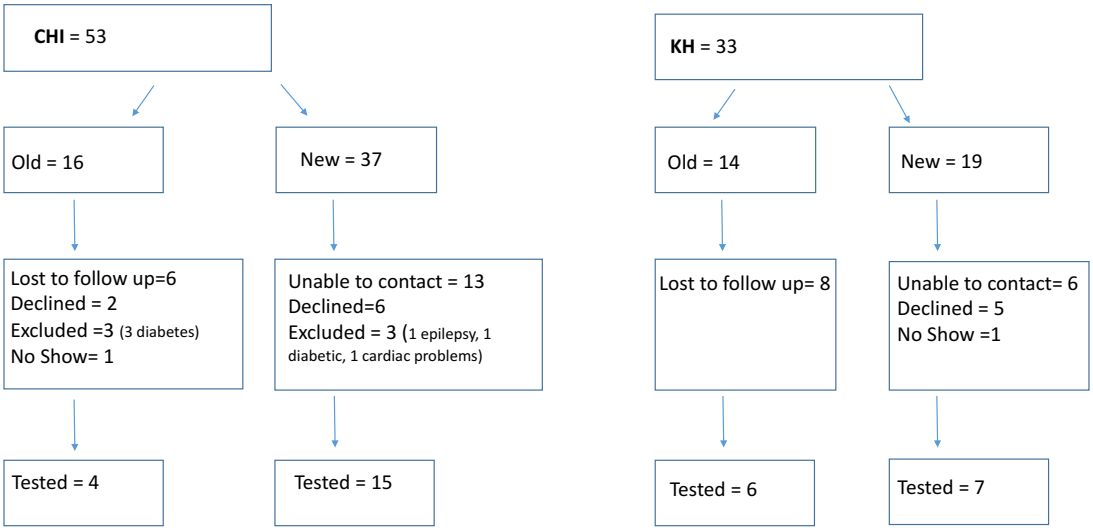

Figure 2.1 Identification and recruitment of patients in the CNH study.

2.1.2 Healthy controls

Data from typically developing children were available from two separate studies, one investigating cognitive outcome of patients who had suffered a cardiac arrest in infancy or early childhood (Cardiac Arrest (CA) study, n=9), and another investigating cognitive outcome associated with prolonged febrile seizures (STEPSOUT, n=17). A third group of healthy controls was specifically recruited for the current CNH study (n=18). Recruitment of controls for the current CNH study was achieved through advertisement in schools, with further invitations extended to the healthy siblings of recruited patients. Table 2.1 gives the age and gender of the participants described above, as well as relevant medical variables for patients that will be discussed in the next section (see Section 2.2; patient characteristics)

Table 2.1 Details of Age, Gender, Gestational Age, Birth Weight and Age at Symptom Onset of the CHI and KH Cohorts; also shown are details of Age and Gender of the Controls.

CHI (n=31)	Range	Mean (SD) / Median
Age (years)	8.1-16.7	10.9 (2.5)
Gender		20 male
Gestational Age (weeks)	33.5-42	40
Birthweight (kg)	1.5-6.1	3.38 (0.99)
Age symptomatic (days)	1-589	1
KH (n=21)		
Age (years)	6-14.8	10.2 (2.6)
Gender		12 male
Gestational Age (weeks)	36-42	40
Birthweight (kg)	1.85-4.6	3.37 (0.64)
Age symptomatic (days)	1-1860	713
Controls (n=44)		
Age (years)	6.3-17.6	11.6 (2.58)
Gender		16 male

2.2 Patient characteristics

2.2.1 Medical variables

Of the 31 participants with CHI, 19 presented with symptoms and were classified as hypoglycaemic within the first day of life. Four patients presented with symptoms between four days and two weeks of life. The remaining eight patients presented between three and 19 months of life. Three of the 31 patients were from multiple births and had intrauterine growth restriction (IUGR). Seven patients were born before 38 weeks gestation.

Eighteen of the patients with KH presented before the age of three. Four presented with symptoms of hypoglycaemia within the first 48 hours of life, while seven presented between 12 to 23 months. The remaining seven presented between 25 to 36 months. The remaining patients (n=3) showed symptoms after three years of age. One patient was from a multiple birth and had IUGR. Two patients were born before 38 weeks gestation. Of the medical variables detailed in Table 2.1 (age at symptom presentation, gestational age, and birthweight) plus IUGR, only the mean age at symptom presentation was significantly different between groups (Mann-Whitney U test, $z=4.6$, $p<0.001$). This showed that patients with CHI typically presented with symptoms of hypoglycaemia at a younger age than those with KH. This is expected given the differing aetiologies of hypoglycaemia.

Forty-five percent of patients with CHI and 52% of patients with KH experienced seizures due to hypoglycaemia (either as an initial presenting symptom or subsequent

to initial diagnosis). Importantly, none of the patients whose data are included in this thesis were diagnosed with epilepsy. In 52% of patients with CHI, hypoglycaemia was ongoing and continued medical management was required. Fifty-seven percent of patients with KH were still experiencing episodes of hypoglycaemia at the time of testing. The rates of seizures as a symptom of hypoglycaemia and disease status (e.g. ongoing or resolved) did not differ between groups (X^2 test p=0.61 and p=0.7, respectively). Twelve patients with CHI had known genetic mutations causing abnormal pancreatic secretion of insulin. Eight had genetic mutations in ABC88, two in KCNJ11, one in SCHADD and one in GLUD1. There were no known genetic causes of hypoglycaemia in the KH group. These characteristics are detailed in Figure 2.2.

2.2.2 Medical management of hypoglycaemia

Ten patients with CHI underwent a near or sub-total pancreatectomy to manage their hypoglycaemia. The age at which the pancreatectomies were performed ranged from one month to four years of age. The remaining 21 patients were, or are currently, medically managed with Diazoxide, Octreotide or Langreotide. For patients with KH, a 'HYPOSTOP' protocol was designed for each child detailing actions to take during episodes of hypoglycaemia; this normally involved giving Maxijul (a high carbohydrate formula).

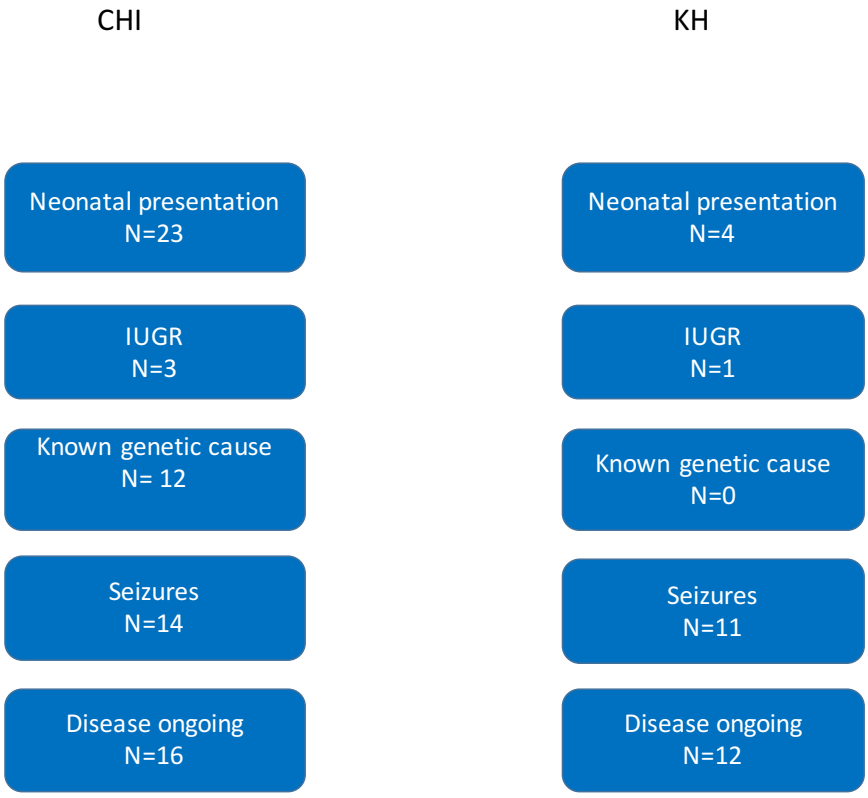

Figure 2.2 Medical characteristics of the patient groups.

2.2.3 Educational support

At the time of assessment all children were attending mainstream school. A number of children in each patient group had been, or were currently, in receipt of learning support in the classroom. Thirty-eight percent of children with KH and 26% of children with CHI required supported learning in the classroom. The level of support ranged from a full statement of special educational needs to support in specific subjects. There were no significant differences in the number of children receiving learning support between patient groups (x^2=1.26, p=0.26).

2.2.4 Socio-economic status

To compare the socio-economic status of participants, English Indices of Deprivation scores were acquired using postcodes that define Lower-layer Super Output Areas (LSOAs). LSOAs are small areas of similar population sizes with approximately 650 households. The indices of deprivation (https://www.gov.uk/government/statistics/english-indices-of-deprivation-2015) provide measures of deprivation in England according to LSOAs. These indices are a compendium of variables indexing levels of deprivation from assessments of income, employment, education, health, crime, housing, services, and living environment. For each participant an index of multiple deprivation (IMD) rank and decile was calculated using data provided by the indices of deprivation. A small area with a rank of 1 is considered the most deprived, whereas a small area with a rank of 32844 is the least deprived. Similarly, LSOAs that fall in the first decile are within the 10% highest deprived areas in Britain. For each LSOA, an Income Deprivation Affecting Children Index (IDACI) that measures the proportion of children living in income-deprived families is also provided. The rank and decile scales are the same as detailed for the LSOAs.

Indices of deprivation were calculated for all patients where postcodes were available. Table 2.2 describes the mean rank and scores for both IMD rank and deciles, and IDACI rank and deciles. There were no significant differences between groups on these measures of socioeconomic status.

Table 2.2. Mean estimates of SES based on postcode for healthy controls, patients with CHI and patients with KH. *Data missing for 12 healthy controls (STEPSOUT cohort) **Data only available for patients living in England

Group	IMD rank (SD)	IMD Decile (SD)	IDACI rank (SD)	IDACI decile (SD)
Control (n= 32)*	16701 (8834)	5.7 (2.7)	16203 (10475)	5.4 (3.1)
CHI (n=29)**	16507 (8393)	5.4 (2.5)	1744 (9052)	5.7 (2.8)
KH (n=21)	19666 (10091)	6.5 (3)	17978 (9338)	6 (2.7)

2.3 Division of dataset

2.3.1 Small and large cohort

Within the experimental chapters of this thesis (Chapters Three, Four, Five, Six and Seven), reference is made to a 'large cohort' and a 'small cohort'. The 'large cohort' is composed of all patient data from the previous HBI study and from the current CNH study. The 'large cohort' of control data also includes data from healthy controls from the CA, STEPSOUT, and the CNH study (see Table 2.1). The small cohort is composed of all data collected for the current CNH study.

The large patient cohort includes cognitive and behavioural data that was obtained from children recruited under the HBI study, and children recruited for the CNH study. The 'small cohort' is exclusive to patient and control data collected by the author for the CNH study. The small patient cohort had these same tests administered, but additional tests were added to the protocol in order to address the aims described in in Chapter Three (to elucidate impairment in executive functions and motor skills). In order to ensure reliability of results, particularly those relating to brain structure-

function relationships, in each imaging chapter (Chapters Four, Five, and Six), analyses were performed first on the neuroimaging data of the large cohort, and subsequently on the data set of the small cohort. Figure 2.3 provides a graphical representation of participants included in the large and small cohorts.

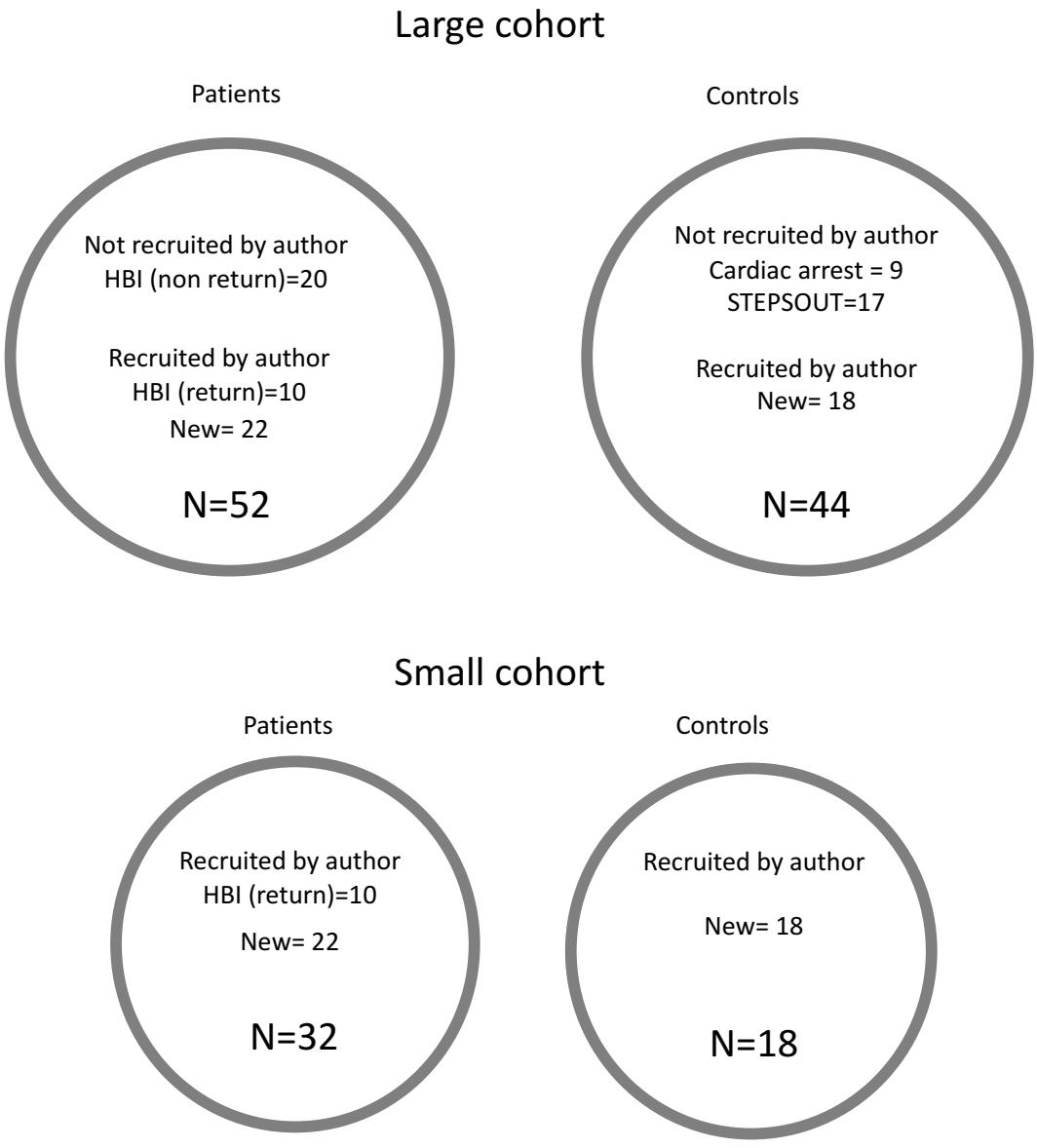

Figure 2.3 Description of composition of large and small cohorts.

2.3.2 Age and gender differences within the large and small cohorts

Within the large cohort, there was a significant difference in the proportion of males to females between patients with CHI and healthy controls (x^2=6.35, p=0.012) with more

males in the patient group compared to controls. There were no significant differences in gender between patients with KH and healthy controls (x^2=2.5, p=0.144), and the two patient groups did not differ from each other in the proportion of males to females (x^2=0.288, p=0.592). Groups did not differ in age (F=2.329, p=0.1). Within the small cohort no significant differences existed in the proportion of males to females in any of the groups, nor were there any significant age differences between groups.

2.3.3 Cohorts included in each chapter

The following section briefly outlines the cohorts whose data are included in each chapter; details of the number, ages and gender of participants included within each analysis can be found in the relevant chapter.

Chapter Three includes patient data from both the large and small cohorts. These are compared to control data from the small cohort, and, separately, to standard population means where these are available. Chapters Four (Voxel-based Morphometry) and Six (Tract-based Spatial Statistics) include analyses on both the large and small cohorts of participants as outlined in Figure 2.3. Chapter Five (Subcortical Volumes) differs slightly in that the analysis of manually-measured hippocampal volumes is conducted on the data from the small cohort of controls (Figure 2.3) as well as data acquired from a large control group (n = 64 from a database collected for an MRC study *Hypoxia/ischaemia in children: Patterns of neuropathology and associated memory impairment*), for whom hippocampal volume measurements by the same researcher (Prof. David Gadian) were available. Data from the large cohort of patients were compared to this combined control group (MRC controls + small

cohort controls, n=82), and this analysis was repeated in the small cohort of patients and controls. For the analysis of automatically segmented volumes of the basal ganglia and thalamus, analyses on both the large and small cohorts as outlined in Figure 2.3 was performed. Chapter Seven (structure-function relationships) analyses patient data only, with analyses performed in both the large and small cohorts.

2.4 Summary

In this section relevant medical and socioeconomic variables of both patient groups and the control cohort have been described. Patients with CHI are symptomatic at a significantly earlier age than patients with KH, which is expected given the aetiology of the disease. The only other significant difference between groups lies in the large cohort of patients, where there a significantly higher proportion of males in the CHI group compared to healthy controls is observed. The subdivision of patient and control groups into large and small cohorts has been described. The age and gender of participants included in each analysis will be given in Chapter Three (Cognitive and Behavioural), Chapter Four (Voxel-based Morphometry), Chapter Five (Subcortical Structures), Chapter Six (Tract-based Spatial Statistics) and Chapter Seven (Structure-function Relationships).

Chapter 3

Cognitive and Motor Outcome After Early Hypoglycaemia

3 Cognitive and motor outcome after early hypoglycaemia

This chapter focuses on the late effects of hypoglycaemia on cognitive and motor abilities in children with CHI and those with KH. Participants completed a comprehensive series of neuropsychological tests that aimed to uncover impairments in executive functions and motor skills within the context of cognitive abilities and educational attainments. This chapter reports on the neuropsychological performance of patients with KH and those with CHI relative to the standards of healthy controls, and, separately, relative to standard population means. Finally, results of an exploratory principle components analysis are described.

3.1 Introduction

3.1.1 Background

Hypoglycaemia is known to have deleterious consequences for the immature brain and to pose a risk of developmental adversities. A review of the literature indicates that early hypoglycaemic episodes (especially when experienced because of an abundance of insulin) are associated with developmental delay (Ağladıoğlu et al., 2013; Al-nassar, Sakati, Al-ashwal, & Bin-abbas, 2006; Avatapalle et al., 2013; Burns, Rutherford, Boardman, & Cowan, 2008; Harken, Filler, Avruskin, & Crigler, 1971; Ismail & Werther, 2005; Kerstjens, Bocca-Tjeertes, de Winter, Reijneveld, & Bos, 2012; Lucas, Morley, & Cole, 1988; Ludwig et al., 2011; Mazor-Aronovitch et al., 2007; Steinkrauss et al., 2005) and are detrimental to later cognitive and psychomotor functioning (Cresto et al.,

1998; Harken et al., 1971; Ludwig et al., 2011; Meissner et al., 2003; Menni et al., 2001; Rother, Matsumoto, Rasmussen, & Schwenk, 2001), even when occurring outside of the neonatal period (as with diabetes). However, studies examining homogenous groups of children are lacking, and few published studies have assessed later outcome in children with neonatal hypoglycaemia. Fewer studies still have assessed outcome using formal psychometric assessment in patients with CHI, while only one published study (Kogut et al., 1969) has applied formal psychometric testing in patients with KH.

3.1.2 Results of the HBI study

An introduction to the HBI study (Kumaran, 2012) has been given in Chapter One. Briefly, this study compared the cognitive and motor profiles of patients with CHI against those with KH. Those were KH were used as a healthy control group owing to the protection of ketone bodies. It was hypothesised that children with CHI would be impaired across all domains of cognition that were assessed compared to the standard population means, as well as their KH counterparts, and that children with KH would show complete preservation of functioning.

In patients with CHI, comparison of performance to standard population means revealed impairments in verbal and non-verbal intelligence, working memory and processing speed, academic attainment (spelling, reading and mathematical ability), sustained attention and divided attention and memory ability. Motor scores were not compared to the standard population mean, but patients with CHI performed significantly below patients with KH on a measure of manual dexterity. Other (gross)

motor skills were unaffected. Patients with CHI also had significantly lower scores than patients with KH in non-verbal intelligence and full scale IQ, on all academic attainment subtests and all memory assessments. Relative to standard population means, patients with KH showed preserved functioning in intelligence, working memory and processing speed, academic attainments and memory, but contrary to hypotheses they were impaired on tests of attention measuring attentional control, and selective attention. They did not perform significantly better than patients with CHI on any attention measure, or on measures of working memory and processing speed. In addition to this, the range of scores on these tests of attention, working memory and processing speed were very similar between the two patient groups. This suggests that patients with KH may not have an entirely preserved cognitive profile. Table 3.1 gives a summary of the results.

Table 3.1 Summary of HBI study cognitive and motor results

	IQ	Academic attainment	Selective attention	Sustained attention	Attentional Control	Dual task	Working memory	Processing speed	Memory	Gross Motor	Fine Motor
CHI < standard	✓	✓	×	✓	×	✓	✓	✓	×		
KH< standard	×	×	✓	×	✓	×	×	×	×		
CHI<KH	✓	✓	×	×	×	×	×	×	✓	×	✓

This was the first study to assess patients with CHI and KH using a battery of standardised assessments, and the sample of patients in both groups was large enough to draw some tentative statistical inferences. However, there are some limiting factors to this study. The first is the inclusion of patients with epilepsy, which is a limiting factor in interpreting the results in patients with CHI. Impairments reported in CHI, which cover a wide spectrum, may be exaggerated due to the inclusion of patients with epilepsy, a neurological condition that can have significant consequences for cognitive functioning. The second is the lack of a healthy control group against which to compare both patient groups.

Despite these limitations, consistent with reports of impairment in attention in children with CHI (Rother, Matsumoto, Rasmussen, & Schwenk, 2001) and in children with EODM (Bjørgaas et al., 1997; Hannonen et al., 2003; Lin et al., 2010; Northam et al., 2001; Rovet & Ehrlich, 1999) the HBI study points towards impairments in the executive domain following hypoglycaemia.

Typical brain development in childhood and the emergence of higher order skills

By the age of six, total cerebral volume is around 95% of an adult's volume, although peak volume is reached at 11 years in females and 14 years in males (Lenroot & Giedd, 2006). The developmental trajectories of grey and white matter are different; while white matter increases fairly linearly across regions into the fourth decade of life, reflecting myelination of axons (Gogtay et al., 2004) grey matter volume follows an inverted U-shaped developmental trajectory which is regionally specific (Lenroot &

Giedd, 2006). Grey matter volume tends to increase at younger ages, reflecting a dramatic increase in synaptic density, followed by a sustained loss of grey matter volume which begins around puberty and varies amongst different regions, such that the peak of frontal lobe volume occurs at around 12 years of age and the peak of temporal lobe volume occurs at around 16 years of age (Gogtay et al., 2004; Lenroot & Giedd, 2006). In the frontal lobe the dorsolateral prefrontal cortex is one of the last regions to mature, with grey matter volume reduction evident at the end of adolescence accompanied by its later myelination (Gogtay et al., 2004). It's thought that the loss of grey matter volume largely reflects heterochronic synaptic elimination - a pruning process whereby synapses that have failed to make functional connections are discarded - which has been postulated to reflect better functional specialisation and improved information processing (Blakemore & Choudhury, 2006; Giedd et al., 1999; Gogtay et al., 2004; Otsby, Tamnes, Fjell, & Walhovd, 2011).

3.1.3 Executive functions

Executive functions are those skills that allow purposeful and goal directed activity (Anderson, 1998), and have been argued to be domain general, influencing all aspects of behaviour (Lezak, 1993). While there are many different models of executive functions, most consider executive functions an umbrella term, encompassing skills that are interrelated. According to many influential models, executive functions can be categorized into distinct but interacting components, with important subcomponents that include (but are not limited to) inhibition, set-shifting, working memory and planning, with measures of attention also falling under the remit of executive functioning (Anderson, 2002; Diamond, 2014; Manly et al., 2001). These

subcomponents of executive functions have been identified from various factor analysis studies as well as structural equation modelling approaches (Anderson, 2002; Manly et al., 2001; Miyake et al., 2000). The inter-dependent but distinct components are proposed to work together to act as a top-down supervisory control system through which proficient executive functioning is achieved (Stuss & Alexander, 2000). One such model has been proposed by Anderson (2002; Figure 3.1). Cognitive flexibility is the ability to shift between two tasks, successfully divide attention, process two streams of information simultaneously and store and update information (working memory). Attentional control is defined as the ability to focus attention for long periods of time, to suppress irrelevant or unwanted stimuli (sometimes termed selective attention) and to inhibit a pre-potent response. Information processing is the ability to fluently and efficiently process incoming information and the speed at which output is reached. Finally, goal setting is the ability to develop new concepts and to implement new initiatives (Anderson, 2002; Diamond, 2014; Manly et al., 2001). Children with executive dysfunction might present with impairments in any one of these domains; with poor working memory, planning and organisational problems or mental inflexibility (Anderson, 1998). The nature of impairments in patients with CHI that have been identified by Kumaran (2012) and suggested by Rother et al. (2001), and the relatively selective impairment identified in patients with KH (which appears to be related specifically to attentional control), indicate that compromised executive functions may be one of the hallmarks of outcome following early hypoglycaemia.

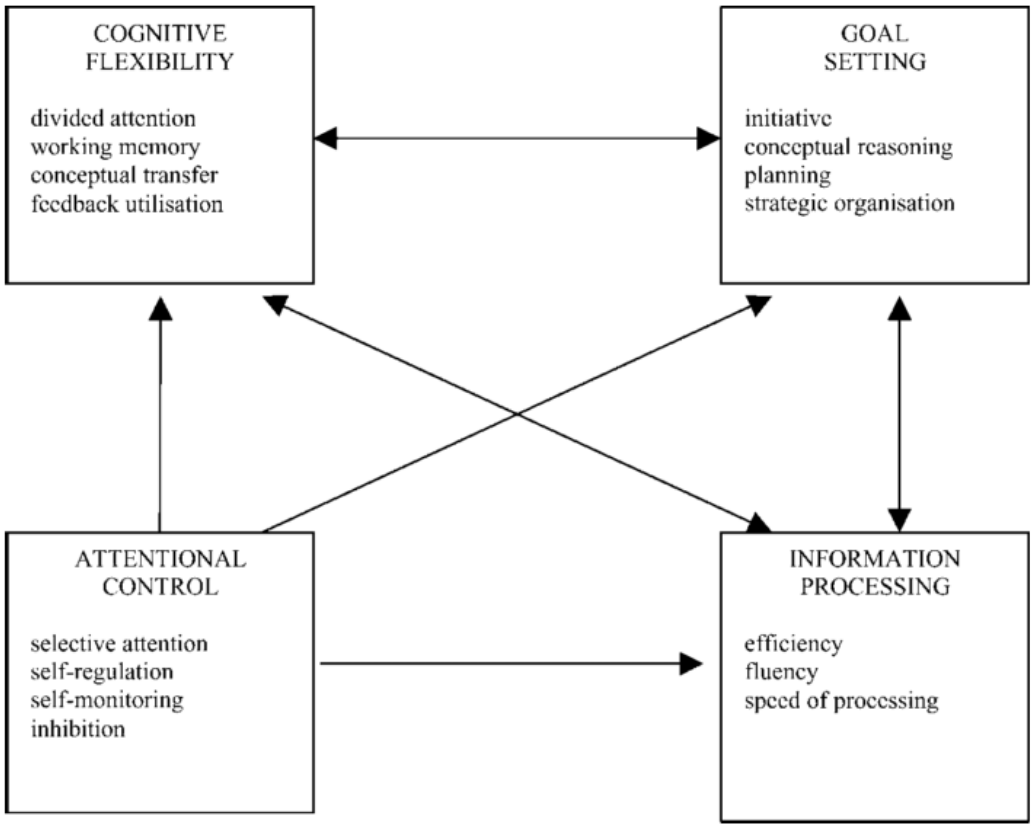

Figure 3.1 Model of executive functions. From Anderson 2002.

In line with the protracted development of the frontal lobe, later emerging higher order functions (particularly components of executive functions) have been shown to emerge and mature at different rates. Various studies have provided evidence of different executive skills coming online at different ages, and with continued improvement into adolescence (Anderson, 2002). Improvements in executive functions mediate the acquisition of other skills, with most executive skills improving rapidly between early and late childhood, reaching a plateau during early adulthood (McAuley & White, 2011). The greatest increases in executive functions have been observed in inhibition of perseverations and planning abilities between the age of five to eight years, while notable increases are observed amongst most executive functions between eight to 11 years of age (Romine & Reynolds, 2005).

Skills in attentional control (inhibition, selective attention, self-regulation and self-monitoring) emerge as early as 12 months of age, continuing to improve dramatically until around 9 years of age, after which performance gains have been found to plateau, reaching adult levels by 11-14 years of age (Anderson, 2002; Romine & Reynolds, 2005). Cognitive flexibility (e.g. switching, working memory) emerges at around 3-4 years of age and ability increases throughout middle childhood and into adolescence. Goal setting abilities emerge at around 4 years of age, although improve rapidly between seven and ten years of age, with improvements reflecting refinement of strategies and better decision making, which continues into adolescence. Information processing gains are made at around 11-12 years of age, with limited gains made after the age of 15 years. Therefore by middle childhood, attentional control abilities are fairly mature, and by 12 years of age, information processing, cognitive flexibility and goal setting are relatively mature, although gains are made into adolescence (Anderson, 2002; Blakemore & Choudhury, 2006).

As previously mentioned, working memory capacity increases dramatically with a typical increase of two to three fold in ability from childhood to adolescence (Gathercole, 1999; Isaacs & Vargha-Khadem, 1989). Working memory can be further broken down into individual components, according to the model of working memory proposed by Baddeley and Hitch (1974). In this model, performance is dependent on the functioning of three slave systems, which are coordinated by a supervisory controller, the 'central executive'. The controlling 'central executive' is domain-general in nature, coordinating information from the slave systems, while two of the three

slave systems are domain-specific, for verbal (the phonological loop) and visual (visuo-spatial sketchpad) information. The episodic buffer is the third and newest slave system to be added the model and is able to integrate information across modalities while acting as an interface between the two slave systems, and is directly controlled by the central executive (Baddeley & Hitch, 1974; Baddeley, 1996, 2000). The central executive is poorly specified but within this model is used as a reminder of the importance of attentional control functions to working memory (Baddeley, 1996). Thus, successful working memory performance is contingent on functions that are executive in nature, but working memory impairment may also be due to deficits in the domain specific components of working memory.

Because the executive skills mentioned above come 'online' at different time points, difficulties in these domains can remain undetected until middle childhood, and impairments in any one of these domains has the propensity to interfere with development and educational progress (Mateer & Williams, 1991). There are few studies assessing components of executive functioning in children who experience early and recurrent episodes of hypoglycaemia, but in those who have (both in CHI and EODM) there is evidence that these skills are compromised (Bjørgaas et al., 1997; Hannonen et al., 2003; Kumaran, 2012; Lin et al., 2010; Northam et al., 2001; Rother et al., 2001; Rovet & Ehrlich, 1999).

3.1.4 Study Aims

The studies reviewed in Chapter One and in this chapter have highlighted a number of shortcomings relating to confounding variables, small sample size, direct measurement

versus questionnaire-based assessment methods, and lack of methodological rigour. This study aims to address these issues in a number of ways.

First, studying a large sample of children who experienced hypoglycaemia because of congenital hyperinsulinism or ketotic hypoglycaemia addresses the issue of previously reported small sample sizes and cohorts that have a wide range of aetiologies. Second, including only children with no known neurological diagnoses (e.g. epilepsy) addresses the issue of confounds due to known neurological dysfunction. Although many children included in this sample experienced seizures because of hypoglycaemia, often as a presenting symptom, none had a confirmed diagnosis of epilepsy (i.e., recurrent, unprovoked seizures unrelated to blood sugar levels; Cross, 2015). By including only those who are neurologically normal, it will be possible to determine whether any previously identified impairments are truly specific to hypoglycaemia. Finally, a group of healthy controls also completed the assessments used in this thesis and the majority of tests were standardised and have population means available. This allows for a comparison of performance in each patient group against a healthy control group, as well as against a population mean. In this study, patients are compared to both healthy controls and standard population means to ensure that impairments do not arise as a function of a high performing control group.

Based on the literature and the results of the HBI study, a neuropsychological protocol was designed. Tests of intelligence were administered in order to ascertain a general level of cognitive ability. In order to give a comprehensive executive profile of both groups, levels of functioning in skills that fall under the umbrella of executive functions

were assessed using appropriate tests. The tests that were chosen assess working memory, cognitive flexibility, planning, attentional control and information processing. Previous studies have reported motor delays and deficits in motor skills. In light of this, different components of visual-motor coordination were examined to determine whether deficits are related to problems in fine motor skills, gross motor skills, coordination, visual perception, or visual-motor integration. Academic attainment was assessed and, given the report of difficulties in mathematics, a test of internal number representations was given to assess the specificity of this impairment. Immediate and delayed memory ability was also assessed.

3.1.4.1 Aims and hypotheses

This study aimed to determine what (in the absence of any known neurological diagnoses) the long-term outcomes of cognitive and motor function of children with a diagnosis of CHI and KH are. In addition, this study examined whether deficits in components of executive function and motor skill are selective (i.e. over and above a reduction in intelligence or learning ability).

It was hypothesised that patients with CHI would show deficits in executive functions and motor skills against the background of lower than normal intelligence, memory and academic attainments. Patients with KH were predicted to show selective deficits in executive functions (excluding working memory and processing speed), but no impairments in other domains of cognitive and motor functions. Accordingly, it was hypothesised that patients with KH would outperform those with CHI on most measures, except on those assessing executive functions.

3.2 Methods

3.2.1 Neuropsychological protocols

To test the above hypotheses, two types of assessment were carried out; questionnaire-based and formal psychometric assessment. Parents completed a set of questionnaires in order to obtain an indication of functioning within the home environment. Each participant completed a battery of neuropsychological tests, allowing a direct measure of ability. Assessments were completed at the Wolfson Centre, UCL Institute of Child Health. Sessions were completed during one day, with plenty of breaks, or divided over multiple testing sessions (usually over consecutive days).

3.2.1.1 Emotional and Behavioural functioning

Parental Questionnaires: The Strengths and Difficulties Questionnaire (SDQ [Goodman, 1998]) and Child Behaviour Checklist (CBCL [Achenbach, 2003]) provided parental ratings of social emotional functioning.

The SDQ is a 25-item parental questionnaire for children between the ages of four and 17. The questionnaire measures symptoms relating to emotional regulation, conduct, hyperactivity and peer relations, and also has a scale of measurement for pro-social behaviour. Parents are asked to assess how true statements relating to these behaviours are (e.g. 'complains of stomach aches': not true, sometimes true, certainly true). Scores on the emotional regulation, conduct, hyperactivity and peer relations

scales are summed to create a 'total difficulties' score. Higher scores indicate poorer functioning, except on the pro-social behaviour scale, where lower scores indicate poorer functioning.

The CBCL is a 120-item parental questionnaire for children aged between six and 18 that measures emotional, behavioural and social tendencies. Responses to questions about the tendency for a child to display a particular behaviour take the form of a Likert scale (0=never, 1=sometimes, 2=often), and scores for various behavioural domains (e.g. aggression, anxiety) are produced. Scores are also provided for internalising and externalising behaviours and a total problems score is derived from these. A T-score above 65 is considered to be in the clinical range.

3.2.1.2 Intellectual Ability

Intelligence was assessed using the Wechsler Abbreviated Scale of Intelligence (WASI [Wechsler, 1999]). This test provides verbal and performance intelligence quotients (IQ). Verbal IQ (VIQ) reflects semantic knowledge and performance IQ (PIQ) reflects nonverbal abilities, visual perception and visual-motor/coordination skills. Patients who were seen as part of the HBI study, and who did not return to take part in the CNH study, were assessed with the Weschler Intelligence Scale for Children (WISC-IV [Weschler, 2004]), producing measures of verbal comprehension (VCI; likened to VIQ) and perceptual reasoning (PRI; likened to PIQ). As the WASI uses only VIQ and PIQ to generate a Full Scale Intelligence Quotient (FSIQ), and the WISC-IV uses VCI, PRI, Working Memory (WM) and Processing Speed (PS), a General Ability Index (GAI) was calculated for each participant. GAI is a measure of intellectual functioning derived

from VIQ and PIQ only, making a comparison of overall intellectual ability derived from the two intelligence tests possible.

3.2.1.3 Executive Functions

Parents completed the Behaviour Rating Inventory of Executive Functioning (BRIEF [Goioa, Isquith, Guy and Kenworthy, 2000]); a questionnaire designed to measure children's executive abilities, according to ratings of the occurrence of behaviours in everyday situations at home. Responses generate ratings of the child's ability to inhibit, shift and regulate emotions (which form the behavioural regulation scale), and of the child's working memory capacity, their ability to initiate, to plan and organise, to organise materials, and to monitor. These five items form the metacognitive scale. The global executive scale is a composite of both these scales. T-scores above 65 are considered within the clinical range.

3.2.1.3.1 Attention

Conners 3 Questionnaire

To obtain reports of everyday attention functioning, parents completed the Conners questionnaire (3rd edition [Conners, 2012]) a 110-item questionnaire for children and adolescents ranging from age six to 18. Primary measures derived from responses are inattentiveness, hyperactivity, learning problems, peer problems and executive dysfunction, with additional measures of aggression problems, peer relationships and family relationships. In addition, accordance with DSM-IV-TR symptomatic criteria is measured.

Test of Everyday Attention for Children

The Test of Everyday Attention for Children (TEA-Ch, [Manly, Robertson, Anderson, Nimmo-Smith, 1998]) assesses various dimensions of attention. Descriptions of the subtests used and the corresponding dimension of attention measured are outlined in Table 3.2. Broadly, this test captures ability in selective attention, sustained attention, attentional control and dual task monitoring (Manly et al., 2001). The same four core subtests as those used in Kumaran's study (2012) were administered, and a further subtest (opposite worlds) was given to provide further insight on attentional control (inhibition) capabilities.

Table 3.2 Dimensions of attention measured by the TEA-Ch

Dimension of attention	Test	Description	Outcome scores
Selective attention	Sky Search	Participants must visually scan a large array of stimuli for 'targets' as quickly as possible.	1. Number of correctly identified targets 2. Time per target 3. Attention score
Sustained attention	SCORE!	Participants listen to string of audio 'beeps' that vary in number and length of inter-stimulus intervals. Between 1-15 beeps are presented, and the participant must keep track of the number of beeps presented and verbally report the total to the examiner at the end of each trial. Ten trials.	1. SCORE sustained attention
Attentional control: set switching and inhibition	Creature counting	Participants must count the number of visually presented targets, according to the direction of counting implied by an explicit cue (arrow).	1. Creature counting total correct 2. Timing score
	Opposite worlds	Participants make their way around a path of squares labelled '1' and '2'. This test consists of two conditions; the 'same world' condition, where subjects must say what they see, and the opposite world condition, where subjects must resist the overlearned response, and say the opposing number to the one that is presented. A time penalty is incurred when subjects give the wrong response.	1. Same world score 2. Opposite worlds score
Dual Task Proficiency	Sky Search DT	Participants must visually scan for targets (as in Sky Search) whilst counting a string of beeps over a number of trials (as in SCORE). The outcome variable reflects the time and accuracy cost of performing two tasks at the same time.	1. Dual task score

Conners Continuous Performance Task

To further examine deficits in attention a computerised assessment of attention was administered. The Conners *Continuous Performance Test*, 3rd edition, (CPT-III [Conners, 2014]) is a 14-minute task that uses a 'not x' paradigm to assess attention-related problems. Participants are required to respond to a visual array of stimuli (letters) by pressing the space bar- the participant must inhibit their responses when an X appears. The main measures of attention examined are inattentiveness, (detectability, omissions, commissions, Hit Reaction Time (HRT), HRT standard deviation, variability), impulsivity (HRT, commissions, perseverations), sustained attention (HRT block change, omissions by block, commissions by block) and vigilance (HRT inter-stimulus interval change, omissions by inter-stimulus interval [ISI], commissions by ISI). Table 3.3 describes how these measures are calculated in further detail. The CPT is a clinical assessment tool that is used to diagnose the likelihood of ADHD.

Table 3.3 CPT-III subtests and their inclusion in measures of aspects of attention.

Dimension	Score	Description
Inattentiveness	Detectability (d')	Ability to discriminate between targets and non-targets
	Omissions	Missed targets
	Commissions	Incorrect responses to non-targets
	Hit Reaction Time (HRT)	Response speed
	HRT standard deviation	Response speed consistency
	Variability	Variability of response speed consistency
Impulsivity	HRT	Response speed
	Commissions	Incorrect responses to non-targets
	Perseverations	Random or anticipatory responses
Sustained Attention	HRT block change	Change in response speed across blocks of trials
	Omissions by block	Missed targets by block
	Commissions by block	Incorrect responses to non- targets by block
Vigilance	HRT Inter-Stimulus Interval (ISI) change	Change in response speed at various ISIs
	Omissions by ISI	Missed targets by ISI
	Commissions by ISI	Incorrect responses to non-targets by ISI

3.2.1.3.2 Working memory

WISC-IV

Forward and backward digit span and letter-number sequencing subtests from the WISC-IV were used to assess working memory (the ability to hold and manipulate information in short term memory).

Self-Ordered Pointing Task

To further explore deficits in working memory, the Self Ordered Pointing Task (SOPT) was administered. The test is an adaptation of a paradigm designed by Petrides and Milner (1982) to assess frontal lobe dysfunction in patients with surgical excisions for epilepsy. The abstract designs version of the SOPT is a self-generated visual working memory task with increasing memory load. Performance is dependent on successful self-generation, monitoring and updating of responses. Subjects are shown an array of abstract designs that are difficult to maintain in working memory using verbal labels. There are five blocks of increasing memory load, with three trials in each block. In the first block (4 designs), participants are asked to point to one of four designs, and then, on the next page, to point to a different design, and so on, until they have pointed to all designs (Figure 3.2). Importantly, participants are instructed not to point to the same spatial location twice in a row. The designs increase in numbers from four to 12, in two design increments. Although the time taken to complete a trial was explicitly recorded, participants were told that accuracy was more important than speed, so reaction time was not a variable of interest here. Patients with lesions in the frontal lobe as well as patients with large right temporal lobe removals including the

hippocampus have been shown to be impaired on this self-monitored test of working memory (Petrides, & Milner, 1982). Interestingly, patients with early-onset bilateral damage to the hippocampus are also impaired on this test but only when the memory load is increased, suggesting that this test covers aspects of both working memory and long-term memory (Geva, Cooper, Gadian, Mishkin, & Vargha-Khadem, 2016).

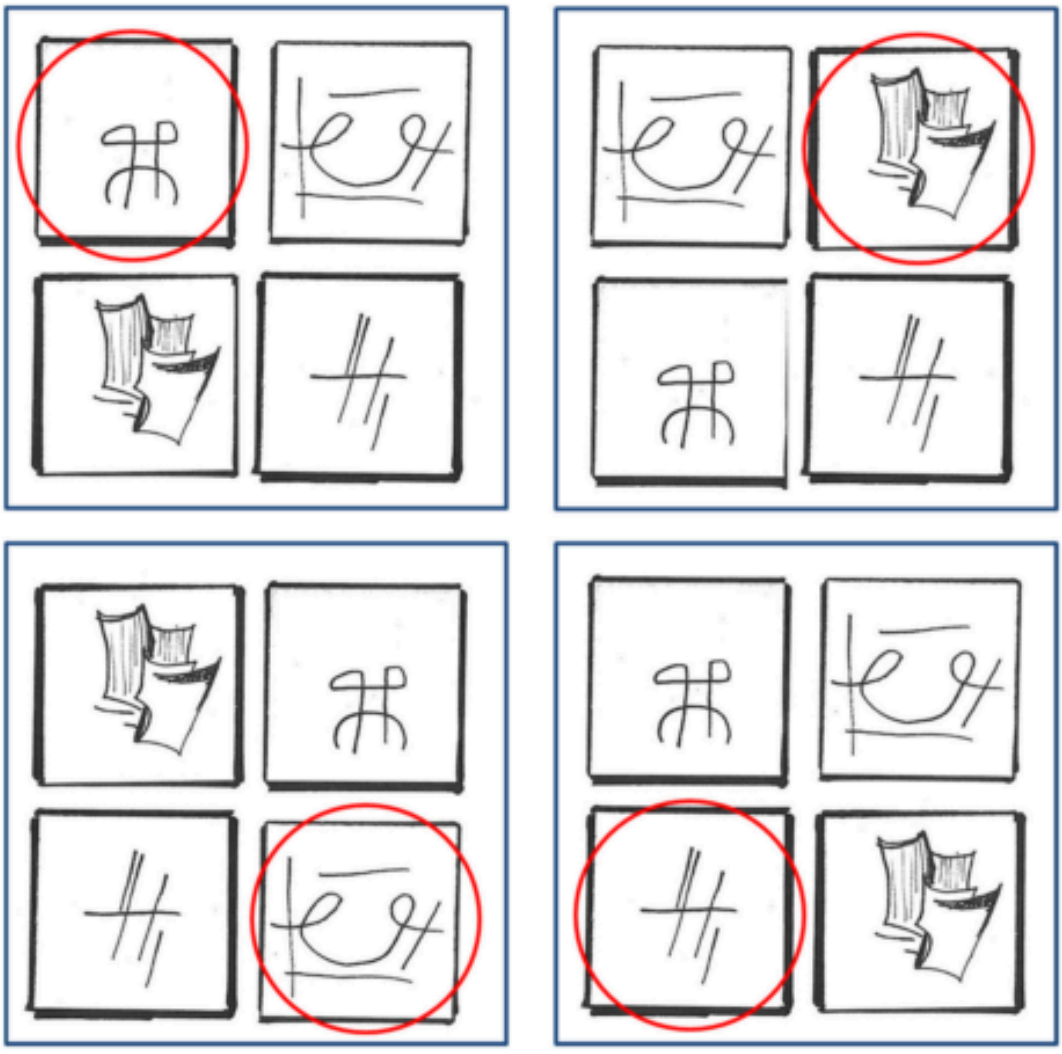

Figure 3.2 Example of a correct trial in the SOPT 4 item condition (adapted from Geva et al., 2016). Red circles indicate a possible sequence of correct response.

3.2.1.3.3 Processing speed

Coding and Symbol Search subtests from the WISC-IV were used to assess processing speed (psychomotor skills and timed responses to abstract visual information).

3.2.1.3.4 Planning

The Tower (a subtest from the Delis Kaplan Executive Function System) was administered to assess the planning aspect of executive functions. (D-KEFS, [Delis, Kaplan, Kramer 2001]). The Tower assesses participant's spatial planning skills, the ability to establish a rule set and the ability to follow a set of instructions.

3.2.1.4 Memory

Sunderland Memory Questionnaire

Parents completed The Sunderland Memory Questionnaire (Sunderland, Harris & Baddeley, 1983), a 45-item questionnaire that measures everyday memory functioning. Z-scores are created from responses on items one to 27, based on in-house data from a large group of aged-matched typically developing controls (n=65).

Children's Memory Scale

Memory was assessed using the Children's Memory Scale (CMS), Cohen, 1997. This assessment provides an index score for verbal and visual immediate and delayed memory, as well as a general memory quotient. In addition, index scores for learning, attention/concentration, and delayed recognition are obtained.

3.2.1.5 Academic attainment

WIAT-II

The Wechsler Individual Achievement Test, 2nd edition (WIAT-II [Wechsler, 2005]) is a large battery from which specific tests were chosen based on the findings of Kumaran (2012). Achievement in mathematics was assessed using the numerical operations and mathematical reasoning subtests. The test of numerical operations is a non-timed paper and pencil test that assesses proficiency of solving problems requiring operational solutions. The mathematical reasoning test involves solving a series of real life mathematical problems. Single word spelling to dictation was also assessed.

Mathematical Estimation Test

In light of previously observed deficits in mathematical ability (Kumaran, 2012), The Mathematical Estimation Test (MET [Wolke, Schulz, & Meyer, 2001]) was administered to assess numerical representations. The MET is a 12-item test of integrity of domain-specific numerical representations. Participants are asked to estimate length, relative positions on number lines, number of dots in a visual array, and magnitude of distances. These measures have been shown to correlate with performance on WIAT-II measures of mathematical ability in extremely preterm children (Simms et al., 2013).

3.2.1.6 Motor skills

Movement Assessment Battery for Children

Manual dexterity, gross motor co-ordination and balance were assessed using the Movement Assessment Battery for Children, 2nd edition (MABC-2 [Barnett, Henderson

and Sugden, 2007]). This test examines fine and gross motor skills using age-appropriate tasks. Norms for three age bands are provided (although only two are of interest here), four to six years, seven to ten years and 11-16 years. Motor functions assessed include:

1. Manual dexterity (e.g. turning pegs in-between fingers, or accurately placing pegs into a board, as well as a drawing task where participants must precisely trace in between a 'path' that gets progressively thinner). This measures fine motor ability.
2. Gross Motor Co-ordination (Aiming and Catching) skills include tasks of throwing and catching a ball with one or two hands, and throwing at a wall target. This task is designed to assess the proficiency of coordinated movement.
3. Static and Dynamic balance skills are also assessed. Static balance involves balancing on one/two boards depending on the age band. Dynamic balance involves heel-toe walking along a line and hopping ability.

A total motor proficiency score is calculated based on these subtests. Scores at or below the 16th percentile indicate a 'risk' for a movement difficulty, while scores at or below the 5th percentile denote a significant movement difficulty.

Beery-Buktenica Developmental Test of Visual Motor Integration

To further examine fine motor abilities, the Beery-Buktenica developmental test of visual motor integration, sixth edition (Beery VMI [Beery & Beery, 2010]) was

administered. This is a non-verbal assessment of pure motor skills, pure visual perception skills, and successful integration of these skills. The visual-motor integration form requires the subject to copy line drawings, which increase in complexity. The visual perception form requires the participant to identify an identical shape from an array of visually similar shapes. The motor coordination form measures the ability to accurately execute fine motor movements through a precise drawing task, where participants must draw a shape by connecting dots, without straying outside of some imposed boundaries.

Tables 3.4 and 3.5 give a summary of the assessments used in this thesis, the subtests of each assessment, the type of score that is obtained and the number of healthy controls, patients with KH and patients with CHI that completed each assessment. Tables 3.4 and 3.5 outline the classification of standard scores and T-scores (for standardised tests).

Table 3.4 Summary of cognitive and motor assessments

Domain	Test	Scores	Measures	Type of score	N
Intelligence	WASI WISC-IV	Verbal IQ (VIQ) Non-verbal IQ (NVIQ) General ability Index (GAI)	Verbal ability Non-verbal ability General Ability	Standard score	Controls= 18 KH= 20 CHI= 31
Executive functions	TEA-Ch	Selective attention Sustained attention Attentional control Attentional control[18] Dual task	Process information, select targets Maintain focus for a long period Efficacy of switching between sets Inhibiting a verbal response Ability to monitor and complete two tasks simultaneously	Standard score	Controls= 18 KH= 20 CHI= 31

[18] Data for opposite worlds available for 17 healthy controls, 19 patients with CHI and 12 patients with KH

Domain	Test	Scores	Measures	Type of score	N
Executive functions	CPT	Inattentiveness Impulsivity Sustained attention Vigilance	Discriminate targets, processing speed Suppression of incorrect responses Task performance over time Responsiveness to task demand	T-score	Controls=16 KH= 11 CHI= 19
Executive functions	WISC-IV	Working memory Processing speed	Hold and manipulate information in mind Speed of psychomotor responses	Standard score	Controls= 17 KH= 20 CHI= 31
Executive functions	SOPT	Working memory	Monitoring and updating self-generated responses	Sum of errors	Controls= 18 KH= 11 CHI= 19

Domain	Test	Scores	Measures	Type of score	N
Executive functions	Tower	Total achievement	Tower completion and number of moves	Standard score	Controls= 17
		First move time	Planning		KH= 12
		Time-per-move	Planning and speed		CHI= 17
		Move accuracy ratio	Efficacy of moves made		
		Rule violations	Ability to establish a rule set, error monitoring		
Memory	CMS	Visual immediate Verbal Immediate	Recall of visual (faces, dot locations) or verbal (stores, word pairs) info after short delay	Standard score	Controls= 17
					KH= 14
		Visual delayed Verbal delayed	Recall of visual or verbal info after short delay		CHI= 24
		General memory	Composite of above memory scores		
		Attention/Concentration	Working memory		
		Learning	Improvement in successive trials		
		Delayed recognition	Recognition of visual/verbal info		

Domain	Test	Scores	Measures	Type of score	N
Academic attainment	WIAT-II	Numerical operations Mathematical reasoning Spelling	Solving problems with learnt mathematical processes Solving real life mathematical problems Spelling from dictation	Index score	Controls= 18[19] KH= 20 CHI= 30
Number skills	MET	Length Number line Dots Distance	Estimate length Estimate position on number line Estimate number of dots Estimate distance	Sum of correct responses	Controls= 18 KH= 11 CHI= 19

[19] Mathematical reasoning data available for 10 healthy controls and 25 patients with CHI. Numerical operations data available for 19 patients with KH. Spelling data available for 16 healthy controls.

Domain	Test	Scores	Measures	Type of score	N
Motor skills	MABC-2	Manual dexterity Aiming and catching Balance Total motor score	Fine motor skills Gross motor co-ordination Balance Overall motor proficiency	Standard score	Controls= 18 KH= 20 CHI= 28
Motor skills	Beery VMI	Visuomotor integration Pure motor Visual Perception	Visual perception and motor integration Fine motor coordination Perception of visual forms	Standard score	Controls= 18 KH=12 CHI=19

Table 3.5 Summary of parental questionnaires

Domain	Questionnaire	Scores	Measures	Type of score	N
Emotional and behavioural functioning	SDQ	Emotional Conduct Hyperactivity Peer relations Pro-social	Emotional regulation Conduct disorder Inattentive/hyperactive behaviour Relationships with others of similar age Helpful, pro-social behaviour	Simple sum	Controls= 14 KH= 11 CHI= 18
Emotional and behavioural functioning	CBCL	Internalising Externalising Total problems	Anxiety, depression, somatic complaints Aggression, rule-breaking behaviour	T-score	Controls= 16 KH= 11 CHI= 17
Executive functions	BRIEF	Metacognitive Behavioural Regulation Executive composite	Working memory, planning, organising Inhibit, shift, regulate emotions Combination of the above	T-score	Controls= 17 KH= 11 CHI= 16
Executive functions	Conners 3	Inattentiveness Hyperactivity Learning problems Executive functioning Defiance/aggression Peer problems Family relationships	Concentration, distractibility High activity, impulsivity Academic struggles Planning and prioritising Argumentative, rule breaking Social skills, difficulties with friendships Feeling unloved, criticised	T-score	Controls= 15 KH= 11 CHI= 17
Memory	Sunderland	Everyday memory	Memory ability in home environment	Sum of responses 1-27	Controls= 16 KH= 11 CHI= 18

Table 3.6 Standard scores and indication of function

Standard Score	Classification
>130	Very superior
121-129	Superior
111-120	High average
90-119	Average
80-89	Low average
70-79	Borderline
<69	Extremely low

Table 3.7 T-Scores and indication of function

T-Score	Classification
>65	Clinical range
60-64	Borderline range
<59	Normal range

3.2.2 Participants

All participants in the large and small cohorts underwent assessments of intelligence, academic attainment, attention and motor skills; thus the large cohort of patients (as outlined in Chapter Two) completed these assessments. Tests that were unique to the neuropsychological protocol devised for this thesis were completed only by the participants in the small cohort. Therefore, the small cohort completed the entire neuropsychological testing battery as outlined above. All healthy controls from the small cohort completed this same neuropsychological test battery. There were no significant differences in age between groups in either the large or small cohorts. In addition, there were no significant differences in the ratio of males to females between groups, in either large or small cohorts. Tables 3.8 and 3.9 provide the ages and genders of participants for each cohort.

For VIQ, PIQ, WM, PS, WIAT-II subtests, TEA-Ch selective attention, sustained attention, creature counting and dual task, all MABC-2 subtests and the CMS, data from the large cohort of patients were available. Variations of group numbers in individual tests are given in the results section. For TEA-Ch opposite worlds, SOPT, MET, the CPT and Beery VMI, and all parental questionnaires, data from the smaller cohort of patients outlined in Table 3.9 were available. Variations of group numbers in individual tests are given in the results section.

Table 3.8 Data available for large cohort of patients

Group	N	Age in months (sd)	Gender
Controls	18	131 (26)	9 male
KH	20	121 (34)	11 male
CHI	31	131 (30)	20 male

Table 3.9 Data available for small cohort of patients

Group	N	Age in months (sd)	Gender
Controls	18	131 (26)	9 male
KH	12	139 (31)	6 male
CHI	19	131 (32)	13 male

3.2.3 Statistical analyses

Data were inspected for normality using the Shapiro-Wilk test and estimates of skewness/kurtosis. Univariate analyses (ANOVAs, or, where data were not normally distributed, Kruskal-Wallis tests) were conducted to examine differences between the means of controls, patients with CHI and patients with KH. The control group

performed significantly above the standard population mean in many of the cognitive and behavioural assessments, indicating a generally better level of emotional, behavioural and cognitive functioning compared to the standard population on which these tests are normed. To avoid false positives arising as a result of the generally inflated performance of the control group, each patient group was compared against the standard population as well as the control group. One-sample tests (t-tests for normally distributed data and Wilcoxin-signed rank tests for non-normally distributed data) were conducted to compare patient groups to the standard population mean. Unless otherwise stated, *p*-values were adjusted for multiple comparisons (within test) via Bonferroni correction for multiple comparisons.

3.3 Results

3.3.1 Emotional and behavioural functioning

Univariate analyses of parental ratings from the Strengths and Difficulties Questionnaire (SDQ) showed significant group differences in ratings on the subscales of hyperactivity (H[2]=9.7, p=0.008 and total problems (H[2]=9, p=0.011). Bonferroni corrected pairwise comparisons showed that in the hyperactivity scale, controls had lower ratings (M=2.4, range= 0-7) than patients with KH (M=5, range=1-9, p=0.02) and patients with CHI (M=4.8, range=1-9, p=0.022), indicating that healthy controls displayed fewer behaviours related to hyperactivity than patients. In the total problems scale, healthy controls (M=5.5, range =1-11) had significantly lower total problems than patients with KH (M=12.5, range=5-32, p=0.023) and patients with CHI

(M=11.6, range=2-25, p=0.037, Figure 3.3). There were no significant differences between patient groups. No other main effects were significant.

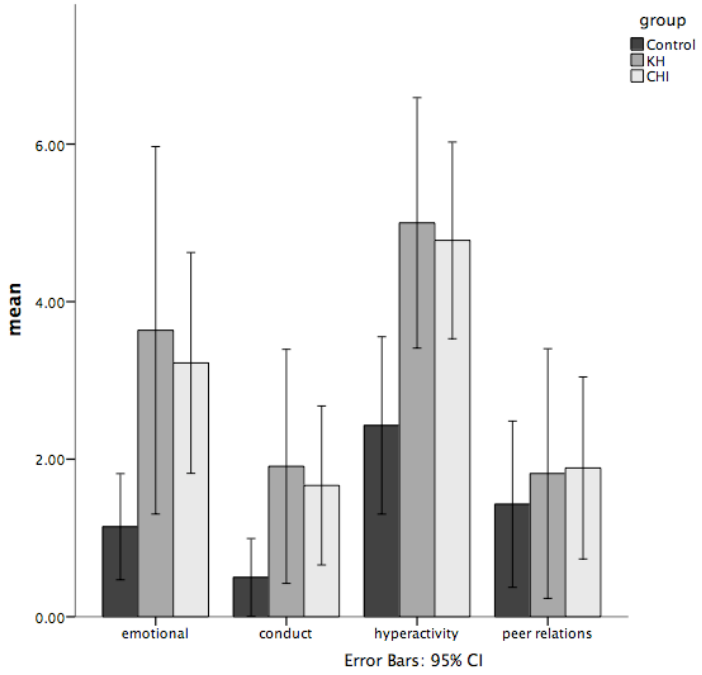

Figure 3.3 Ratings of emotional and behavioural functioning from the SDQ. Error bars represent 95% confidence intervals. Relative to healthy controls, both patient groups had higher ratings of hyperactivity.

Analysis of parental ratings from the Child Behaviour Checklist (CBCL) showed that significant differences existed between groups on the scales of externalising behaviour (H[2]=14.93, p=0.001), with post-hoc Bonferroni corrected analyses showing that both patients with KH and patients with CHI had significantly higher ratings than controls (p=0.001 and p=0.032 respectively, Table 3.10), indicating significantly greater risk for patients compared to controls. Main effects of group were also observed on the scale of internalising behaviour ($F_{(2,41)}$=5.2, p=0.01), with post-hoc Bonferroni corrected comparisons showing that patients with KH had significantly higher ratings than

controls (p=0.008, Table 3.10). Indeed, the mean rating of internalising behaviour in children with KH falls just short of being within the 'borderline' range. No other comparisons were significant. Unsurprisingly, given the combination of higher rates of internalising and externalising behaviours, a main effect of group was found for the CBCL 'total problems' score ($F_{(2,41)}$=10.1, p<0.001) with controls having a lower score than patients with KH (p<0.001) and patients with CHI (p=0.017).

Comparison to the standard mean of 50 showed that patients with KH have higher instances of internalising behaviours (t=2.5, p=0.034) and higher total problems (t=2.9, p=0.015) than the standard population mean, but no significant difference between patients with KH and the standard population mean on the externalising scale was observed. No significant differences between ratings of patients with CHI and the standard mean were observed.

In summary, parental reports of both patient groups indicate concerns about attention (hyperactivity). While group comparisons of internalising and externalising behaviours yielded significant differences between groups, one sample t-tests showed that only parental reports of those with KH were higher than the standard population mean.

Table 3.10 Mean and range of scores on CBCL subscales

	Externalising	Internalising	Total problems
Healthy controls	M= 40.5 range 33-51	M=46.5 range 33-65	M=42.3 range 29-53
KH	M=54.3 range 34-71	M=59.7 range 34-75	M=58.7 range 37-77
CHI	M=50 range 34-71	M=53.6 range 33-71	M=52 range 31-74

3.3.2 Intelligence

A MANOVA examining differences between controls, patients with CHI and patients with KH in non-verbal IQ (performance IQ; PIQ), verbal IQ and GAI was carried out. This revealed a significant main effect of group ($F_{(6, 130)}$=4.4, p<0.001). Follow-up univariate analyses (considered significant at 0.017 after correction for multiple comparisons) showed significant main effects of group for all variables, despite mean scores falling within the average range (Table 3.11; see Table 3.6).

Verbal, non-verbal and general ability

Post hoc Bonferroni-corrected comparisons showed that patients with KH had significantly lower VIQ than controls (p=0.002), and that patients with CHI also had significantly lower VIQ scores than controls (p<0.001). Patients with KH had comparable PIQ to controls, while patients with CHI had significantly lower PIQ than controls (p=0.001). No significant differences in verbal and non-verbal IQ were

observed between the two patient groups. The GAI was significantly higher in controls compared to both patient groups (KH p=0.012 and CHI p<0.001, Table 3.11).

The scores obtained by healthy controls ranged from 97-136 for VIQ, 95-135 for VIQ, and 98-133 for GAI. Thus, no healthy control scored below one standard deviation (1 SD) from the population mean on any measures of intelligence. In children with KH, VIQ scores ranged from 74-130, PIQ scores ranged from 65-133, and GAI score ranged from 68-126. Three individuals with KH obtained VIQ scores more than 1 SD below the mean, two obtained PIQ scores greater than 1 SD below the mean, and three obtained GAI scores that were greater than 1 SD below the mean. In children with CHI, VIQ scores ranged between 75-131, PIQ scores ranged between 75-135, and GAI scores ranged between 76-136. Five individuals obtained VIQ scores greater than 1 SD below the mean, while 11 obtained PIQ scores greater than 1 SD below the mean. Five obtained GAI scores greater than 1 SD below the mean.

Table 3.11 Verbal, non-verbal and general ability in each group

Test	N	Mean (SD)	ANOVA F, P	Pairwise comparisons
VIQ				
Controls	18	115 (10.5)	F=9.1, p=0.001	Control>KH p=0.002 Control>CHI p<0.001
KH	20	99 (14)		
CHI	31	99 (15.7)		
PIQ				
Controls	18	109 (10.2)	11.3, p<0.001*	Control>CHI p=0.001
KH	20	100 (19.4)		
CHI	31	93 (13.6)		
GAI				
Controls	18	114 (9.3)	H=18.6, p<0.001	Control>KH p=0.012 Control>CHI p<0.001
KH	20	100 (16.5)		
CHI	31	95 (13)		

One sample t-tests against the standard population mean of 100 (considered significant at p=0.017 after Bonferroni correction for multiple comparisons) were conducted to verify the performance of both patient groups. These indicated that patients with KH perform in line with the standard population on measures of non-verbal and verbal intelligence, as well as overall general ability (p>0.1 for all). In contrast, patients with CHI show significantly impaired non-verbal IQ (t=-2.83, p=0.004), but verbal abilities and the general ability index were in line with the reference mean of 100 (p>0.05 for both).

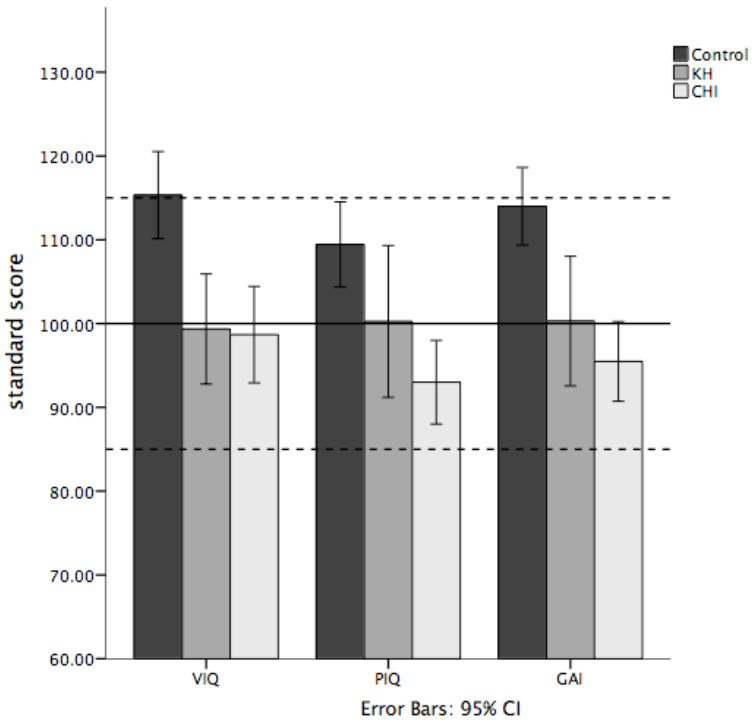

Figure 3.4 Bar chart showing mean VIQ (verbal IQ) PIQ (performance IQ) and GAI (general ability index) scores from intelligence tests in healthy controls, patients with KH and patients with CHI. Solid line indicates the standard population mean, dotted lines indicate one standard deviation from the mean. Error bars represent 95% confidence intervals. Patients with CHI have significantly lower non-verbal IQ (PIQ) relative to the standard population mean of 100.

In summary, when compared to the healthy control group, both patient groups show deficits in verbal and non-verbal abilities. However, the inflated performance of the control group (Figure 3.4) makes it difficult to be certain of the validity of these results. Comparison to the standard population mean, which may be composed of a more thoroughly representative sample of healthy children, allows for verification of results and permits patterns of strengths and weaknesses to be drawn with more confidence. Here, patients with CHI do show significantly lower non-verbal intelligence than standard population means, and indeed over one third of the cohort obtained scores greater than 1 SD below the mean of 100. In contrast, verbal intelligence and general ability are comparable to the standard population mean. Patients with KH do not show significant differences from the standard population mean. Importantly, these analyses did not identify significant differences between patients with CHI and patients with KH.

3.3.3 Executive Functions

Parental reports of executive functioning

Two healthy controls obtained scores in the clinical range on the Behavioural Regulation Index (BRI), while one obtained scores in the clinical range in the Global Executive Composite. One child with KH obtained scores in the clinical range on the BRI, while five obtained scores in the clinical range on the Metacognitive Index scale, and five obtained scores in the clinical range on the Global Executive Composite. Three children with CHI obtained scores in the clinical range for Behavioural Regulation Index, Metacognitive Index and the Global Executive Composite. Univariate analyses were conducted on these three summary scores from the BRIEF; the Metacognition

Index, the Behavioural Regulation Index and the Global Executive Composite. Main effects (considered significant at 0.017 after Bonferroni correction for multiple comparisons) were observed in the Metacognition Index ($F_{(2,41)}$=9, p=0.001) and the Global Executive Composite ($F_{(2,41)}$=6.5, p=0.004). The Metacognition Index represents the child's ability to initiate, plan, organise, and sustain future-oriented problem solving in working memory. The Global Executive Composite is a score that incorporates all of the clinical scales from the BRIEF. Post-hoc Bonferroni corrected comparisons showed that healthy controls had lower ratings of metacognitive difficulties than patients with KH (p=0.001) and patients with CHI (p=0.03, Figure 3.5). Similarly, controls had lower ratings of global executive dysfunction than patients with KH (p=0.003) while only a trend for lower ratings in controls than patients with CHI was observed (p=0.096). Main effects in the behavioural regulation index did not survive correction for multiple comparisons (H[2]=6.2, p=0.044).

One-sample tests against the standard population mean of 50 confirmed that patients with KH have significantly elevated ratings for problems related to metacognition (t=3.22, p=0.009) and global executive function (t=3.1, p=0.01). Compared to the standard population, trend level differences in ratings of metacognitive function in children with CHI were observed (t=1.8, p=0.09). No significant differences were observed in the global executive component (p=0.21).

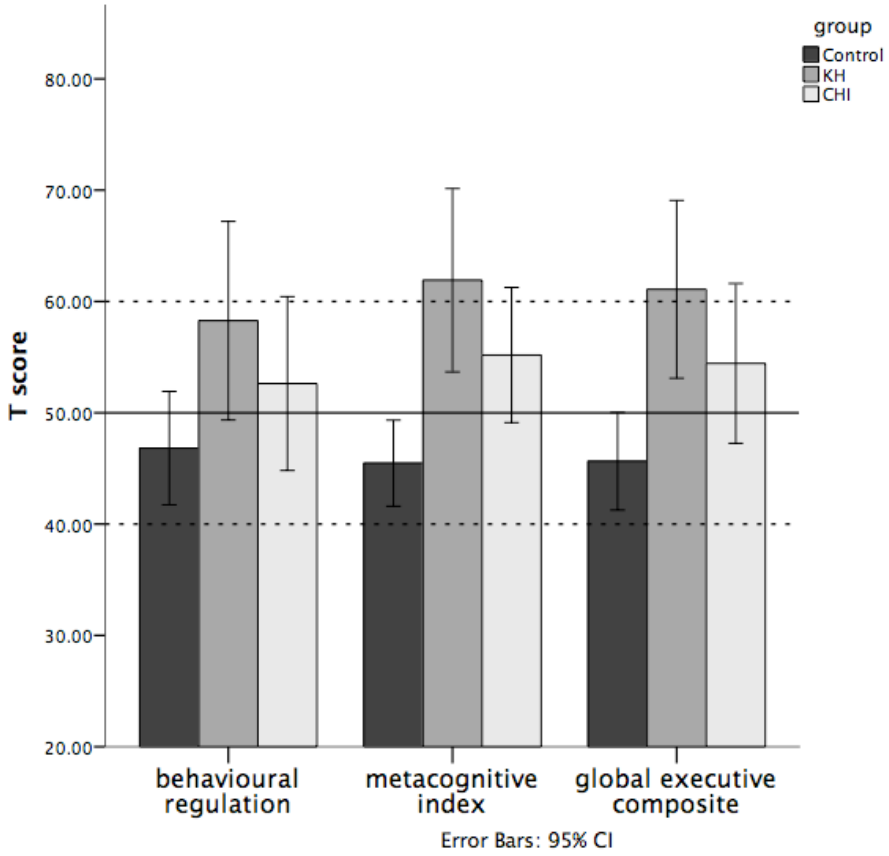

Figure 3.5. Parental ratings of executive function in the form of behavioural regulation, metacognition and the global executive composite derived from the BREIF. T-scores are shown for healthy controls, patients with KH and patients with CHI. Higher T-scores indicate increased reports of dysfunction. Solid line indicates the standard population mean, dotted lines indicate one standard deviation from the mean. Error bars represent 95% confidence intervals. Relative to the standard population mean, patients with KH have significantly higher T-scores in the metacognitive index and global executive component, while patients with CHI show trends for significantly increased T-scores in the metacognitive index.

Working memory

One healthy control did not complete the WISC-IV Working Memory subtest; therefore data for 17 healthy controls are available here. Univariate analyses revealed a significant main effect of group $F_{(2,65)}$=4.8, p=0.01, with post-hoc Bonferroni corrected comparisons indicating that patients with CHI performed significantly worse than healthy controls (p=0.009, Figure 3.6).

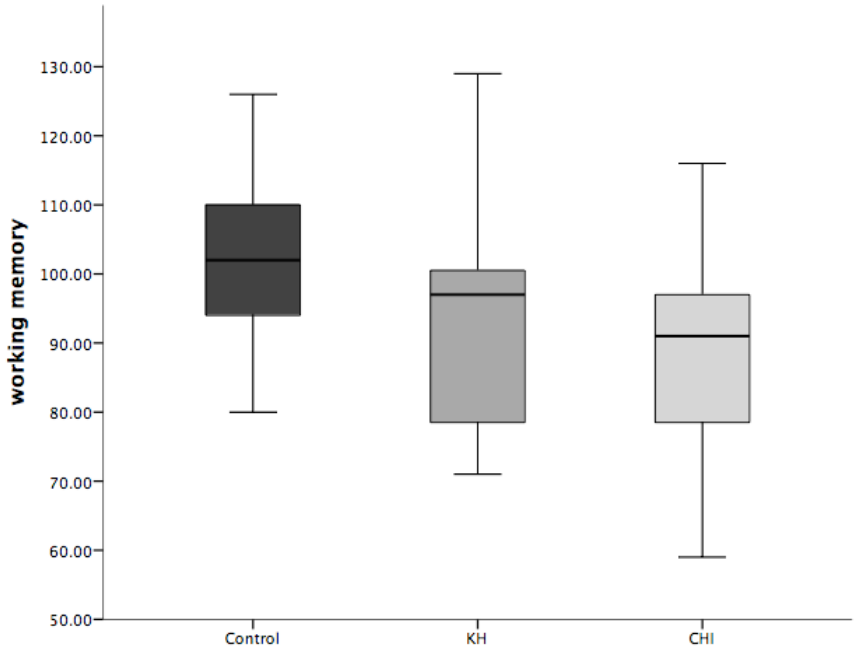

Figure 3.6 Box-and-whisker plot showing WISC-IV Working Memory performance in healthy controls, patients with KH and patients with CHI. Boxes represent upper and lower quartiles of scores. Solid line within box represents the median. Whiskers represent the range of scores. Patients with CHI have significantly lower scores on this working memory assessment compared the standard population mean of 100. Trends for significantly lower scores relative to the standard population mean were observed in patients with KH.

One-sample t-tests against the standard population mean of 100 were conducted to verify the performance of both patient groups. In patients with KH trends were observed for lower working memory ($t=-2$, $p=0.055$), in contrast to patients with CHI who showed significantly lower working memory scores ($t=-4.8$, $p<0.001$).

Exploring working memory difficulties

To explore decrements in working memory, a comparison of group performance on WISC-IV Working Memory subtests was conducted. Main effects were considered significant at 0.025 after Bonferroni correction for multiple comparisons. Univariate analysis of Digit Span (combined forward and backward) performance indicate a

significant main effect of group $F_{(2, 65)}$=7.2, p=0.002. Bonferroni corrected post-hoc comparisons showed that patients with CHI performed significantly worse than healthy controls (p=0.001). No significant main effect of group was observed for performance on the letter-number sequencing subtest (H[2]=2.6, p=0.272).

To follow up the significant differences observed in digit span, a comparison of performance between digit span forwards and digit span backwards was conducted[20.] In traditional models of working memory, digit span forward performance is thought to reflect the capacity of the phonological loop, while digit span backwards is considered to rely on the central executive component of working memory, requiring executive control to hold information online while performing a mental operation (Alloway, Gathercole, Kirkwood, & Elliott, 2009; Baddeley & Hitch, 1974; Gathercole et al., 2008). Main effects were considered significant at 0.025 after Bonferroni correction for multiple comparisons. A trend for differences between groups on the digit span backward subtest was observed (H[2]=5.7, p=0.058). However, a significant difference between group means was observed for digit span forward (H[2]=9.7, p=0.008). Post-hoc Bonferroni corrected pairwise comparisons showed that patients with CHI performed significantly worse on the digit span forwards subtest than healthy controls (p=0.006), while no other comparisons were significant (Table 3.12, Figure 3.7).

[20] Data for DSF and DSB were available for the smaller cohort of patients (CHI n=19 and KH n=12)

To verify these effects one-sample t-tests against the standard population mean of 10 (considered significant at 0.025) were conducted. Here, patients with KH show a trend for lower scores on the letter-number sequencing subtest (t=-2, p=0.06), but otherwise have preserved ability in digit span performance and its components. In contrast, patients with CHI had scores significantly below the standard population mean on the digit span subtest (t=-5, p<0.001), and also on the letter number sequencing subtest (t=-3.2, p=0.003). Further analyses of the digit span subtest confirmed a significantly lower score in digit span forward (t=-5, p<0.001) and a trend for a significantly lower mean score in digit span backwards (t=-2.2, p=0.043).

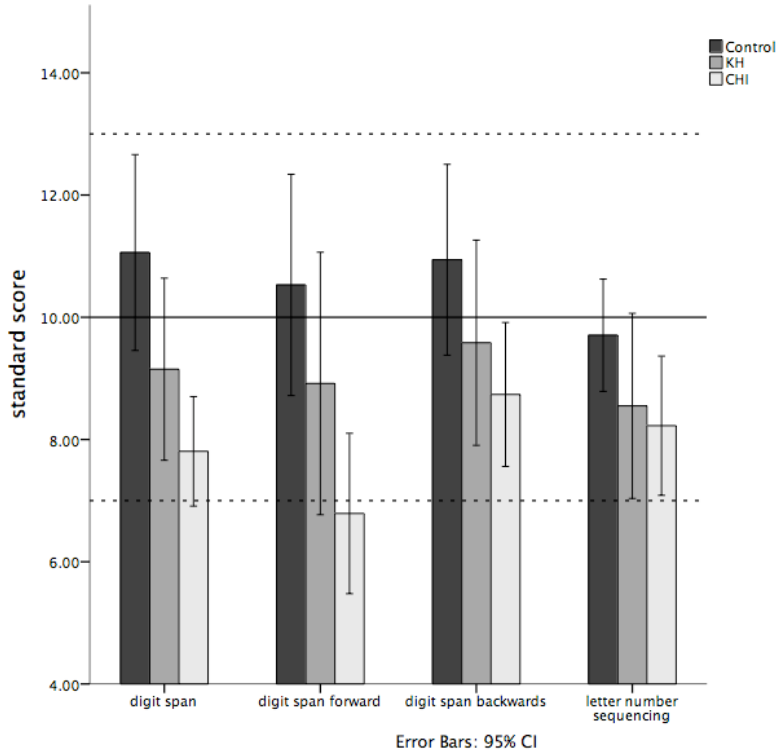

Figure 3.7 Working memory subtest performance in healthy controls, patients with CHI and patients with KH. Solid line indicates the standard population mean, dotted lines indicate one standard deviation from the mean. Error bars represent 95% confidence intervals. Relative to the standard population mean, patients with CHI have significantly lower scores on the digit span subtest and its components, and the letter number sequencing subtest. Patients with KH show trends for significantly lower scores on the letter number sequencing subtest.

Table 3.12 Mean and SD of working memory subtests.

Test	Controls (mean, SD)	KH (mean, SD)	CHI (mean, SD)
Digit span	N=17, 11.1 (3.1)	N=20, 9.2 (3.2)	N=31, 7.8 (2.4)
Letter-number sequencing	N=17, 9.7 (1.8)	N=20, 8.6 (3.2)	N=31, 8.2 (3.1)
Digit span forward	N=17, 10.5 (3.5)	N=12, 8.9 (3.6)	N=19, 6.8 (2.8)
Digit Span backward	N= 17 10.9 (3.1)	N=12, 9.6 (2.8)	N=19, 8.7 (2.5)

Self-Ordered Pointing Task

To further explore difficulties in working memory, the SOPT was administered to the small cohort of patients[21]. The SOPT is a complex visual working memory task (Cragg & Nation, 2007; Geva, Cooper, Gadian, Mishkin, & Vargha-Khadem, 2016; Petrides, Milser, Petrides, & Milner, 1982). Here, a number of variables were analysed. Following on from published procedures (Geva et al., 2016) for analysis of this adaptation of the original test, the following variables were analysed (a) average error score: number of errors in each condition, averaged over three trials, divided by the number of items -1; (b) error rate: average error according to trial order, resulting in the error rate in trial one of all conditions (4-, 6-, 8-, 10- and 12- item conditions), error rate in trial two of all conditions, and error rate in trial three of all conditions (as specified in Geva et al., 2016). Therefore, the average error score represents performance on a particular memory load, while error rate represents the effect of trial order.

[21] Data were unavailable for one patient with KH, therefore 11 patients with KH are included in this sample

Average error score

Data were analysed using a mixed-design ANCOVA with a within-subjects measure of condition (five levels) and a between-subjects factor of group (control, KH or CHI), with age added as a covariate (to control for the likely development in working memory ability across childhood). For condition, Mauchly's test indicated that the assumption of sphericity had been violated ($x^2(9)=33.9$, $p<0.001$); therefore degrees of freedom were corrected using greenhouse-geisser estimates of sphericity. Main effects of condition $F_{(2.7, 120.8)}=3.28$, $p=0.027$, and group $F_{(2,44)=}3.85$, $p=0.029$ were found to be significant, but no interaction between condition and group was observed.

Main effect of group

Post-hoc Bonferroni corrected comparisons showed that overall patients with CHI performed significantly worse than controls ($p=0.03$), while no differences were observed between healthy controls and patients with KH. Therefore, further comparisons were only conducted between healthy controls and those with CHI. Post-hoc independent t-tests comparing patients with CHI to healthy controls showed that there was a trend for higher error scores in patients on the 4-item trial ($t=-1.8$, $p=0.084$), and significantly higher error scores on 6 ($t=-2.5$, $p=0.017$), 8 ($t=-2.2$, $p=0.034$), 10 ($t=-2$, $p=0.048$), and 12 ($t=-2.48$, $p=0.019$) item conditions (Figure 3.8, Table 3.13). These results were replicated when controlling for age using an ANCOVA.

Main effect of condition

Post-hoc comparisons examining the main effect of condition (in all groups) showed that compared to the 4-item condition, significantly more errors were made on 6-

(p=0.007), 10 (p=0.007) and 12 (p=0.001) item conditions. Performance on the 8-item condition was not significantly different to performance on any of the other item load conditions, although a trend for worse performance on the 12-item condition compared to the 8-item was observed (p=0.076).

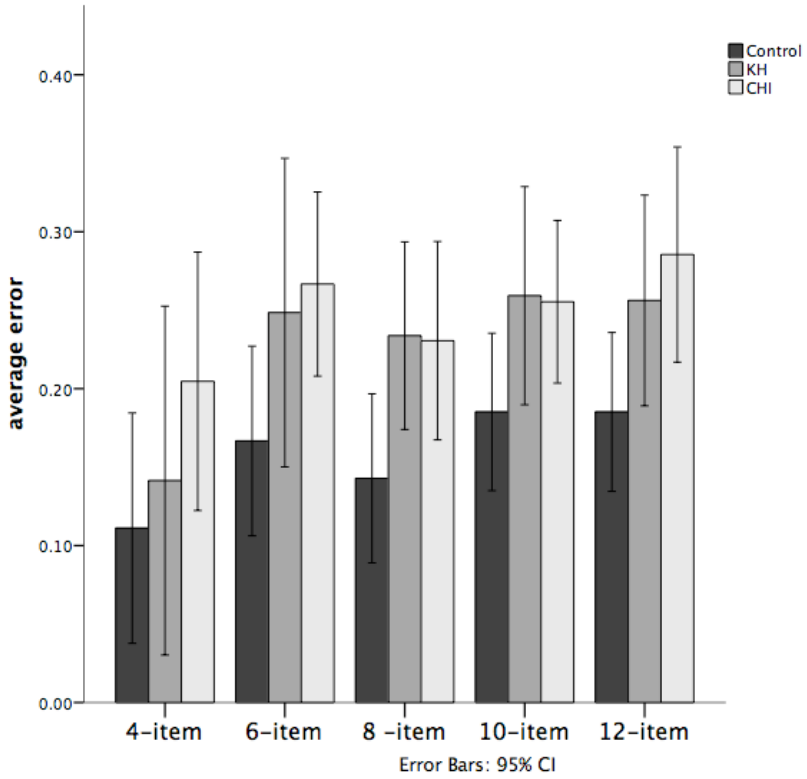

Figure 3.8 Average error scores for each item load in healthy controls, patients with KH and patients with CHI. Error bars represent 95% confidence intervals. Patients with CHI had significantly higher error scores compared to healthy controls on 6-, 8-, 10- and 12-item conditions, while trends for significantly higher error scores on the 4 item condition was observed.

Table 3.13 average error score in each condition of the SOPT. $^{+}$ significant at trend level ($p<0.09$)

Items	Group	Average error score (SD)	Significance
4	Control	0.11 (0.15)	Control<CHI^{+}
	KH	0.14 (0.17)	
	CHI	0.2(0.17)	
6	Control	0.17 (0.12)	Control<CHI
	KH	0.25 (0.14)	
	CHI	0.27 (0.12)	
8	Control	0.14 (0.11)	Control<CHI
	KH	0.23 (0.09)	
	CHI	0.23 (0.13)	
10	Control	0.19 (0.10)	Control<CHI
	KH	0.26 (0.10)	
	CHI	0.26 (0.11)	
12	Control	0.19 (0.1)	Control<CHI
	KH	0.26 (0.1)	
	CHI	0.29 (0.14)	

Effect of trial number

To examine whether proactive memory interference was occurring, error rates across trials (i.e. all errors in the first trial across condition, all errors in the second trial across conditions, etc.) were compared in a 3 x 3 multivariate test with age added as a covariate (trial, three levels and group, three levels). This revealed a significant main effect of group ($F=4.05$, $p=0.024$) but no significant effect of trial ($F<1$) and no significant group by trial interaction ($F<1$). Post-hoc pairwise comparisons corrected for multiple comparisons using the Bonferroni method showed that patients with CHI performed significantly worse that controls across trials ($p=0.02$, Figure 3.9.) but that their error rates did not differ between trials. No significant differences between healthy controls and patients with KH were observed.

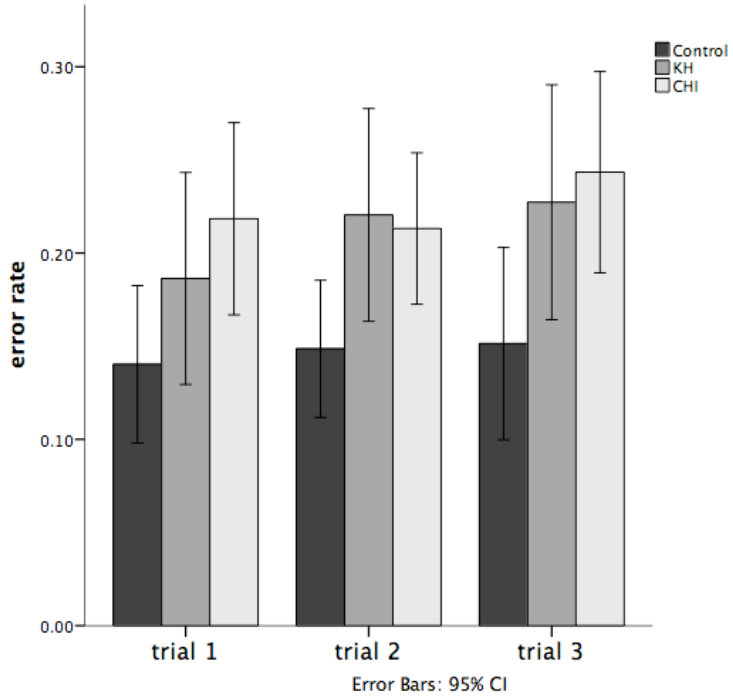

Figure 3.9 Error rates by trial in controls, patients with KH and patients with CHI. Error bars represent 95% confidence intervals. Patients with CHI performed significantly worse than healthy controls across trials, but error rates did not significantly differ between trials.

To examine the contribution of WISC-IV general cognitive ability (GAI) and working memory (WM) ability to SOPT performance, a hierarchical linear regression was conducted across patient groups and also in the control group. General ability, WISC-IV WM scores and age were entered as predictors to explain variance on the total error score of the SOPT. The final model was significant (R^2=0.43, $F_{(3,27)}$=6.67, p=0.002). Examination of coefficients indicated that working memory ability as measured by the WISC-IV significantly predicted the number of errors made on the SOPT, but that neither GAI or age were significant predictors of performance. The same model applied in healthy controls was unable to predict performance on the SOPT (R^2=0.19, $F_{(3,13)}$=0.7, p=0.41). Results are presented in Table 3.14.

Table 3.14 linear model of predictors of patient performance on the SOPT

	b	SEB	β	P
Constant	56.29	11.88		<0.001
GAI	-0.32	0.12	-0.44	0.014
Constant	69.24	10.87		<0.001
GAI	-0.03	0.13	-0.04	0.822
WM	-0.46	0.14	-0.63	0.002
Constant	68.76	16		0.001
GAI	-0.03	0.15	-0.04	0.861
WM	-0.46	0.14	-0.63	0.003
Age	0.002	0.058	0.007	0.968

In summary, patients with CHI show pervasive executive working memory deficits that are identifiable on this experimental working memory paradigm, success of which is dependent on monitoring and updating a sequence. They perform below the level of healthy controls on every load above the elementary 4-item load. Although not significantly different from controls, patients with KH have very similar error rates compared to those with CHI on all item load conditions apart from the 4-item load (Figure 3.8, Table 3.13.), and they show considerable variability in their performance (Figure 3.8). Linear regression showed that working memory as measured by the WISC-IV significantly predicted error rates on the SOPT, but that general cognitive ability was not a significant predictor of error scores.

No evidence of proactive memory interference was observed, with comparable performance on trials one, two and three. This was an effect that was observed in each group. Cragg et al. (2007) observed a similar effect in their study of typically developing children between the ages of five and 11, where no difference in error rates were observed between trials in an abstract designs condition. In contrast to the lack of effect of trial number on abstract designs, Cragg et al. (2007) found an effect of trial number in the object condition. They suggested that this could be the effect of verbalising, resulting in the creation of stronger memory traces and therefore leading to proactive interference. As well as a lack of proactive memory interference, these data do not show evidence of worse performance with increasing memory load. With the exception of the 4-item condition, error scores on six, eight, 10 and 12 item conditions were similar (note that when calculating the error score the number of items within each condition was controlled for).

These analyses have shown that children with CHI have profound working memory deficits across all working memory subtests, both standardised and experimental. While children with KH do not show detectable impairment on the subtests of the WISC-IV working memory assessment, there is a trend towards impairment on the task as a whole as well a trend toward impaired performance on letter-number sequencing. In addition, although not statistically significant, compared to healthy controls children with KH have higher error scores on every condition of the SOPT. This suggests that working memory ability is not entirely preserved in patients with KH, especially the component of working memory that is considered to depend on the central executive.

Attention

Parental reports of attention

Analysis of parental ratings from the Conners 3 (main effects considered significant at $p<0.007$) showed main effects of group on the Inattention scale ($H[2]=10.7$, $p=0.005$) and Executive Functioning scale ($H[2]=10.1$, $p=0.006$). Trends were observed for significant main effects on the Learning scale ($p=0.03$) and the Global Index scale ($p=0.016$). Bonferroni-corrected post-hoc comparisons indicated that controls had lower ratings on the Inattentiveness scale than patients with KH ($p=0.03$) and patients with CHI ($p=0.01$). Controls also had significantly lower ratings on the Executive Functioning scale than patients with KH ($p=0.005$), while no differences were observed between patients with CHI and controls (Figure 3.10). Analysis of the DSM-IV Symptom scale scores showed a main effect of group on symptom ratings for ADHD Inattentive Type ($H[2]=14.2$, $p=0.001$), with controls having lower scores and therefore showing fewer symptoms related to ADHD (inattentive type) than patients with KH ($p=0.001$) and patients with CHI ($p=0.018$) (Figure 3.11).

One-sample tests against the standard mean of 50 confirmed that as a group, patients with KH had higher scores on the Inattentive scale ($t=3.5$, $p=0.006$) and Global Index Scale ($t=3.5$ $p=0.003$), indicating problems in these areas. They also showed significantly increased scores on the Symptom Scale of ADHD inattentive type ($t=3.4$, $p=0.006$). In contrast to the comparison with healthy controls, patients with KH also showed trends for higher scores on the Hyperactivity Scale ($Z=2.3$ $p=0.02$) and the Symptom Scale of ADHD Hyperactive/Impulsive Type ($Z=2.1$ $p=0.032$). Furthermore, patients with KH showed trends for higher scores on the Oppositional Defiant Disorder

(ODD) Symptom Scale (t=2.3, p=0.048). One sample t-tests in patients with CHI also confirmed significantly higher scores on the inattentive scale (t=3.4, p=0.004) and, corroborating this, trends for significantly increased scores on the DSM-IV ADHD (inattentive type) symptom scale (t=2.8 p=0.014).

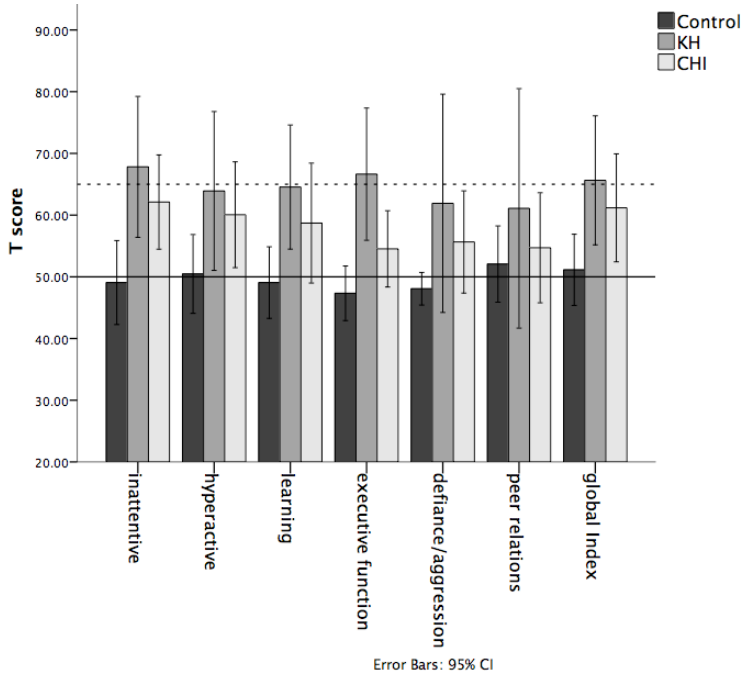

Figure 3.10 Conners 3 content scale T-scores. Scores are shown for healthy controls, children with KH and children with CHI. Higher scores indicate increased rating of dysfunction. Error bars represent 95% confidence intervals. Dotted line indicates cut off for clinical significance. Scores above 65 are clinically significant.

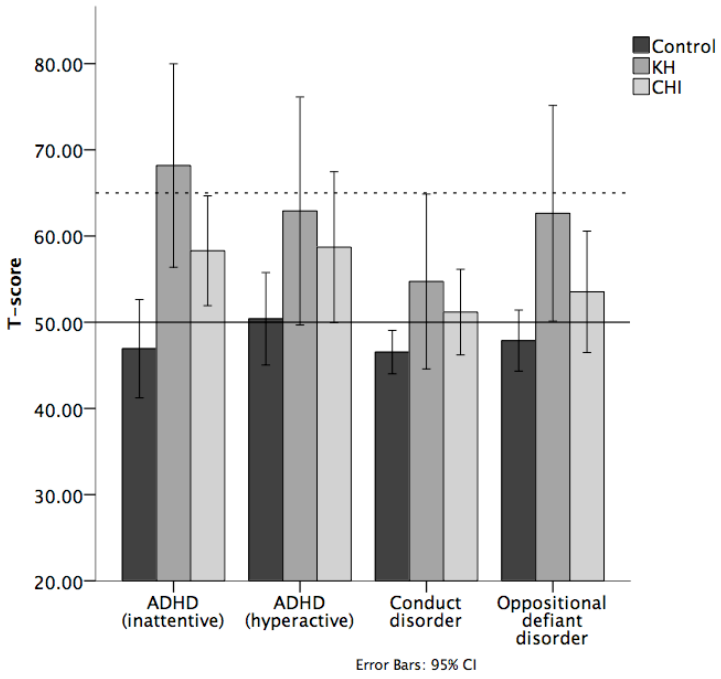

Figure 3.11 Symptom scale T-scores from the Conners 3. Solid line represents the standard population mean of 50. Dotted line represent cut off point for clinical significance; scores above 65 are in the clinical range. Error bars represent 95% confidence intervals. Scores are shown for healthy controls, patients with KH and patients with CHI. Relative to the standard population mean of 50, patients with KH have significantly increased scores on ADHD (inattentive) and ADHD (hyperactive) symptom scales. Relative to the standard population mean of 50, patients with CHI have significantly increased scores on the ADHD (inattentive) symptom scale.

Test of Everyday Attention for Children (TEA-Ch)

Univariate Kruskal-Wallis tests were carried out and main effects for the core subtests were considered significant at p=0.01 after correction for multiple comparisons. Post-hoc tests were corrected for multiple comparisons using the Bonferroni method. Significant differences between groups were observed in attentional control: set switching (creature counting) and attentional control: inhibition (opposite worlds), with both patient groups obtaining lower scores than healthy controls (Table 3.15). Significant effects were also observed on the dual task measure, driven by significant differences between patients with CHI and controls. These results are shown in Figure 3.12.

Analysis of performance on supplemental measures (speed of identifying visual targets in the selective attention paradigm 'time-per-target'; the accuracy of responses in the creature counting attentional control/set switching subtest 'creature counting accuracy') revealed significant differences between groups. A main effect of group was found on the 'time-per-target' measure ($H[2]= 7.2$, $p=0.028$), and Bonferroni corrected post-hoc comparisons showed that patients with KH took significantly longer to identify targets than controls ($p=0.042$) with a trend for differences between controls and patients with CHI ($p=0.065$). In addition, significant differences between groups were observed on the accuracy of performance on the attentional control (creature counting) subtest ($H[2]=6.4$, $p=0.041$). Bonferroni corrected post-hoc comparisons showed that patients with CHI made significantly more errors on this test than healthy controls ($p=0.035$).

One sample t-tests (considered significant at $p=0.01$ for core measures) were conducted to confirm the results indicated above. These showed that patients with KH perform significantly below standard population means on the test of selective attention ($t=-3$), attentional control: set switching ($Z=-2.9$) attentional control: inhibition ($t=-4$), as well as the on the dual task measure ($Z=-3.3$, $p<0.008$ for all tests). Comparison of patients with CHI against the standard population mean indicated significantly lower scores in selective attention ($t=-2.8$) sustained attention ($t=-5.7$), attentional control: set switching ($t=-3.7$), attentional control: inhibition ($t=-7.1$) and dual task performance ($Z=-4.8$, $p<0.01$ for all tests).

Table 3.15 Performance on the TEA-Ch

Test	N	Mean (SD)	ANOVA F, P	Pairwise comparisons
Selective attention				
Controls	18	99 (8)	H=4.8, p=0.12	
KH	20	93 (10.9)		
CHI	31	94 (11)		
Sustained attention				
Controls	18	97 (14.1)	H=5, p=0.08	
KH	20	93 (18.9)		
CHI	31	86 (13.9)		
Attentional control : Set Switching (creature counting)				
Controls	17	100 (10.9)	H=9.7, p=0.008	
KH	19	88 (12)		Controls>KH, p=0.039
CHI	26	89 (15.3)		Controls>CHI, p=0.009
Attentional control : Inhibition (opposite worlds)				
Controls	17	101 (12.7)	H=19.7, p<0.001	
KH	12	82.5 (15.2)		Controls>KH, p=0.005
CHI	19	78.4 (13.2)		Controls>CHI, p>0.001
Dual task				
Controls	17	89 (10.8)	H=8.8, p=0.013	
KH	19	79.7 (18.7)		Controls>CHI, p=0.01
CHI	31	73.1 (16)		

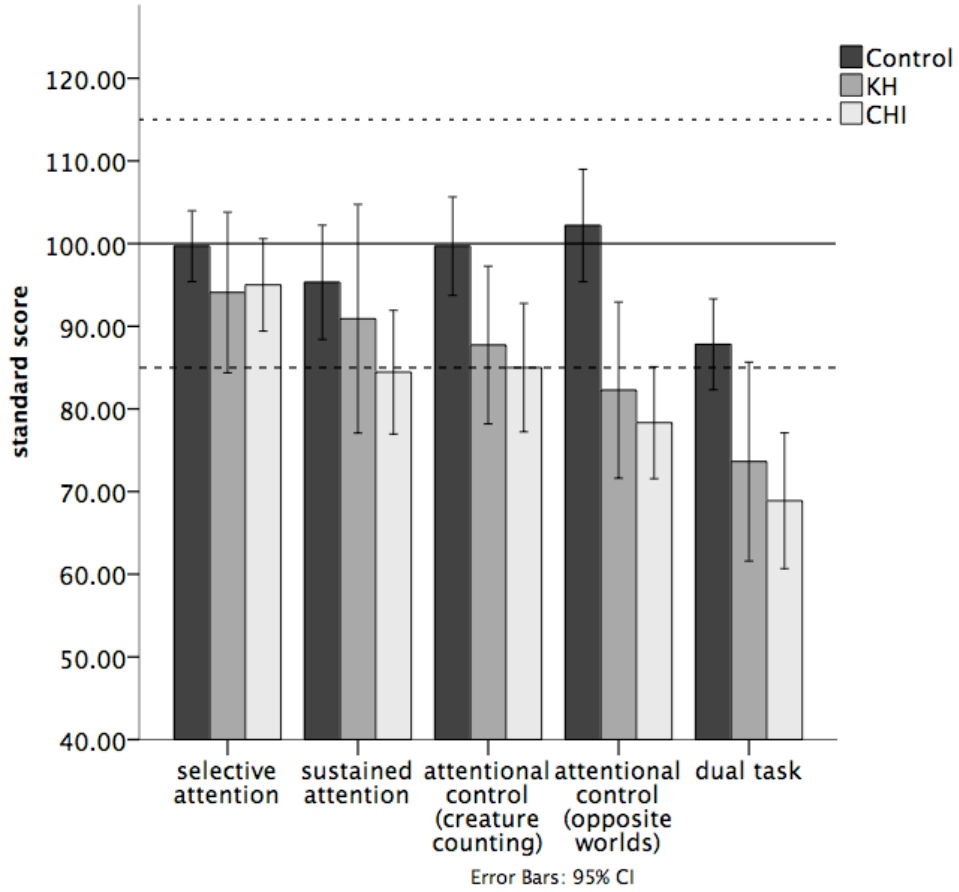

Figure 3.12 Performance on the subtests of the TEA-Ch. Mean scores of healthy controls, patients with KH and patients with CHI are shown. Solid line indicates the standard population mean, dotted lines indicate one standard deviation from the mean. Error bars represent 95% confidence intervals. Compared to the standard population mean of 100, patients with KH show significantly lower scores on measures of selective attention, attentional control and dual task ability. Patients with CHI had significantly lower scores than the standard population mean on all of the subtests of the TEA-Ch.

Continuous Performance Test (CPT)

Kruskal-Wallis tests (main effects considered significant at 0.006 after Bonferroni correction for multiple comparisons) showed no significant main effects that survived correction for multiple comparisons. However, trends for significant differences between groups in Detectability (D') ($F_{(2,46)}$=6.5, p=0.038), and Commissions, ($F_{(2,46)}$=8.2, p=0.016), were observed. Post-hoc Bonferroni corrected comparisons

indicated that patients with KH had significantly higher t-scores (indicating worse performance) than controls in D' (p=0.032) and Commissions (p=0.012, Table 3.9). No other comparisons were significant. These results can be seen in Table 3.16. Figures 3.13 and 3.14 show performance of all three groups.

To confirm these results, comparison to the standard population mean of 50 was conducted using one-sample tests. No results survived correction for multiple comparisons (p=0.006), but patients with KH showed trends for significantly higher t-scores in D' (t=2, p=0.041) Omissions (t=2.7, p=0.024), Commissions (t=2, p=0.05), Hit Reaction Time (HRT) standard deviation (t=2.6, p=0.009) and HRT Inter-Stimulus Interval (t=2.5, p=0.033). In patients with CHI trends for significantly higher t-scores were observed for D' (t=2.2, p=0.039) and Hit RT (t=2.3, p=0.032). A weak trend for differences in HRT block change was observed (t=1.9, p=0.058).

Table 3.16 Variables obtained from the CPT

Test	N	Mean (SD)	ANOVA F, P	Pairwise comparisons
Detectability (D')				
Controls	16	49 (9.9)	H=6.5, p=0.038	Control<KH, p=0.032
KH	11	58 (10.5)		
CHI	19	54 (7.6)		
Omissions				
Controls	16	52 (13)	H=3.8, p=0.15	
KH	11	61 (14)		
CHI	19	54 (13.5)		
Commissions				
Controls	16	48 (8.6)	H=8.2, p=0.016	Control<KH, p=0.012
KH	11	56 (8.1)		
CHI	19	52 (7)		
Perseverations				
Controls	16	52 (12.4)	H=1.4, p=0.489	
KH	11	56 (13.3)		
CHI	19	52 (10.4)		
Hit Reaction Time				
Controls	16	53 (9.3)	H=0.5, p=0.784	
KH	11	55 (14.3)		
CHI	19	55 (9.1)		
Hit reaction time standard deviation				
Controls	16	54 (13.4)	H=2.9, p=0.23	
KH	11	61 (14.7)		
CHI	19	55 (12.9)		
Hit reaction time block change				
Controls	16	51 (12.4)	H=1.8, p=0.41	
KH	11	58 (16.2)		
CHI	19	58 (14.5)		
Hit reaction time inter stimulus interval				
Controls	16	51 (9.9)	H=4.6, p=0.103	
KH	11	62 (16.1)		
CHI	19	53 (9.6)		
Variability				
Controls	16	55 (15.5)	H=1.9, p=0.388	
KH	11	56 (9.6)		
CHI	18	52 (12.4)		

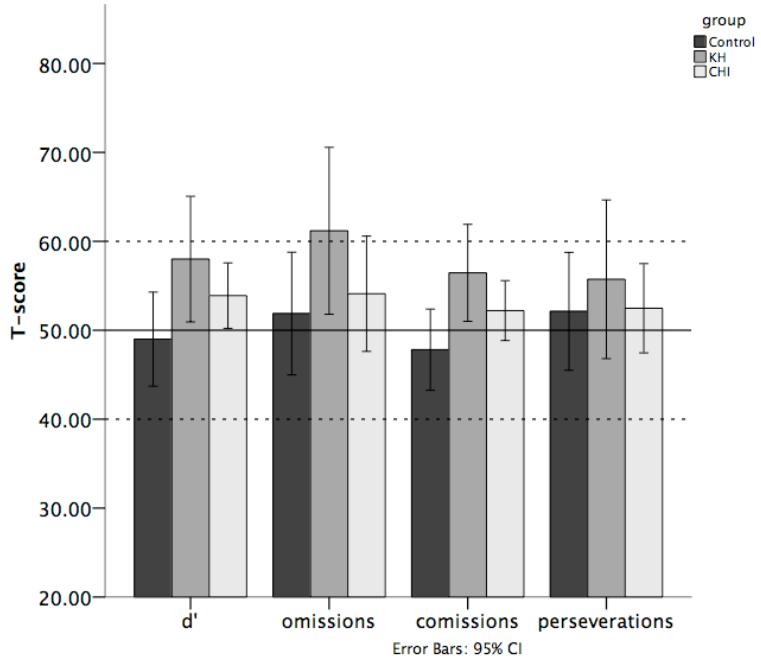

Figure 3.13 Performance on four main measures of the Conners CPT. Solid line indicates the standard population mean, dotted lines indicate one standard deviation from the mean. Error bars represent 95% confidence intervals. Relative to the standard population mean, both patient groups showed trends towards elevated scores on d'.

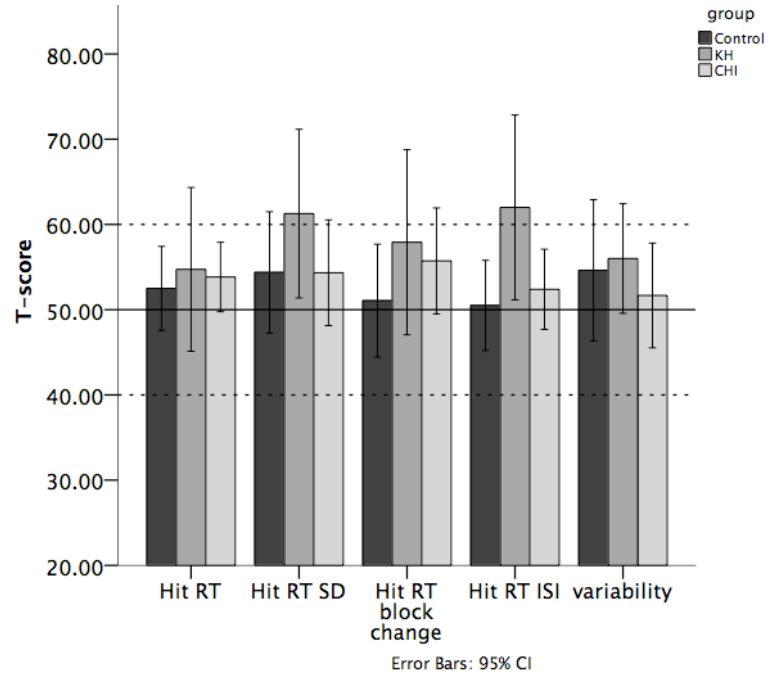

Figure 3.14 CPT performance measures dependent on reaction time. Solid line indicates the standard population mean, dotted lines indicate one standard deviation from the mean. Error bars represent 95% confidence intervals.

In summary, both patient groups exhibit significant impairments in attention as measured by the TEA-Ch, with shared impairments in selective attention, attentional control (set switching and inhibition) and executive (dual task) attention. Furthermore, both patient groups obtained significantly elevated scores on the detectability measure of the CPT. While this did not survive correction for multiple comparisons, given the attentional impairment identified on the TEA-Ch, these results are not considered to be false positives. Indeed, the results of the CPT seem to corroborate those of the TEA-Ch; in patients with CHI only, a decrement was observed on the sustained attention task, accompanying trends for a decrement in the sustained attention measure (HRT block change) of the CPT, while patients with KH appear to have preserved functioning on these two tests of sustained attention. In addition, in accordance with the results from both the assessments of attention (pointing to attentional deficits in both patient groups), parental ratings of items in the inattentive content scales were elevated in both patient groups (Figure 3.10), as well as reporting a higher occurrence of ADHD inattentive type symptoms (Figure 3.11). These findings were replicated when comparison to the standard population mean was made, indicting the robustness of these effects.

Processing speed

A Kruskal-Wallis test showed a significant main effect of group ($H(2)=16.8$, $p<0.001$) in WISC-IV Processing Speed scores. Post-hoc Bonferroni corrected t-tests showed that patients with CHI performed significantly worse than controls ($p<0.001$) as did patients

with KH (p=0.01; Figure 3.15). One-sample t-tests against the standard population mean of 100 were conducted to verify the performance of both patient groups. In patients with KH trends were observed for significantly lower processing speed scores than the standard population mean (t=-1.9, p=0.079). Patients with CHI show significantly lower scores than the standard population mean (t=-3.6 p<0.001).

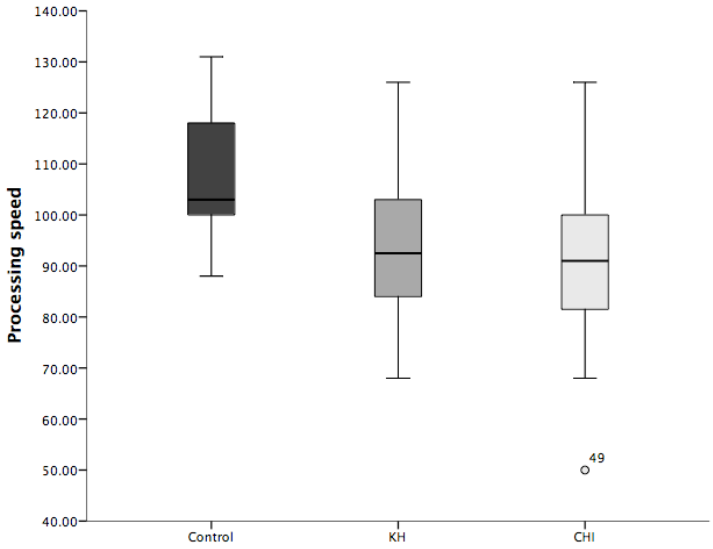

Figure 3.15 Box-and-whisker plot showing processing speed scores in each group. Boxes represent upper and lower quartiles of scores. Solid line within box represents the median. Whiskers represent the range of scores. Patients with CHI have significantly lower scores compared to the standard population mean of 100. A trend towards significantly lower scores compared to the standard population mean was observed in patients with KH.

Relationship between working memory, processing speed and attentional processes

To examine the associations between scores on these three tests, and their dependence on general cognitive ability, a partial correlation controlling for GAI was conducted. This was conducted in the healthy control group, and then across a collapsed patient group (in the large cohort of patients). The subtests of the TEA-Ch are assumed to tap into different executive functions (Manly et al., 2001); selective attention is proposed to represent attentional control, as is the creature counting (set

switching) measure. Sustained attention falls under cognitive flexibility and is dependent on the ability to maintain and update a count. Dual task ability also falls under cognitive flexibility. In patients, working memory and processing speed abilities were associated with performance on selective attention and set switching ability independent of general intellectual ability, while sustained attention performance was related to working memory ability independent of general intellectual ability (Table 3.17). In healthy controls no significant relationships between variables remained after controlling for general intellectual ability. These results are suggestive of a domain general executive dysfunction in patient groups that is independent of cognitive ability.

Table 3.17 Correlations between WM, PS and TEA-Ch variables in patients after controlling for general ability *=significant at 0.05 **=significant at 0.01 $^{+}$=trend ($p<0.09$)

	Working memory	Processing speed	Selective attention	Sustained attention	Set switching	Dual task
Working memory	r=1	r=0.23	r=0.35*	r=0.40**	r=0.38**	r=0.23
Processing speed	r=0.23	r=1	r=0.45**	r=0.15	r=0.37*	r=0.28^{+}

Planning

D-KEFS Tower

Univariate analyses (ANOVA or Kruskal-Wallis tests) were considered significant at 0.01 after Bonferroni correction for multiple comparisons. No significant differences were observed between groups on the total achievement score, first move time, time per move, or move accuracy ratio subtests. The only significant difference between groups was in the rule violations subtest ($H(2)=12.3$, $p=0.002$), with post-hoc Bonferroni corrected comparisons showing that controls made significantly fewer rule violations than those with KH ($p=0.038$) and those with CHI ($p=0.003$), Table 3.18).

One sample t-tests against the standard population mean showed that there was a trend for quicker first move times in patients with KH ($t=2.02$, $p=0.014$) but no other comparisons were significant. Similarly, significantly quicker first move times were observed in patients with CHI when compared to the reference mean ($t=2.48$, $p=0.011$), while no other comparisons were significant.

Table 3.18 Performance on the Tower subtest

Test	N	Mean (SD)	ANOVA F, P	Pairwise comparisons
Tower Achievement				
Controls	17	101 (13.2)	ns	
KH	12	106 (14.5)		
CHI	17	101 (7.7)		
First move time				
Controls	17	103 (11.7)	ns	
KH	12	108 (10.1)		
CHI	17	106 (8.6)		
Time-per-move				
Controls	17	106 (6.3)	ns	
KH	12	106 (7.4)		
CHI	17	103 (6.3)		
Move accuracy ratio				
Controls	17	96 (10)	ns	
KH	12	90 (13.7)		
CHI	17	94 (14.6)		
Rule violations				
Controls	17	104 (2.2)	H=12.3, p=0.002	
KH	12	98 (7.5)		Control>KH (p=0.038)
CHI	17	97 (7.1)		Control>CHI (p=0.003)

In summary, of all the scores of the tower task, the only measure on which patients deviated significantly from the standard population is the first move time, with patients showing significantly higher index scores than controls. This means that in both patient groups, initiation of the first move was quicker than in controls. A fast first move time is could reflect a lack of planning; these results could suggest that in both patient groups planning abilities might be compromised. In addition, while this was not replicated in the one-sample t-tests, both patient groups made significantly more rule violations than the healthy control group, pointing to a deficit in establishing

a rule set. Together with parental reports of impaired metacognition in both patient groups, this could indicate dysfunction in the area of planning.

3.3.4 Memory

Parental ratings of everyday memory function

Parental ratings[19] of everyday memory functioning, measured by the Sunderland memory questionnaire, were transformed into z-scores based on in-house data from 65 healthy controls ranging in age from eight to 16 years. High z-scores indicate a greater incidence of everyday memory problems as reported by parents. An ANOVA was conducted to examine differences between groups. As Levene's test was significant (indicating that assumption of homogeneity of variance had not been met) Welch's statistic is reported here. A main effect of group was observed $F_{(2, 21.8)}=11.6$, $p<0.001$. Post-hoc Bonferroni corrected comparisons indicated that patients with KH had significantly higher parental reports of everyday memory problems than controls ($p=0.001$), as did patients with CHI ($p=0.046$). There were no differences between the two patient groups.

To verify these results one-sample t-tests were used to compare scores against the mean of 0. These indicated that parents of patients with KH report a significantly increased incidence of everyday memory problems ($t=3.7$, $p=0.004$). In contrast, no

[19] Parental reports of everyday memory functioning were available for the small cohort of patients only

significant difference was observed between the ratings of those with CHI and the standardised mean of zero (results at trend level t=1.7, p=0.099) (Figure 3.16).

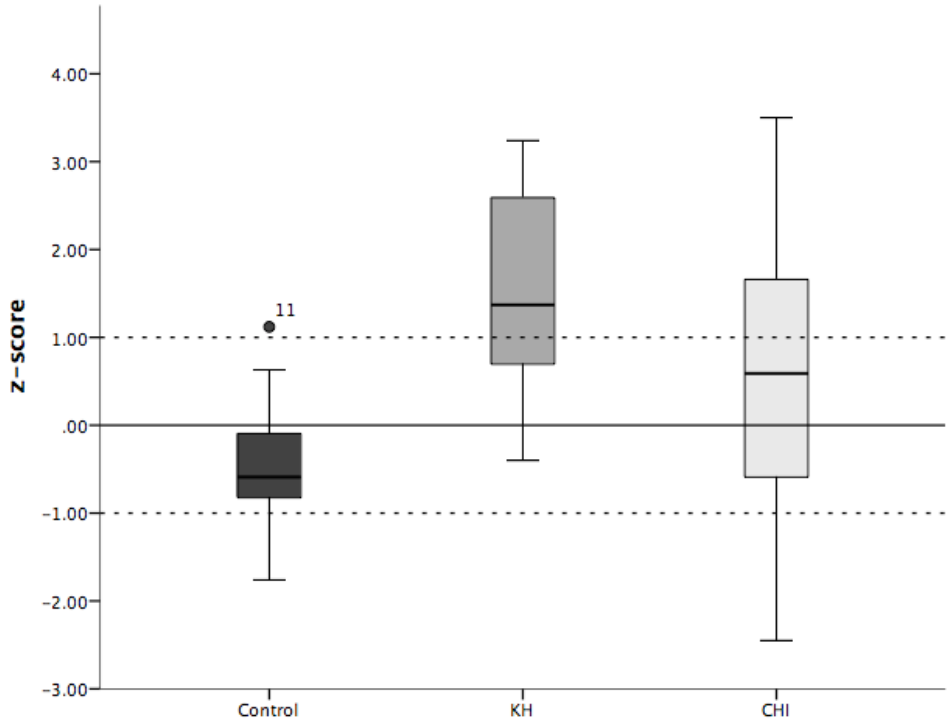

Figure 3.16 Box plot showing parental ratings of everyday memory functioning from the Sunderland. Boxes represent upper and lower quartiles of scores. Solid line within box represents the median. Whiskers represent the range of scores. Solid line in the graph represents the standard mean of 0. Dotted lines represent one standard deviation from the mean. Patients with KH have significantly greater reports of memory problems compared to the standard population

Children's Memory Scale

A MANOVA conducted on Visual Immediate, Visual Delayed, Verbal Immediate, Verbal Delayed and General Memory Index scores revealed a significant main effect of group ($F_{(5, 49)}$=3.7, p=0.006). Follow up separate univariate ANOVAs considered significant at p=0.01 showed main effects of group in visual immediate memory and general

memory (Table 3.19). Trends were observed for main effects in visual delayed and verbal delayed memory. Bonferroni corrected post-hoc comparisons showed that patients with CHI had significantly lower Visual Immediate memory scores than controls (p=0.012) and significantly lower Verbal Delayed memory scores compared to healthy controls (p=0.031). Patients with CHI also had significantly lower Verbal Immediate memory scores than controls (p=0.018), and lower General Memory scores than healthy controls (p<0.001) while a trend for differences in General Memory scores between patients with KH and healthy controls was observed (p=0.092). These results are presented in Table 3.19.

Analysis of the supplemental tests of the CMS (attention / concentration, learning and delayed recognition) using univariate ANOVAs (considered significant at 0.017 after correction for multiple comparisons) showed main effects for group differences in attention/concentration ($F_{(2,52)}$=8.3, p=0.001). Bonferroni corrected post-hoc comparisons showed that patients with KH had significantly lower scores compared to healthy controls (p=0.02), and that patients with CHI also had significantly lower scores on this measure than healthy controls (p=0.002).

Table 3.19 Memory scores in controls and patients

Test	N	Mean (SD)	ANOVA F, P	Pairwise comparisons
Visual Immediate				
Controls	17	107 (12.2)	F=5, p=0.011	Control>CHI p<0.01
KH	14	103 (12.6)		
CHI	24	95 (12.5)		
Visual Delayed				
Controls	17	106 (12.4)	H=8.2, p=0.038	Control>CHI, p=0.053
KH	14	104 (13.3)		
CHI	24	96 (10.5)		
Verbal Immediate				
Controls	17	112 (12.8)	H=6.6, p=0.017	Control>CHI, p=0.018
KH	14	98 (21)		
CHI	24	96 (15.4)		
Verbal Delayed				
Controls	17	112 (11.4)	F=4.98, p=0.014*	Control>CHI p=0.031
KH	14	102 (20)		
CHI	24	98 (17)		
General memory				
Controls	17	114 (12.5)	F=8.3, p=0.001	Control>KH p=0.092
KH	14	101 (18.8)		Control >CHI p<0.001
CHI	24	94 (15.8)		

*Homogeneity of variance violated: Welch's statistic reported here

The healthy control group performed above standard population means on all of the composite measures derived from the CMS (Figure 3.17). To verify the observed memory deficits in patients, one-sample tests (considered significant at p=0.01 for main measures and 0.017 for supplemental tests) were conducted. These showed that for all tests the average performance of patients in both groups was comparable to the reference mean of 100 (p>0.1 for all).

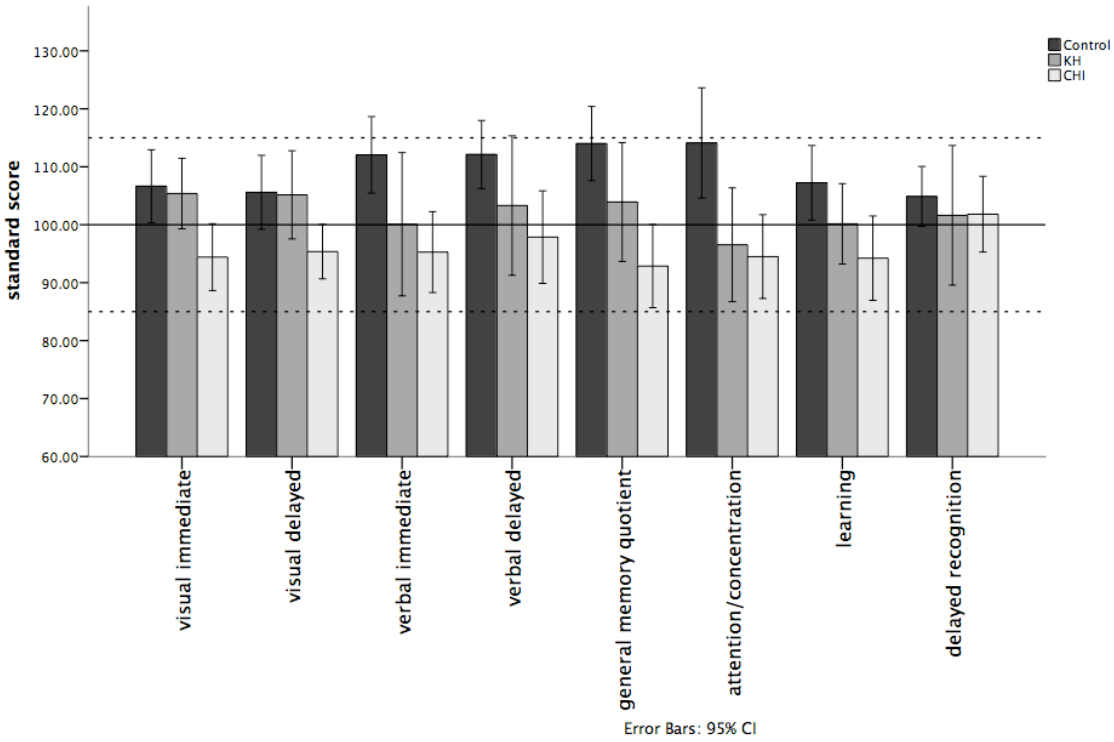

Figure 3.17 Memory performance on all subtests of the CMS. Solid line indicates the standard population mean, dotted lines indicate one standard deviation from the mean. Error bars represent 95% confidence intervals. Memory performance in both patient groups was comparable to the standard population mean.

Individual memory impairment

Predicted verbal and general memory scores are available from the WISC-IV based on a child's verbal intellectual ability, and criteria from the WISC-III can be used to determine whether the difference between predicted and actual memory scores is significant. Significance levels are not provided for WISC-IV data, but the predicted scores are highly similar between the two tests (Muñoz-López et al., 2017). Here, VIQ was used to predict verbal memory abilities and General Memory, and the difference between the actual-predicted score quantified. Despite this group-level preservation of memory abilities, in both patient groups there were individuals with significantly lower memory scores than predicted from their VIQ. In 25% of patients with CHI and 36% of patients with KH, Verbal Immediate memory scores were significantly lower

than predicted values from verbal IQ, while in healthy controls no participants had a lower Verbal Immediate memory than predicted by their verbal IQ (Table 3.20). Fisher's exact test confirmed that there was an association between group membership and whether Verbal Immediate memory performance was the same as, below or above predicted scores from IQ (f=11.9, p=0.01), with controls having less of a tendency to obtain scores significantly below their predicted scores. In both patient groups a considerable proportion of children obtained scores that were significantly below their predicted scores in Verbal Delayed memory (25% of CHI and 29% of KH compared with 6% of controls) and MQ (25% of CHI and 21% of KH compared with 6% of controls) but Fisher's exact test did not show a significant association between group membership and memory status (p>0.05).

Table 3.20 Predicted-actual memory discrepancies in each group

	Controls			KH			CHI		
	% above	% below	% no diff	% above	% below	% no diff	% above	% below	% no diff
Verbal Immediate	12	0	88	28	36	36	8	25	67
Verbal Delayed	23	6	71	21	29	50	21	25	54
General Memory	29	6	65	29	21	50	12	25	63

To summarise, no reliable evidence of group-level memory impairment has been observed in these two patient cohorts. However, the number of patients with memory functioning significantly below that predicted from the verbal IQ was greater than in the healthy comparison group. Combined with indications of impaired everyday memory functioning (an effect that is particularly pertinent in patients with KH) these results indicate that memory functioning is not completely preserved in patients with hypoglycaemia, and that immediate (short term) memory might be particularly vulnerable in these patients.

3.3.5 Academic attainment

Three children with KH obtained scores greater than 1 SD below the mean in the Numerical Operations subtest, and six obtained scores outside of this normal range on the test of Mathematical Reasoning. Ten children with CHI obtained scores greater than 1 SD below the mean in the Numerical Operations subtest, while seven obtained scores greater than 1 SD below the mean in the Mathematical Reasoning subtest. In the spelling subtest, three healthy control participants obtained scores greater than 1 SD below the standard population mean, while six children with KH obtained scores greater than 1 SD below the mean. Eleven children with CHI obtained scores greater than 1 SD below the mean in this spelling subtest.

Owing to missing data in healthy controls (on the mathematical reasoning subtest) a MANOVA was not performed. Univariate analyses were conducted and main effects were considered significant at 0.017 after correction for multiple comparisons.

Significant group differences in performance on the numerical operations subtest were observed $H(2)=9.46$, $p=0.009$ with Bonferroni corrected post-hoc tests showing that patients with CHI performed significantly worse on the numerical operations subtest than controls ($p=0.009$) as did patients with KH ($p=0.051$). Main effects for spelling and mathematical reasoning subtests did not survive correction for multiple comparisons, although trends for differences between groups were observed on both subtests (Table 3.21, Figure 3.18).

One-sample t-tests were carried out for confirmation of these observed effects. These indicated that patients with KH perform in line with the standard population on all assessments of academic attainment ($p>0.05$ for all). In patients with CHI, contrary to the comparison with healthy controls, the only significant difference observed was in the spelling subtest ($t=-2.8$ $p=0.009$).

In summary, despite significant differences between patients and controls on the numerical operations subtest, compared to standard population means the only significant group level impairment exists in spelling abilities in patients with CHI. However, in both patient groups there were individuals with scores that fell significantly below the average range.

Table 3.21 Academic attainment from the WIAT-II. [+] significant at trend level

Test	N	Mean (SD)	ANOVA F, P	Pairwise comparisons
Mathematical reasoning				
Controls	10	107 (9.9)	[+]F=3.2, p=0.048	[+]Control>KH, p=0.076 [+]Control>CHI, p=0.066
KH	20	93 (16.2)		
CHI	25	93 (16)		
Numerical operations				
Controls	18	113 (17.6)	H=9.46, p=0.009	Control>KH, p=0.051 Control>CHI, p=0.009
KH	19	99 (19.5)		
CHI	30	96 (19.4)		
Spelling				
Controls	16	104 (14.4)	[+]H=7.23 p=0.027	Control>CHI, p=0.027
KH	20	96 (10.5)		
CHI	30	93 (14.3)		

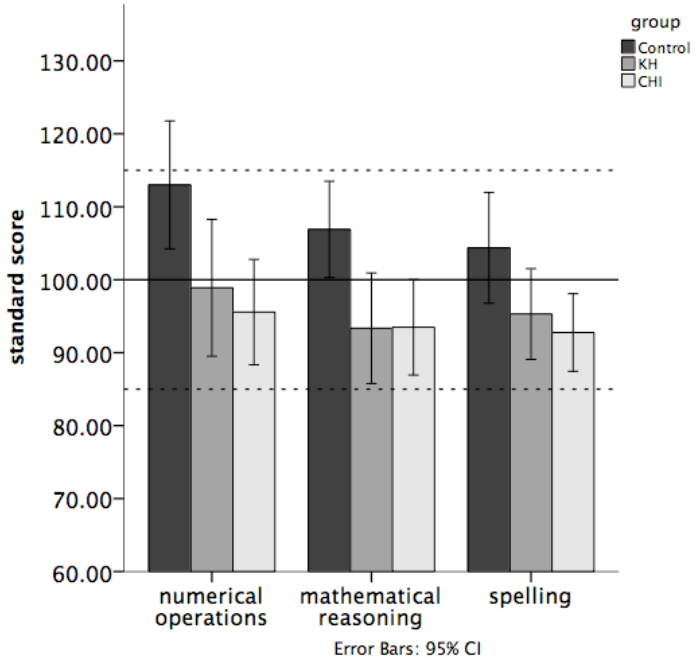

Figure 3.18 Academic attainment scores. Solid line indicates the standard population mean, dotted lines indicate one standard deviation from the mean. Error bars represent 95% confidence intervals. Patients with CHI have significantly lower spelling ability compared to the standard population mean.

Mathematical estimation subtest

The mathematical estimation subtest is a non-standardised measure of internal numerical representations. A one-way ANCOVA with age added as a covariate was conducted to compare performance between groups on the subtests and composite total score of the Mathematical Estimation Subtest. Analysis of the 'total score' showed a significant main effect of group ($F_{(2,44)}$=4.03, p=0.025). Post-hoc analyses corrected for multiple comparisons using the Bonferroni method indicated that patients with CHI performed significantly worse than controls (p=0.021). Other group comparisons were not significant. Of the subtests (dots, number line, distance and length) a main effect of group was observed in performance on the 'dots' subtest ($F_{(2,44)}$=3.18, p=0.05). However, Bonferroni corrected post-hoc comparisons did not reveal significant differences between groups.

Predictors of mathematical ability

Next, the contribution of executive functions, general cognitive ability and numerical representations to mathematical ability were examined. There is evidence suggesting that mathematical ability (particularly during childhood) depends on executive functions, particularly working memory and processing speed (see for example Berg, 2008; Cragg & Gilmore, 2014). In light of the findings that both patient groups show compromise to these functions, the contribution of executive functions, general ability and numeric ability (as measured by the MET) to scores on maths attainment tests was examined, to determine whether a restriction in domain general executive functions, cognitive ability or a deficit in specific numeric ability were making significant contributions to mathematical achievement. A stepwise hierarchical linear regression

model was built, with each standardised mathematical assessment as a dependent variable and working memory, processing speed, the MET and general ability as predictors. This analysis was conducted in the small cohort of patients and was run across a collapsed patient group (CHI plus KH) in order to allow for the addition of predictor variables. Of specific interest here was the independent contribution of working memory, processing speed and mathematical ability after the contribution of general cognitive ability. In the numerical operations subtest, this model explained over 70% of the variance in patient's scores $F(4,29)= 16$, $p<0.001$, $R^2=0.72$. General ability was found to be a significant predictor of scores in the numerical operations subtest, but working memory ability also explained a unique portion of the variance (Table 3.22). Processing speed and performance on the MET did not make a significant contribution to the model when all variables were held constant. This model was unable to predict values in the cohort of healthy controls.

Scores in mathematical reasoning were significantly predicted by GAI, but adding the other predictors to the model did not significantly improve the ability of the model to predict scores (R^2 change=0.11, p=0.076, Table 3.23). This model was not applied to the control group owing to many missing scores on the mathematical reasoning subtest. These results suggest that mathematical ability is dependent on a combination of general cognitive ability as well as working memory ability.

Table 3.22 Predictors of scores in numerical operations in patients

		b	SEB	β	P
Step 1	constant	-3.3	18.3		0.859
	GAI	1.05	0.19	0.73	<0.001
Step 2	constant	-33.37	19.27		0.005
	GAI	0.58	0.22	0.4	0.014
	WM	0.73	0.22	0.5	0.003
	PS	0.13	0.19	0.09	0.5
	MET	-0.45	1.1	-0.05	0.69

Table 3.23 Predictors of scores in mathematical reasoning in patients

		b	SEB	β	P
Step 1	constant	15.7	13.8		0.265
	GAI	0.81	0.14	0.74	<0.001
Step 2	constant	19.55	16.35		0.243
	GAI	0.52	0.19	0.47	0.01
	WM	0.36	0.19	0.32	0.062
	PS	-0.08	0.17	-0.07	0.64
	MET	1.45	0.95	0.21	0.139

3.3.6 Visual-Motor skills

To examine visual motor coordination participants were assessed with the movement assessment battery for children (MABC) and the Beery Buktenica developmental test of visual-motor integration (Beery VMI).

Movement ABC-2

For analysis of the MABC-2 main effects were considered significant at 0.0125 after correction for multiple comparisons. A main effect of group was observed on the 'total' motor score of the MABC ($p=0.003$). Post-hoc Bonferroni corrected comparisons showed that patients with CHI had significantly lower scores than controls ($p=0.002$) while a trend was observed for a lower total motor score in patients with KH relative to controls ($p=0.065$). A main effect of group was observed on the measure of manual dexterity ($p=0.003$), with post-hoc Bonferroni corrected comparisons showing that patients with CHI had significantly poorer manual dexterity skills than healthy controls ($p=0.004$). Finally, a main effect of group was observed for the gross motor coordination subtest (aiming and catching). Post-hoc Bonferroni corrected comparisons showed that patients with KH had significantly lower scores than healthy controls. No other comparisons were significant. A trend was observed for main effects of group on the balance subtest, with post-hoc analysis showing that patients with CHI performed significantly more poorly than healthy controls ($p=0.032$, Table 3.24, Figure 3.19).

One sample tests (considered significant at 0.0125) corroborated the results above, showing that patients with KH had significantly lower total motor scores compared to

the reference mean (t=-3.2 p=0.004), as well as impaired gross motor coordination (aiming and catching) skills (t=-3.5, p=0.001) and a trend for impaired manual dexterity (t=-2.1 p=0.054). In patients with CHI a lower total motor score was observed (t=-3.2, p<0.001), as was a lower manual dexterity score (t=-4.3, p<0.001).

Beery VMI

Main effects were considered significant at p=0.017. A significant effect of group was observed on the assessment of visual-motor integration (p=0.004), and post-hoc Bonferroni corrected comparisons showed that controls performed significantly better than patients with KH (p=0.05) and patients with CHI (p=0.004). A main effect of group was also observed on the visual perception subtest (p=0.005), with post-hoc Bonferroni corrected comparisons showing that patients with CHI perform significantly below controls (p=0.003). Finally, a significant main effect of group was also observed on the motor subtest (p<0.001), with both patient groups performing significantly below controls on this test of fine motor skills (Table 3.24, Figure 3.19).

One-sample tests were conducted to verify the results above. Scores on the visual-motor integration form were found to be comparable to the standard mean in patients with KH (p>0.05) but below the standard mean in patients with CHI (t=-4.1, p<0.001). Fine motor skills, measured through performance on the motor subtest, were found to be significantly below the reference mean in patients with KH (t=-3.4 p<0.001) and patients with CHI (t=-8.1, p<0.001). In patients with CHI, performance on the visual perception subtest was significantly below that of the standard population mean (t=-3, p=0.001). These results perfectly corroborate the comparison with the healthy control

group, with the exception of a lack of significance between patients with KH and controls on the assessment of visual-motor integration.

Table 3.24 Motor performance in patients and controls. [+]significant at trend level

Test	N	Mean (SD)	ANOVA F, P	Pairwise comparisons
MABC Total Motor score				
Controls	18	100 (11.7)	F=6.53, p=0.003	[+]Control>KH p=0.065 Control>CHI p=0.002
KH	20	89 (14.9)		
CHI	28	85 (15.6)		
MABC Manual Dexterity				
Controls	18	96 (15.3)	H=11.7, p=0.003	Control>CHI, p=0.004
KH	20	91 (19)		
CHI	28	78 (14)		
MABC Aiming and Catching				
Controls	18	101 (13.4)	H=9, p=0.011	Control>KH, p=0.008
KH	20	87 (10.9)		
CHI	28	93 (21.2)		
MABC Balance				
Controls	18	106 (13.4)	H=7, p=0.031	Control>CHI, p=0.032
KH	20	97 (17)		
CHI	28	95 (15.6)		
Beery Visual-Motor Integration				
Controls	18	102 (10.9)	F=6.35, p=0.004	Control>KH p=0.05 Control>CHI p=0.004
KH	12	90 (13.9)		
CHI	19	88 (15.6)		
Beery Visual				
Controls	18	105 (10.5)	H=10.7, p=0.005	Control>CHI, p=0.003
KH	11	95 (22)		
CHI	19	92 (11.2)		
Beery Motor				
Controls	18	97 (8.7)	F=13.1, p<0.001	Control>KH p=0.023 Control>CHI p<0.001
KH	12	84 (15.8)		
CHI	19	77 (12.6)		

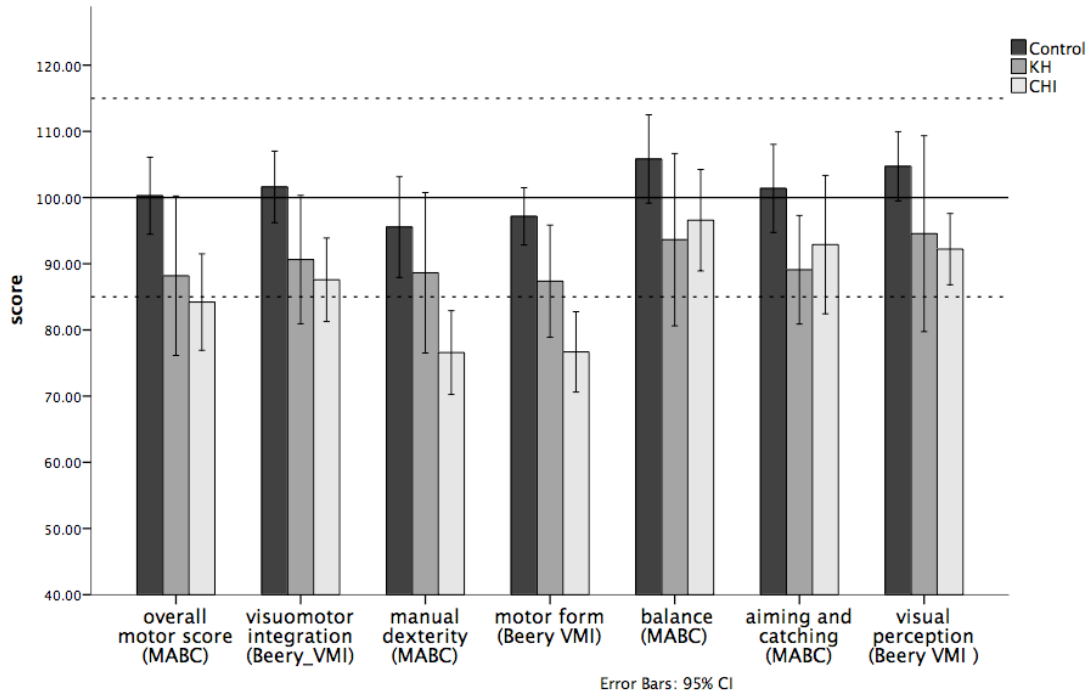

Figure 3.19 Motor profile of patients compared to controls. Solid line indicates the standard population mean, dotted lines indicate one standard deviation from the mean. Error bars represent 95% confidence intervals. Relative to the standard population mean of 100, both patients show significantly fine and gross motor coordination motor skills.

Clinical classification of motor skill impairment

The MABC-2 provides a 'traffic light' system for assessing the likelihood of a clinical movement disorder based on the total motor score. A score at or below the fifth percentile (red zone) indicates a significant movement disorder, a score at the sixth to 16th percentile (amber zone) indicates a risk of movement disorder while scores above the 17th percentile (green zone) indicate no movement disorder. In controls, all participants apart from one (who scored in the red zone) scored above the 17th percentile. In patients with KH, 12 of 20 patients were at or below the 16th percentile; with 11 of these being classified in the amber zone and one being classified in the red zone. In patients with CHI, of 28 patients who were assessed, 16 were at or below the

16th percentile. Eleven of these were classified as being in the red zone, while five were classified as being in the amber zone (Figure 3.20).

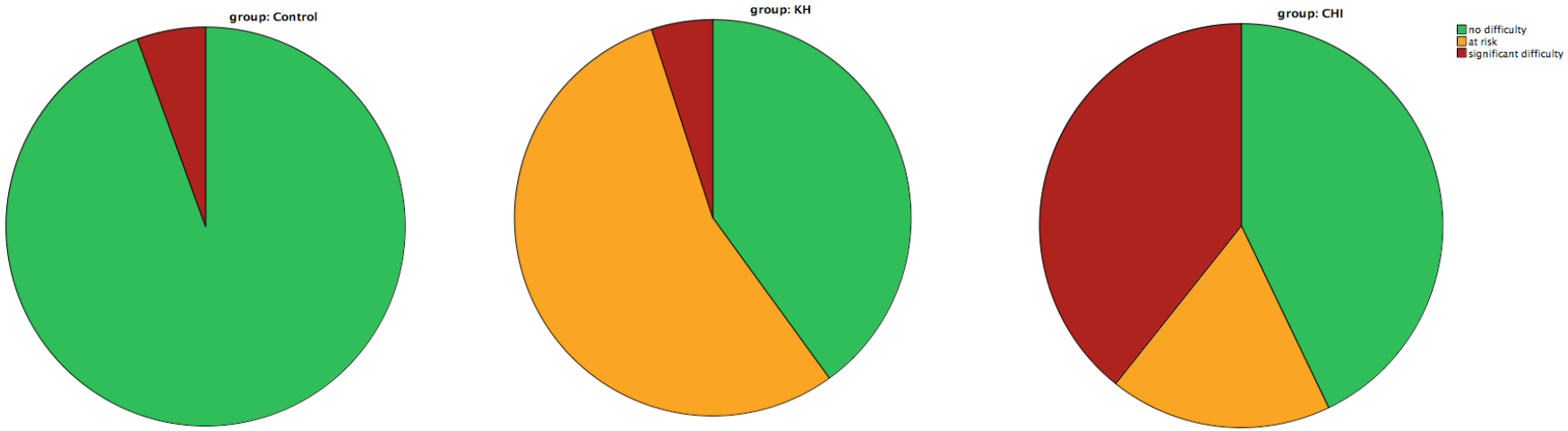

Figure 3.20 Percentages of movement disorder likelihood as identified by the MABC-2 in controls, patients with KH and patients with CHI. Over half of patients with KH and patients with CHI are classified as having or being at risk if having a motor difficulty.

In summary, both patient groups show significantly compromised motor skills. This manifests as a reduction in overall motor proficiency, with over half of patients assessed in each group classified as having, or at risk of having, a movement difficulty (Figure 3.20). Fine motor skills are affected in both patient groups, although in patients with CHI this effect seems to be more robust, surviving correction for multiple comparisons on both assessments of fine motor ability (MABC-2 Manual Dexterity and the Beery Motor form). Gross motor coordination appears to be impaired only in those with KH. Patients with CHI perform worse than controls and the standard population mean on the visual-motor integration form of the Beery, indicating a specific weakness in this area, and they also show impairments in visual perception abilities compared to the healthy controls and the standard population mean. This points to a possible impairment in perceptual abilities, affecting the success of visual-motor integration.

3.3.7 Exploratory principal components analysis

This thesis employed an extensive battery of tests which revealed impairments in attention and motor skills in both patient groups, as well as impairments in working memory, processing speed and non-verbal intelligence that were particular to patients with CHI. Despite intra-test correction for multiple comparisons, the risk of false positives is elevated with such a large testing battery. In addition, it cannot be statistically determined which variables rely on the same processes, and therefore are intrinsically correlated and reflect an underlying 'latent' variable. A latent variable is

one that cannot be measured directly but is measured indirectly through several observable variables (DiStefano, Zhu, & Mîndrilă, 2009). In order to expose underlying latent variables and to reduce this large dataset, an exploratory principal components analysis was conducted.

3.3.7.1 Methods

This analysis was conducted in the small patient cohort only. This was in order to ensure that the breadth of neuropsychological data collected (rather than the volume) was maximised and that the latent variables reflected constructs specific to the underlying patient profile, not the profile of the healthy controls. To allow for the likely correlation between factors, oblique rotation (direct Oblimin) was applied. Variables entered in to the initial analysis were screened for suitability and those that did not meet a certain criterion (KMO > 0.5) criterion were removed from the analysis (Field 2013). Note that the separate subtests of working memory variables, rather than a composite score, were entered in order to allow abilities relating to different executive functions to be separated. To ensure that any single participant was not driving the clustering of variables, a sensitivity analysis was performed (employing a leave-one-out approach). Variables that consistently loaded onto the same factor were carried forward in creating factor-based components. This approach was adopted to ensure that only the most reliable variables were used in creating factor-based scores. However, it resulted in data dropout, as some variables did not consistently load on to the same component.

Creating factor-based scores

Factor based scores were created by summing the Z-scores for each variable that loaded onto a component. Summing the variable scores has the advantage of preserving the original variation in the data, and Tabachnick and Fidell (2001) state that this is acceptable for an exploratory factor analysis. Factor-based scores were created for patients and for healthy controls (based on the PCA performed on the patients). One healthy control participant was excluded from all analyses of factor-based scores owing to many missing data points in the variables that contribute to each component. A significant proportion of healthy controls did not complete the WIAT mathematical reasoning subtest, therefore predicted scores from IQ were used here.

3.3.7.2 Results

After data screening, 20 variables were included in the final analysis. The Kaiser-Mayer-Olkin measure verified the sampling adequacy for the analysis (KMO=0.75). Five factors had eigenvalues over the value of 1, in combination explaining 72% of the variance in scores. Table 3.25 shows the factor loadings of each variable. Loadings greater than 0.3 are highlighted in bold. The sensitivity analyses revealed that four components were consistently represented by the same variables, and factor based scores were created for each participant (Table 3.26). These will now be described.

Table 3.25 Initial solution from PCA with all 31 subjects, with factor loadings

Variable		Literacy and numeracy	Executive processes	Motor skills	Complex working memory
Sustained attention	**0.73**	**-0.46**	-0.24	0.11	-0.07
Digit span backwards	**0.6**	0.09	-0.16	0.14	0
Selective attention	**0.57**	0.12	0.14	0.09	**-0.41**
VIQ	-0.21	**0.89**	-0.18	0.09	-0.06
Spelling	0.09	**0.85**	-0.14	0.07	0.11
Opposite worlds	-0.05	0.11	**-0.88**	0.04	0.03
Creature counting	0.19	0.1	**-0.76**	0	0.01
Processing speed	0.17	0.09	**-0.51**	0.2	-0.25
Balance	0.14	-0.05	0.16	**0.8**	0.01
Manual dexterity	0.16	0.09	-0.08	**0.8**	0.15
Tower achievement	**-0.34**	-0.17	**-0.33**	**0.65**	-0.18
Beery motor	**0.39**	0.12	-0.04	**0.59**	-0.13
Berry VMI	**0.39**	0.23	-0.13	**0.51**	-0.03
Letter number sequencing	0.25	**0.3**	0	**0.37**	**-0.3**
SOPT	-0.14	-0.02	0.12	0.05	**0.75**
Dual task	-0.11		-0.07	0.24	**-0.71**
Mathematical reasoning	0.21	**0.43**	0.16	0.09	**-0.63**
Digit span forward	0.02	-0.02	**-0.5**	-0.16	**-0.58**
PIQ	-0.01	0.26	0.091	**0.5**	**-0.49**
Numerical operations	0.33	**0.39**	**-0.34**	-0.05	**-0.39**
Eigenvalue	8.5	1.8	1.7	1.3	1.1
Variance	42.6	9.1	8.4	6.3	5.4

Component one represented 'complex working memory and attention' and included selective attention, the total number of errors made on the SOPT, digit span forwards, mathematical reasoning and numerical operations.

Component two represented 'executive processes' and included attentional control: set switching from the TEA-Ch (creature counting), attentional control: inhibition from the TEA-Ch (opposite worlds), processing speed, digit span forward and numerical operations.

Component three represented attainment in literacy and numeracy, and included verbal IQ, spelling and mathematical reasoning.

Component four represented motor skills, and included balance, manual dexterity and the pure motor form from the beery.

Table 3.26 Factor-based scores

	Complex working memory	Executive processes	Motor skills	Literacy and numeracy
Control (mean, SD)	0.25 (2.36)	1.73 (2.86)	-0.19 (1.98)	1.86 (1.85)
KH (mean, SD)	-4.19 (4.39)	-2.76 (4.52)	-2.32 (3.06)	-0.92 (2.44)
CHI (mean, SD)	-4.34 (4.03)	-4.11 (4.36)	-3.34 (2.16)	-0.28 (2.66)

Univariate ANOVAs considered significant at 0.0125 after Bonferroni correction for multiple comparisons indicated that all components showed a main effect of group. A significant main effect was observed in the complex working memory and attention[20] component $F_{(2,44)}$=8.6, p=0.001. Post-hoc Bonferroni corrected comparisons showed that patients with KH had significantly lower scores than healthy controls (p=0.008), as did patients with CHI (p=0.001). No significant differences were observed between the patient groups. A main effect of group was observed in the executive processes component $F_{(2,45)}$=10.4, p<0.001, with post hoc Bonferroni corrected comparisons showing that healthy controls had significantly higher scores than patients with KH (p=0.012) and patients with CHI (p<0.001). No significant differences were observed between patient groups. Main effects of group were also seen in the literacy and numeracy component $F_{(2,45)}$=5.9, p=0.005, with post hoc tests (Bonferroni corrected) showing that healthy controls had significantly higher scores than patients with KH (p=0.009) and patients with CHI (p=0.027). No significant differences were observed between patient groups. Finally, main effects were observed on the motor component $F_{(2,45)}$=8.2, p=0.001, with post hoc comparisons (Bonferroni corrected) showing that controls had significantly higher motor component scores than patients with CHI (p<0.001) and a trend for higher scores than patients with KH (p=0.062). Once again, no significant differences were observed between patient groups. Figure 3.21 displays factor based scores.

[20] One patient with KH was removed from this analysis owing missing data

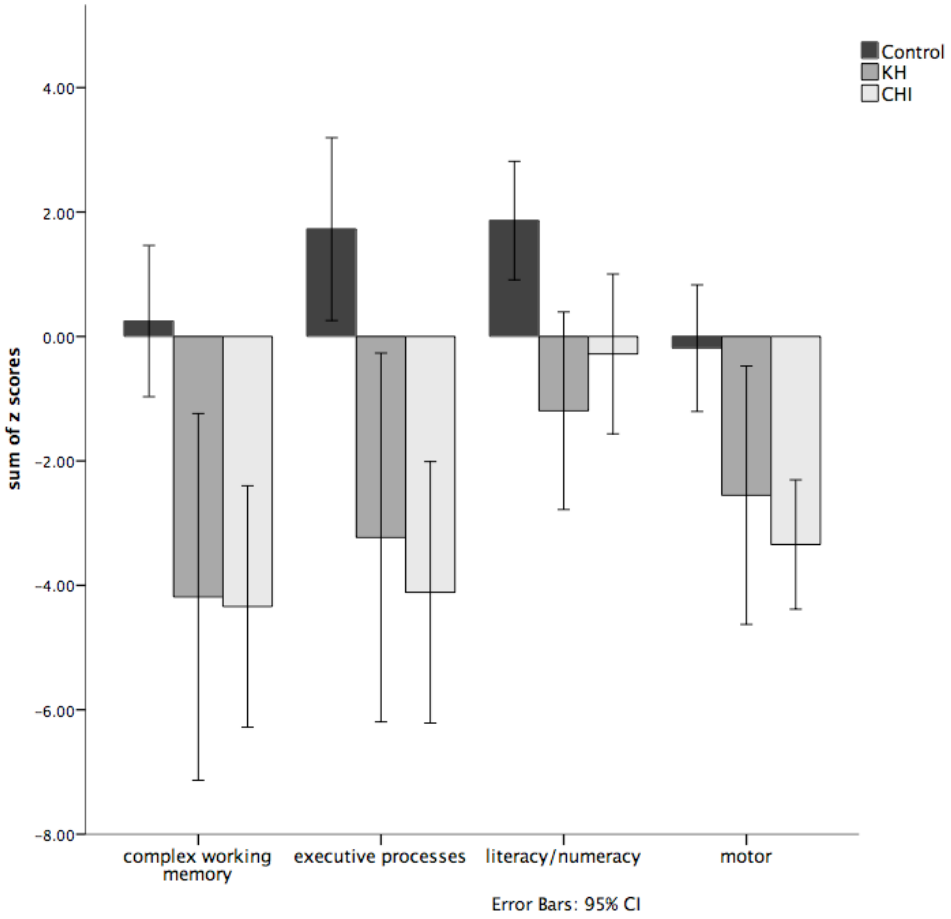

Figure 3.21 Resulting components from exploratory factor analysis. Error bars indicate 95% confidence intervals. Both patient groups had significantly lower scores than controls on all factor based scores.

To summarise, this exploratory factor analyses has revealed clusters of variables that presumably rely on similar processes. Theoretically, all components are composed of variables that should cluster together. All motor measures load on to the same component, while the attentional control measures from the TEA-Ch, processing speed and working memory all fall under the umbrella of executive functions (Figure 3.2). Similarly, the SOPT, digit span forward, mathematical ability and selective attention represent complex working memory tasks which are reliant on the executive network but more specifically related to cognitive flexibility, although there are some aspects of attentional control within this component. Transposing these factors on to the healthy

control dataset and comparing patients with groups has led to very similar conclusions as were presented at the beginning of this chapter; that both patient groups exhibit similar impairments in the domain of executive functioning and motor skills. While these impairments may be more pronounced in patients with CHI, it is clear that they are also evident in patients with KH. This conclusion is supported by the finding that the two patient groups do not differ on any neuropsychological measure, nor on any factor based components. This analysis has revealed underlying latent variables and has replicated the deficits documented at the beginning of the chapter whilst vastly reducing the risk of making a type-1 error (false positive). These factor-based components will be carried forward in establishing the neural basis of these observed impairments.

3.4 Discussion

This chapter has described a comprehensive neuropsychological profile of children with KH and CHI. Compared to the healthy control group (who were very high functioning) both patient groups are impaired on almost all measures of functioning. Therefore, impairments will be discussed in relation to the standardised population mean, which can be considered as reliable. A summary can be seen in Tables 3.27 to 3.30. Where comparisons to the standard population mean were not available impairments will be discussed in relation to healthy controls.

Table 3.27 Deficits in intelligence in patient groups ✓Restricted ✖ Normal

	Verbal Intelligence	Non-Verbal Intelligence	General Ability
KH	✖	✖	✖
CHI	✖	✓	✖

Table 3.28 Restrictions in executive functions in both patient groups ✓Restricted ✖ Normal ✓$^{+}$ Trend level

	Working Memory (WISC-IV)	SOPT	Selective attention	Sustained attention	Set Switching	Attentional control	Dual task	D'	Planning	Processing speed (WISC-IV)
KH	✓$^{+}$	✖	✓	✖	✓	✓	✓	✓	✓	✓$^{+}$
CHI	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Table 3.29 Deficits in scholastic attainment in both patient groups ✓Restricted ✖ Normal ✓+ Trend level

	Numerical Operations	Mathematical Reasoning	Spelling
KH	✖	✖	✖
CHI	✖	✖	✓

Table 3.30 Restrictions in visual-motor coordination in both patient groups. ✓Restricted ✖ Normal ✓+ Trend level

	Manual dexterity	Fine motor (Beery)	Motor coordination	Balance	Visuo-motor integration	Visual Perception
KH	✓+	✓	✓	✖	✖	✖
CHI	✓	✓	✓	✖	✓	✓

3.4.1 Intellectual functioning

As a group, patients with KH have preserved intelligence, with mean scores that do not differ from the standard population mean. In contrast, patients with CHI have impairments in non-verbal IQ although verbal intelligence and general ability are preserved. This deficit in non-verbal IQ has been reported previously (Rother et al., 2001) and could be affected by the restrictions in manual dexterity ability observed in patients with CHI. The block design subtest, which determines a portion of the non-verbal intelligence score, is timed and involves manipulating three-dimensional blocks to re-construct two-dimensional geometrical designs. Indeed, a linear regression with non-verbal IQ as the dependent variable showed that manual dexterity from MABC-2 explained a significant proportion of the variance in scores in patients with CHI ($F_{(1,26)}$=9.9, p=0.004, R^2=0.28). The same model was not significant when applied to patients with KH or healthy controls. By the same token, it is possible that visual-motor and perception difficulties identified in patients with CHI (using the Beery VMI) contributes to this deficit. A correlational analysis identified that Beery VMI abilities were significantly related to non-verbal IQ scores in patients with CHI (r=0.635, p=0.003), but also in patients with KH (r=0.823, p=0.001) and in controls (r=0.46, p=0.055). It is therefore likely that in patients with CHI restricted motor and motor-perceptual abilities do play a role in performance on this test of non-verbal abilities. Importantly, no significant differences in measures of intellectual functioning between patients with CHI and patients with KH were observed, in contrast to the findings of Kumaran (2012).

3.4.2 Working memory, processing speed and attention: executive dysfunction

Parental reports of behaviours related to inattention and poor working memory, planning and inhibition (from the SDQ, Conners 3 and the BRIEF) were elevated in both patient groups. Accordingly, pervasive deficits in working memory, as well as deficits in processing speed, were identified in patients with CHI and, albeit to a lesser extent, in patients with KH.

3.4.2.1 Working memory

The results of this study confirms the finding of impaired working memory in patients with CHI (Kumaran, 2012) and extend this finding to show that working memory impairments prevail even in patients who are free from neurological diagnoses (e.g. epilepsy). Furthermore, the current study examined the specific components of the WISC-IV Working Memory assessment and found that performance in patients with CHI was lowered across all of these subtests. Particularly poor performance was observed on digit span forwards, a task that is thought to rely on the phonological loop in the traditional model of working memory (Baddeley & Hitch, 1974). In addition, patients with CHI showed lowered performance on the SOPT, suggesting poor visual working memory. This version of the SOPT is designed to be difficult to code verbally (although it is possible that participants assigned verbal categories to the abstract designs in order to facilitate monitoring of responses). Importantly, as the performance on the SOPT relies on successful self-generation, monitoring and updating of a sequence, it is considered to depend on the functioning of the central executive, regardless of the modality in which it is presented (Joseph, Steele, Meyer, & Tager-Flusberg, 2005).

Although not as profound in patients with CHI, a trend toward verbal working memory impairment was also identified in patients with KH, with low performance observed on the WISCV-IV working assessment compared to the standard population. Examining the subcomponents of this assessment showed that performance on the Letter-Number Sequencing subtest was driving this decrement. This component of the working memory assessment (much like the backward digit span) is considered to depend on the ability to shift between sets, which is an executive demand (Holmes et al., 2014) and therefore dependent on the central executive, unlike the digit span forward subtest which requires no manipulation or shifting between sets for successful performance and is dependent on the phonological loop.

This impairment in working memory was a novel finding and was unexpected given previous report of preserved working memory functioning in patients with KH (Kumaran, 2012). Furthermore, while differences between controls and patients with KH were not significant, performance on the SOPT 6, 8, 10 and 12-item conditions were very similar between the two patient groups (Figure 3.9), indicating that the ability to self-generate, monitor and update a sequence (demands of an executive nature) is lowered in patients with KH.

As discussed in the introduction to this study, according to Baddeley and Hitch's (1974) model, working memory performance is aided by three slave systems which are supervised by an attentional controller that allocates resources accordingly and functions as a coordinator; the 'central executive' (Baddeley & Hitch, 1974; Baddeley,

1992; Alloway, Gathercole, Kirkwood, & Elliott, 2009; Gathercole et al., 2008; Holmes et al., 2014) . These distinct but interacting cognitive systems (domain-general and domain-specific) are involved in the coordination of higher level attentional control and the short-term storage and manipulation of information (Alloway, Gathercole, & Pickering, 2006; Holmes et al., 2014). The central executive is responsible for multitasking, shifting between tasks, adopting retrieval strategies, controlling and manipulating information. The central executive also coordinates the slave systems (Diamond, 2014; Hubber, Gilmore, & Cragg, 2014) and, although it is not well defined, it can be thought of as a reflection of all executive functions (Baddeley & Hitch, 1994; Baddeley & Hitch, 1974; Baddeley, 1996; Miyake et al., 2000). Thus poor working memory performance could be due to problems in any of the domain-specific (verbal or visual) slave systems, or because of impairments in the central executive. The results of this study suggest that patients with CHI show clear impairments in two of the three components of Baddeley's model of working memory; it remains to be seen whether this impairment extends to the visuospatial sketchpad. The results in patients with KH point to a compromised domain general central executive.

Across patient groups, but not in controls, working memory ability as measured by the WISC-IV significantly predicted scores on the SOPT, explaining a substantial proportion of the variance, over and above general cognitive ability. This is evidence that an impairment in working memory exists over and above a limited general ability. Importantly, in the face of preserved IQ, children with low working memory have been found to have poor performance in other aspects of executive function, such as switching, planning and inhibition (Holmes et al., 2014). Children with low working

memory commonly have attentional deficits (Gathercole et al., 2008) and children with ADHD have similar neuropsychological profiles to children with poor working memory (Holmes et al., 2014; Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005).

3.4.2.2 TEA-Ch and CPT

Patients with CHI show profound deficits in all dimensions of attention measured by the TEA-Ch as well as significant working memory impairments, in accordance with this previously identified co-occurrence. This confirms previous reports of attentional deficits in CHI from the limited number of studies examining this domain (Lord et al., 2015; Rother et al., 2001). This study has shown that these deficits in attention are genuine effects, rising above the methodological limitations of previous studies. Patients with KH showed an almost identical pattern of impairment (with the exception of normal performance on the sustained attention task) and therefore have an extremely similar profile to those with CHI, when assessed with the TEA-CH. Both patient groups show particularly compromised functioning on the measures of the TEA-Ch that rely on cognitive flexibility (dual task) and attentional control (creature counting and opposite worlds). This is despite the fact that a number of patients in both groups did not meet the minimum criteria of accurate responses to calculate a 'switching' score in the creature counting subtests; thus this deficit is apparent even when excluding those who are likely to be the most impaired.

Although performance on the CPT was varied and did not yield the significant differences in the patient groups that were expected (given their profile on the TEA-Ch), both patient groups performed significantly worse than the standard population

on the detectability score of the CPT. Surprisingly, overall patients with KH performed more poorly on more measures of the CPT than patients with CHI; patients with CHI showed deficits in performance only in measures relating to processing speed (HRT) and sustained attention (HRT Block Change). This is consistent with their impairment in processing speed and sustained attention as measured by the WISC-IV and TEA-Ch subtests.

3.4.2.3 Evidence for a disrupted executive network

Planning abilities were also impaired in both patient groups, with higher scores (indicating poor performance) observed on the Tower 'first move time'. This could indicate a lack of planning and strategy when approaching a task. Taken together, the co-occurrence of impairments in working memory, attentional control and cognitive flexibility, as well as planning, provide support for executive dysfunction in these patient groups, which is a novel finding.

Some researchers argue that such broad ranging deficits result from an impairment in one core module, e.g. working memory (Gathercole et al., 2008) or attentional control (Anderson, 2002), which has a knock-on effect on the rest of executive network. Others suggest that the co-occurrence is due to a globally compromised executive network (Holmes et al., 2014). The data presented in this study do not allow for corroboration of either of these theories and it is difficult to establish the direction of causality in these theories. However, from this study it is evident that broad executive dysfunction exists in both patients with CHI and patients with KH, which is an impairment that does not appear to be solely due to compromised cognitive ability; if

it were, then we would at least expect patients with KH to have preserved functioning. Instead, the observed impairments indicate a specifically disrupted executive network. The deficits in both visual and verbal working memory, combined with decrements on other tests that tap into executive functions (attentional control, selective attention, dual task, measures from the CPT and planning), strongly indicate a compromised executive network. Deficits in tasks mediated by the executive network are likely mediated by frontal-striatal and fronto-parietal networks, which may be compromised in both patient groups (Anderson, 2002; Holmes et al., 2014; Stuss & Alexander, 2000) and will be explored in Chapter Seven.

3.4.2.4 Consistency with parental reports

Impairments in attention and executive functions in those with KH are corroborated by parental reports of problems in this domain, while parental reports of difficulties in attention were largely at trend level in patients with CHI. This discrepancy between parental reports of attention results and the formal assessment of these abilities could result from a number of factors.

In patients with CHI, it is possible that their ongoing complex medical needs occlude any difficulties in cognitive functioning that is observed by parents. Indeed, the same pattern of results was seen for the ratings on the BRIEF, where ratings of patients with CHI were not significantly different from the standard population mean, despite clear impairments in aspects of executive functioning. It might be that the two forms of assessment are not ecologically similar enough, with judgement of executive functioning in a home environment being challenging. Given the consistency of

impairment in paper and pencil and computerised tests, it is unlikely that the effects observed are false positives. Teacher ratings of behaviour might have yielded different results here; parents may not have the same opportunity to evaluate executive functioning as teachers do, with successful functioning within a classroom environment depending so greatly on executive functions.

3.4.2.5 Implications

Problems in executive functioning are considered to interfere with the capacity of a child to develop normally and affect their interaction with the environment. Longitudinal data suggest that limitations in working memory capacity will substantially constrain a child's ability to succeed in learning and consolidating complex skills during the period of early formal education (Gathercole, Tiffany, Briscoe, & Thorn, 2005). These executive deficits are thought to result in cognitive, academic, and social disturbance (Anderson, 1998), and therefore can have significant consequences for a child's development.

3.4.3 Academic attainment

Although a number of individuals in both patient groups were in receipt of learning support in the classroom, academic attainments appeared to be largely preserved in both patient groups. Patients with KH showed normal levels of academic attainment in mathematics and spelling. In patients with CHI, the only measure of scholastic achievement that fell significantly below the standard population mean was that of spelling, in contrast to the results of Kumaran, where impairments in mathematical

ability were noted. Finally, there were no significant differences between patient groups, contrary to previous comparisons that yielded significant differences in scholastic achievement (Kumaran, 2012). It is possible that the results reported in the latter study were driven by patients with known neurological conditions, who may have had poorer performance on these measures of academic attainment. Exclusion of patients with epilepsy in the current study may therefore have led to an increased performance in many of the domains assessed.

Despite an overall preservation of mathematical ability, in both patient groups there were individuals with scores on the mathematical attainment tests that fell well below the average range. Patients with CHI also had a significantly lower total number of correct responses on the MET. Regression models show that while general cognitive ability is a significant predictor of mathematical ability, working memory also explains a significant proportion of the variance in scores on numerical operations (with contributions of working memory to mathematical reasoning scores being at trend level). Processing speed and internal numerical representations (as measured by the MET) were not significant predictors of mathematical ability; a result which contradicts the findings of Simms et al. (2013) who found that scores on the MET explained a small but significant amount of variance in mathematical ability scores in extremely preterm children. The lack of association between processing speed and mathematical ability could be a reflection of the task demands; these attainment tests are not timed and so may make less demands on the efficiency of processing information, but more on the coordination and allocation of attentional resources to maintain and update representations (Berg, 2008). However, the results of the mathematics assessment are

in line with previous studies that highlight the strength of the relationship between domain general executive skills (such as working memory) and mathematical ability (Berg, 2008; Cragg & Gilmore, 2014). This indicates that variations in mathematical ability are unlikely to be because of a domain-specific deficit in numerical representations, but because of a combination of general cognitive ability and working memory ability.

3.4.4 Visual-motor skills

Patients with CHI and patients with KH show impairments in motor skills. They show significantly lower total motor proficiency than the standard population, and significantly lower fine motor skills as measured by the Beery. In patients with CHI, the effect on motor skill is particularly marked with regard to fine motor skills, with scores falling significantly below the normal population on both the manual dexterity subtest of the MABC-2 and the pure motor subtest of the Beery VMI. However, there is also a marked level of lowered overall motor proficiency, with more than half of patients being classified as having, or at risk of having, a movement coordination disorder. These results show that reports of motor impairment in infancy and childhood, so often stated in the literature documenting effects of early hypoglycaemia (Burns et al., 2008; Hershey et al., 1999; Lucas, Morley, & Cole, 1988; Meissner et al., 2003; Menni et al., 2001) do persist past the early years and into childhood and adolescence. Of note, patients with CHI achieved lower scores on the assessment of visual-motor integration and visual perception, suggesting that previous reports of visual-perceptual abnormalities (Avatapalle et al., 2013; Burns et al., 2008) may also be a lasting feature of hypoglycaemia. For patients with KH, who are not a well-studied population, this

study shows that gross motor coordination abilities might be at risk, and again a large percentage (more than half) of children were identified as being at risk of having a movement coordination disorder. Combined with a failure to identify differences in motor ability between the two patient groups, these results suggest that motor skills are particularly vulnerable in early hypoglycaemia regardless of the aetiology, and with significant potential for impairment that extends into late childhood.

3.4.5 Memory

Previous studies have suggested that long-term memory impairment and hippocampal damage follow severe hypoglycaemia in diabetes (Hershey et al., 1999, Kauffman et al, 1999, Rovet & Ehrlich, 1999). The results of the memory assessments indicate that while mnemonic deficits are not a characteristic feature of either group of patients, there is a high incidence of memory impairment as implied by significantly lower memory scores than predicted from IQ scores in both groups. Importantly, this effect is present in both immediate and delayed verbal memory. Success on the immediate memory test is not traditionally thought of as relying on working memory capacity, as it exceeds traditional working memory capacity and requires the participant to remember a large amount of information (Geva et al., 2016). However, it is possible that performance may be mediated by the observed executive dysfunction present in both patient groups, especially in light of attentional difficulties, which may affect encoding of information. A relationship between these indices of memory and the integrity of the hippocampus has been shown previously (Cooper et al., 2013; Muñoz-López et al., 2017). Combined with parental ratings of impaired everyday memory, the relationship between memory indices and the hippocampus (thought to be selectively

vulnerable to hypoglycaemia) and other memory relevant structures warrants further investigation and will be explored in Chapter Seven.

3.4.6 Limitations

It is important to recognise the limitations of the present study. This study employed a very large protocol, which could have caused fatigue in participants. In order to ensure that tests of executive functioning were not compromised greatly by mental fatigue, these tests were administered at the beginning of each testing session. As a result of the time constraints imposed by this lengthy protocol, there were some areas of functioning that were not assessed, such as reading ability (both word reading and reading comprehension). Inclusion of these tests would have given a more comprehensive overview of academic attainment. The results described here do not consistently refer to the same number of participants - some data were only available for the small cohort of patients and this may render some conclusions less reliable, as they are drawn from a smaller sample of patients. The stringent levels applied to the exploratory principal components analyses led to some data loss, rendering the components less rich, but more reliable. Finally, and importantly, in the light of the observed executive dysfunction present in both patient groups, it would have been beneficial to have teacher ratings of functioning in the classroom environment, which would provide a rich account of the child's functioning in school, and may be more ecologically valid than parental ratings of behaviour at home.

3.4.7 Conclusion

This study has found that both patients with CHI and patients with KH show pronounced and selective impairments in executive functions and motor skills. Perhaps more striking than this is the lack of statistical difference on any of the neuropsychological measures between patient groups. Aside from the measures of executive functioning described in the Chapter One, in the HBI study (Kumaran, 2012) those with KH outperformed their CHI counterparts on all other measures of functioning. However, it should be noted that the inclusion of patients with epilepsy in the HBI study could have driven the differences observed between patient groups. The finding that patients with KH have such a similar profile to patients with CHI, with impairments restricted to the executive and motor domains, is an indication that regardless of the presence of ketone bodies, repetitive episodes of hypoglycaemia at a young age have long-term adverse consequences on cognitive and motor outcome. The brain-based origins of these impairments, in both patient groups, will now be investigated.

Chapter 4

Voxel-Based Morphometry

4 Voxel-Based Morphometry

This thesis uses T1-weighted and diffusion-weighted MRI scans to assess the effects of early and recurrent hypoglycaemia on various brain structures. This chapter gives an overview of the findings from relevant imaging studies that have examined tissue integrity in populations who experience hypoglycaemia, followed by an introduction to MRI physics. The results of analyses comparing grey and white matter volumes between healthy controls, patients with KH and patients with CHI are then presented. Global volume differences are analysed, followed by a voxel-based morphometry analysis to identify regional differences in tissue volume between groups.

Chapter Five gives an account of the effect of hypoglycaemia on basal ganglia and thalamic volumes, while Chapter Six explores the integrity of core white matter tracts throughout the brain via the analysis of diffusion-weighted scans.

4.1 Introduction

4.1.1 Background

As reviewed in the introduction, there is a wealth of literature indicating that neonates and infants who experience episodes of hypoglycaemia are at risk of subsequent neuropathology, especially in the context of CHI, where hypoglycaemia is persistent and severe (Arnoux et al., 2010). To date, studies of neuropathology following hypoglycaemia have relied on post-mortem analysis or visual inspection of Magnetic Resonance (MR) and Computed Tomography (CT) images to determine the integrity of the cortex, subcortical regions and white matter.

4.1.1.1 Post-mortem analysis of the brain

Post-mortem studies of infants with prolonged hypoglycaemia have shown a varied pattern of damage. Banker et al. (1967) observed microcephaly in three infants, characterised by global widening of the sulci, atrophy of the gyri and severe loss of white matter. They reported effects on grey matter throughout the cortex, as well as thalamic and basal ganglia damage. Corticospinal tracts were reduced in size from the internal capsule through to the spinal cord. In their post-mortem study of six infants with hypoglycaemia, Anderson, Milner and Strich (1967) reported severe brain damage in half their sample, involving grey matter lesions and glial degeneration. The occipital lobes and insula were reported to be severely affected, more so than the frontal lobes, with a relative preservation of the temporal lobes. These studies provide evidence that the immature brain is sensitive to lack of glucose, but do not allow generalisations owing to their small sample sizes. The use of Magnetic Resonance Imaging (MRI) can allow in-vivo identification of a shared pattern of neuropathology secondary to hypoglycaemia in larger groups of patients.

4.1.1.2 Imaging analysis of the brain

Studies using visual inspection of MR and CT images to determine neuropathology after hypoglycaemia during the neonatal period and infancy have often revealed significant damage to the cortex and white matter of the occipital and parietal lobes (Alkalay, Flores-Sarnat, et al., 2005; Aslan & Dinc, 1997; Barkovich et al., 1998; Filan et

al., 2006; Kinnala et al., 1999; Murakami et al., 1999; Spar et al., 1994; Traill et al., 1998), although some report a much more widespread pattern of white matter injury (Burns, Rutherford, Boardman, & Cowan, 2008). Damage to the frontal lobes and subcortical structures (including the thalamus, hippocampus and globus pallidus) has also been reported (Barkovich et al., 1998; Kinnala et al., 1999). These studies include patients with varying aetiologies, and few have focused on patients specifically with CHI. In older children with CHI, studies have reported global brain atrophy and lesions to the basal ganglia (Gataullina et al., 2013; Kara et al., 2007). Avatapalle et al. (2013) studied patients with CHI and found damage to parietal and occipital lobes, white matter atrophy and basal ganglia changes in 70% of cases. Gataullina and colleagues (2013) found that all of the children with CHI included in their study had posterior white matter lesions (Gataullina et al., 2013). Thus, there is considerable evidence to suggest that damage to parietal and occipital regions is common following early hypoglycaemia and that white matter is particularly vulnerable to this type of insult. However, these studies frequently include patients with known neurological conditions (epilepsy), and contain only qualitative reports of brain abnormality secondary to hypoglycaemia at an individual level. As mentioned previously, epilepsy is known to result in structural brain abnormalities (Bernasconi et al., 2004) which makes it hard to draw conclusions about damage specific to hypoglycaemia.

4.1.1.3 Neuroimaging in diabetes

As described in Chapter One, children with early-onset type 1 diabetes mellitus (EODM) can experience episodes of hypoglycaemia owing to the over-administration of insulin. They share the same mechanism of hypoglycaemia as those with CHI, and

neuroimaging profiles may therefore be comparable between the two groups. EODM has been associated with mild brain atrophy (Ferguson, Blane, Wardlaw, et al., 2005), although this has not been explored explicitly in relation to episodes of hypoglycaemia. However, it has been found that children with EODM and a history of hypoglycaemia show white matter volume reduction in the occipital/parietal lobes when compared to those who had not experienced episodes of hypoglycaemia (Perantie et al., 2011). While the inferences that can be drawn from this are limited (owing to the generally dysglycaemic environment characteristic of diabetes treated with insulin) tentative evidence is provided in support of the vulnerability of posterior white matter to hypoglycaemia.

Although these studies point to the vulnerability of posterior brain regions in context of hyperinsulinism, an understanding of damage to the brain that is specific to hypoglycaemia, and arrived at through quantitative neuroimaging analyses, is yet to be established. Importantly, no conclusions can be drawn about what damage might exist at a group level until quantitative neuroimaging methods are applied to a group of patients who share the same aetiology of hypoglycaemia and have no co-morbidities.

4.1.1.4 Neuroimaging in patients with KH

To date, only one study has examined the imaging profile of children with ketotic hypoglycaemia. As described in Chapter One, Kumaran (2012) assessed T1-weighted images of patients with KH and CHI and found that the incidence of white matter lesions was similar in the two groups. This was an unexpected finding, as children with KH were hypothesised to be protected during episodes of hypoglycaemia owing to the

presence of ketone bodies, which are considered to act as an alternative fuel for the brain during episodes of hypoglycaemia (Daly et al., 2003; Grunt et al., 1970). The results of this study dispute this. While the incidence of lesions (as described above) is indicative of neuropathology, details about group-level damage to certain structures and regions in the brain following hypoglycaemia cannot be determined. For this level of inference to be made, data would need to be compared to a healthy comparison group using quantitative imaging methods.

4.1.2 Aims and hypotheses

Using quantitative imaging techniques that are able to distinguish patterns of brain changes at a group level, this study aimed to identify global and regional differences in brain volumes between healthy controls, patients with CHI and patients with KH. First, this study aimed to discern the lasting effects of severe and recurrent hypoglycaemia in children with CHI, as most previous studies have examined outcomes in neonates or infants. It was hypothesised that school-aged patients with CHI will show a primarily posterior pattern of damage compared to healthy controls, with involvement of grey and white matter of the occipital and parietal lobes. However, the impairment in frontally mediated functions that have been presented in Chapter Three suggest that there may also be some pathology in frontal regions of the brain. Second, this study aimed to establish whether children with KH manifest global or focal brain changes on a group level. Given the results of visual inspection of MRI scans in the HBI study (Kumaran, 2012), it was expected that compared to controls, patients with KH might show some differences in white matter volume when compared to controls. As the first study to make such a comparison, no specific hypotheses were made about where

these differences would lie anatomically. Finally, a direct comparison of patients with CHI and patients with KH aimed to determine whether differences exist between these two patient groups. Again, there were no specific hypotheses about where these differences would lie, but considering the severity of CHI and the suspected role of ketone bodies during episodes of hypoglycaemia, it was expected that children with KH would show a relative preservation of brain tissue in comparison to those with CHI, manifesting as volume decreases in brain tissue in children with CHI compared to children with KH.

4.2 Methods

4.2.1 Principles of MRI

Protons and magnetisation in the MRI scanner

MRI utilises the intrinsic magnetic properties of the nuclei of hydrogen atoms (i.e. protons), which are found in abundance in the human body, mainly in water and fat. This nuclear magnetism is associated with a property known as spin, which can be visualised as a spinning motion of the nuclei about their own axis.

The MRI scanner contains a large bore magnet that creates a strong, static, homogeneous magnetic field (B_0 = 1.5 Tesla in our case). When a participant is placed inside the MRI scanner, the nuclear magnets within the body align parallel (spin up) or anti-parallel (spin down) to the field B_0, but the lower energy state (i.e. parallel) is preferred. Because of this preference, there is a slight imbalance in the proportion of nuclei that are aligned parallel compared to anti-parallel and this creates a small

magnetisation along the direction of B_0 (Gadian, 1995). This is referred to as longitudinal magnetisation (M_o).

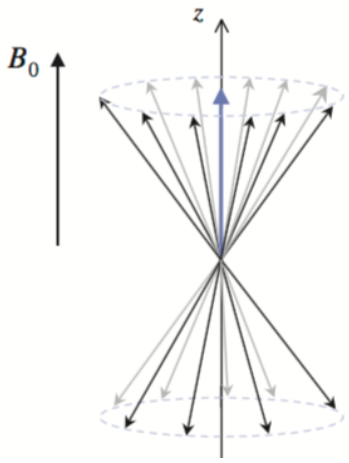

Figure 4.1Shows protons in spin-up and spin-down states. Adapted from MRI: from picture to proton.

The RF pulse and resonance

The nuclear magnetisation can be detected by application of an oscillating (radiofrequency - RF) magnetic field (B_1) transverse to the main magnetic field that satisfies the resonance condition $\Delta E = h\upsilon$ (where *E* is the energy difference between the two magnetic states, *h* is the Planck constant and υ is the frequency of the oscillating field B_1). In the case of a 90° pulse, magnetisation is tilted into the plane perpendicular to the main magnetic field, B_0, thereby creating transverse magnetisation (Gadian, 1995).

Relaxation times, tissue contrast and receiving the MR signal

Once the RF pulse is switched off, protons return to their equilibrium state (aligning parallel to B_0), and the receiver coil records the MR signal that they emit at the resonance frequency (in neuroimaging, this signal is recorded using a head coil). The time taken to return to equilibrium is characterised by two parameters; the spin-lattice (or longitudinal) relaxation time (T1) characterises the return to equilibrium along the direction of B_0, and the spin-spin (or transverse) relaxation time (T2) characterises the return to equilibrium (i.e. 0) in the transverse plane. T2 is generally much shorter than T1. Importantly, MR protocols utilise the differing T1 and T2 relaxation properties of different tissue types and vary the timing of RF pulses to achieve image contrast. For example, white matter has a relatively short T1, while CSF has a very much longer T1. This means that CSF appears dark and white matter bright on a T1-weighted image (Schild, 1990). Changing the interval between the excitatory radiofrequency pulses (i.e. the repetition time TR) in a pulse sequence allows for contrast between different tissue types as it causes the signal to be T1-dependent and hence tissue-dependent.

Acquiring 3D images: spatial localisation and gradient coils

The scanner contains three gradient coils, which produce much smaller magnetic fields than the bore magnet. These gradient coils provide small variations in the strength of the main magnetic field, resulting in spatial variations in the resonance frequency. This makes acquisition of spatial information possible.

Imaging hardware and sequences

All imaging data described in this thesis were acquired using a 1.5T Siemens Avanto (Germany) clinical MRI scanner at Great Ormond Street Hospital, London, using a 12-channel head coil. All participants were scanned unsedated, for a total scan time of 35 minutes.

T1-weighted images were acquired with a 3D Fast Low Angle Shot magnetic resonance imaging (FLASH) sequence, repetition time (TR) = 11 ms, echo time (TE) = 4.94 ms, flip angle = 15°, field of view = 224 × 256 mm, 176 slices, sagittal plane; voxel size: 1 x 1 x 1 mm^3. For Diffusion Tensor Imaging (DTI), echo-planar images were acquired in 60 gradient directions at b=1000 s/mm^2, TR = 7300 ms, TE = 81 ms, field of view: 240 x 240 x 150, 60 slices, axial plane, voxel size: 2.5 x 2.5 x 2.5 mm^3.

4.2.2 Participants

Participant demographics have been described in detail in Chapter Two. In this study, analysis was initially conducted across all cohorts, and was replicated in the smaller cohort collected by the author.

Segmentation failed for three patients (two patients with CHI and one with KH); these scans were not entered into the analysis. One patient with KH had no MRI data available. Table 4.1 gives the age and gender of participants who were included in the final analysis according to group membership, for both the large and small cohorts. In both the large and small cohorts, age did not differ significantly between groups (one-way ANOVA, $p>0.5$ for all). In the large cohort, a trend for significant differences in

gender distribution was observed (χ^2= 5.4, p=0.067), driven by more females in the control group than in either patient group. In the smaller cohort no differences in gender distribution existed (χ^2=0.657, p=0.720)

Table 4.1 Age and gender of large cohort

Group	N	Age in months (SD)	Gender
Controls	44	140 (31)	16 male
CHI	29	130 (30)	18 male
KH	19	125 (33)	11 male

Table 4.2 Age and gender of smaller cohort

Group	N	Age in months (SD)	Gender
Controls	18	131 (26)	9 male
CHI	16	120 (33)	10 male
KH	12	140 (31)	6 male

4.2.3 Analysis of imaging data

4.2.3.1 Global brain volumes

T1-weighted scans were segmented using the 'Segment' tool provided in SPM12. This segments the image into three tissue classes (grey matter, white matter and

cerebrospinal fluid [CSF]). Total tissue volumes were acquired from each of these segmentations using the 'Tissue Volume' utility in SPM12, providing total grey matter, white matter and cerebrospinal fluid volumes. From these, intracranial volume was calculated and each tissue class was divided by the ICV and retained as a proportion of the ICV. In this chapter, analyses of global grey and white matter tissue volumes, as well as CSF volume, were conducted on ICV-corrected tissue volumes.

4.2.3.2 Voxel-Based Morphometry

Voxel-Based Morphometry (VBM; Ashburner & Friston, 2000) is a whole brain quantitative imaging method that can compare the local volume of regional voxels between groups of subjects. It is suitable for comparing groups of patients and healthy participants and it allows the assessment of the integrity of structures throughout the brain, rather than being biased toward a particular structure. In this sense, it is suitable for use where no a-priori hypotheses about where differences may lie between groups, although certain pre-processing decisions (e.g. the choice of smoothing kernel) are usually influenced by hypotheses defined a-priori. VBM also accounts for individual head size and variations that are associated with factors such as age and gender (Ashburner & Friston, 2000).

All analyses reported here were performed in MATLAB 2014b using SPM 12. Unless otherwise stated, default parameters specified by SPM 12 for a VBM analysis were retained. T1- weighted scans were checked for artefact, and the origin manually set to the AC. Images were then segmented into three tissue classes (grey matter, white matter and CSF, determined using default tissue probability maps), registered and bias

corrected simultaneously (Ashburner & Friston, 2005). Next, the deformations that most representatively align the images together were estimated using DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra; Ashburner, 2007). DARTEL increases the accuracy of inter-subject alignment (and therefore the accuracy of spatial normalisation). DARTEL roughly aligns low-resolution versions of the segmented grey and white matter images of each subject with their average. This is an iterative process that generates an increasingly accurate average template (in MNI space) through many iterations of alignment between subjects. This process is repeated six times to achieve the final template to which all original (and in native space) grey and white matter segmentations are warped.

DARTEL produces flow fields that estimate the deformations needed to match each subject's segmented grey and white matter to the template. These flow fields are used to warp the images to the final template, bringing each subject into MNI space. Spatial normalisation in this manner accounts for global differences in brain shape. Grey and white matter images were smoothed using a full width half maximum (FWHM) Gaussian kernel of 8 mm. This kernel size is suitable for detecting differences in white matter and the more variable cortex. When images are smoothed, each voxel represents the average of its surroundings, which is defined by the size of the smoothing kernel, rendering the data more normally distributed (Mechelli, Price, Friston, & Ashburner, 2005). Modulated images, which account for tissue volume, were used in statistical analyses. Figure 4.2 is an example of segmented, smoothed and normalised white and grey matter from one healthy subject.

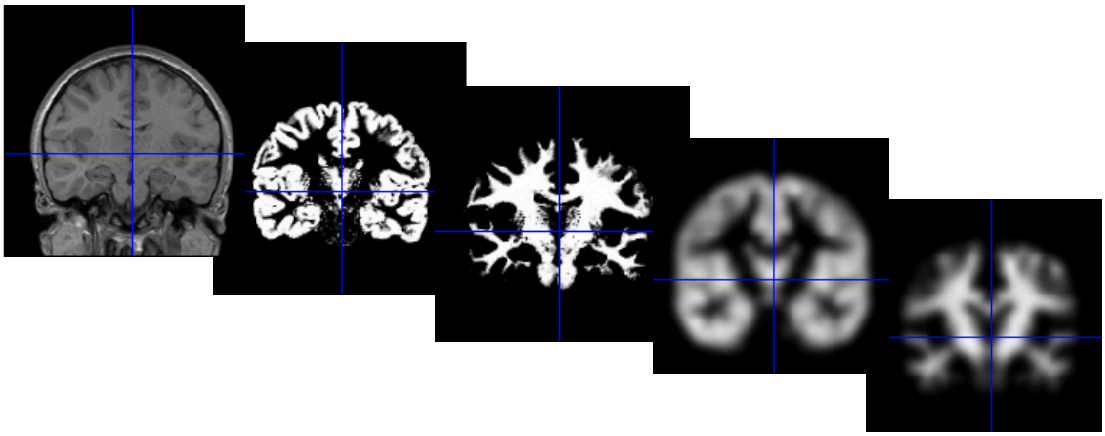

Figure 4.2 Original T1 scan along with segmented and smoothed grey and white matter of a healthy 14-year-old participant.

Statistical analyses were then performed on the smoothed, modulated grey and white matter images. Main effects of group were investigated using an ANCOVA. Age and gender were entered as covariates and were overall mean-centred. To account for differences in overall head size, data were proportionally scaled by intracranial volume (ICV). An absolute mask with a threshold of 0.2 for grey matter and 0.4 for white matter was used to ensure that only voxels that could be truly classified as grey or white matter were included in the analysis. The more stringent threshold was applied to white matter in order to reduce partial volume effects from periventricular regions. Main effects were followed up with two sample t-tests to compare patients with CHI and healthy controls, patients with KH and healthy controls and finally patients with CHI and patients with KH.

All results reported here are significant at cluster-corrected family wise error (FWE) $p<0.05$ unless otherwise stated. This cluster correction was achieved by setting the whole-brain voxel-wise p value to <0.001, and examining clusters in the data that were significant at FWE $p<0.05$. Results were corrected for non-isotropic smoothness (Hayasaka, Phan, Liberzon, Worsley, & Nichols, 2004; Ridgway et al., 2008). Co-ordinates and T-values of local maxima within each significant cluster are tabulated. Identification of anatomical regions was aided by SPM Automatic Anatomical Labelling version 2 (AAL2) and cross-referenced with FSL's Harvard cortical atlas and JHU white matter labels.

4.3 Results

4.3.1 Global brain volumes

A multivariate analysis of covariance (MANCOVA) was conducted to compare ICV, grey matter (ICV-adjusted), white matter (ICV-adjusted), and CSF (ICV adjusted) volumes between groups, while controlling for age and gender. A main effect of group was detected ($F_{(6, 172)}=3.2$, $p=0.006$). Follow-up univariate ANCOVAs were conducted and main effects were considered significant at $p<0.0125$ after Bonferroni correction for multiple comparisons. These showed group differences in white matter volume ($F_{(2, 87)}=5.7$, $p=0.005$) and ICV ($F_{(2,87)}=5.5$, $p=0.006$), but no significant differences in CSF volume ($p=0.952$). Group differences in grey matter volume did not survive correction for multiple comparisons ($p=0.051$, [Figure 4.5]). Post-hoc Bonferroni-corrected pairwise tests showed that patients with CHI have significantly lower white matter

volume compared to controls (p=0.003; Figure 4.4) and lower ICV compared to controls (p=0.009; Figure 4.3). Patients with CHI also had significantly lower ICV than those with KH (p=0.039 [Figure 4.3]). These results remained at trend level in the smaller sample, where a MANCOVA conducted on the same variables showed a trend for a main effect of group ($F_{(6,80)}$ =2.18, p=0.053), and follow up univariate ANCOVAs (considered significant at p=0.0125) showed trends for main effects in white matter $F_{(2,41)}$=3.36, p=0.045) and ICV $F_{(2,41)}$=3.33 p=0.046. Pairwise comparisons show patients with CHI have lower WM volume than controls (p=0.04) and a trend for significantly lower ICV than controls (p=0.062).

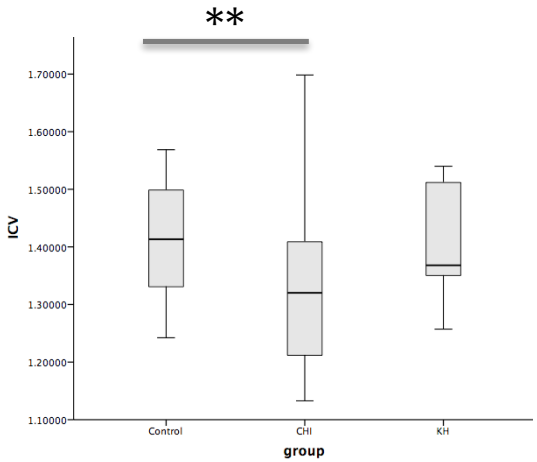

Figure 4.3 Box and whisker plot of ICV (litres) in each group, showing the median, interquartile range and range of ICV values. Patients with CHI have significantly lower ICV than healthy controls

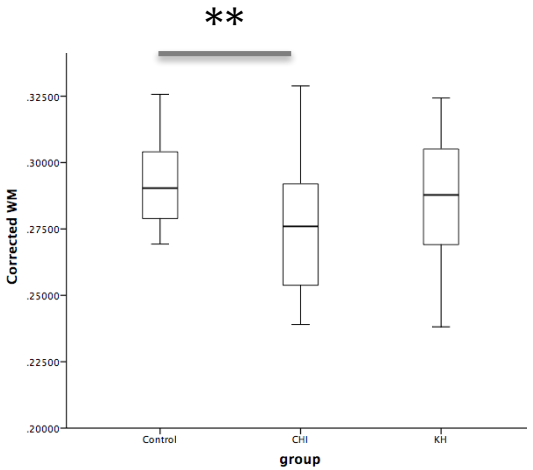

Figure 4.4 Box and whisker plot of ICV-corrected white matter volumes in each group, showing the median, interquartile range and range of corrected white matter. Patients with CHI have significantly lower global white matter volumes than healthy controls

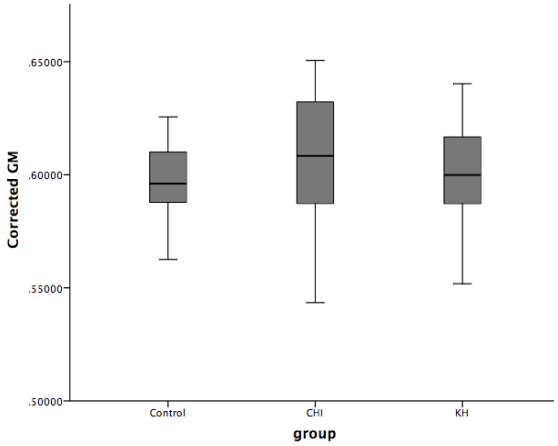

Figure 4.5 Box and whisker plot of ICV-corrected grey matter volumes showing the median, interquartile range and range of corrected grey matter. No significant differences exist between groups

4.3.2 Voxel-Based Morphometry

4.3.2.1 Grey matter volume differences

ANCOVA did not reveal significant difference between groups that survived FWE correction for multiple comparisons. However, group differences were observed in cluster in the left inferior frontal gyrus that was significant at p=0.05, uncorrected. Because of the stringency of the F-test and the correction level employed, as well as observed impairments in executive functioning in patients, post-hoc tests were conducted to examine these differences. These were considered at a more stringent Bonferroni-corrected α of p=0.008, FWE corrected. This revealed a significant difference between patients with CHI and controls, as a large cluster of grey matter volume decrease in the left frontal pole, extending into the left paracingulate gyrus (Table 4.3, Figure 4.6). Volume reduction in the left frontal pole was replicated at uncorrected level in the smaller cohort (cluster-level p-uncorrected= 0.01, k_e=666. Figure 4.7). No other significant volume decreases in either patient group compared to controls were observed, nor were there any significant volume increases in either patient group relative to controls. No regional differences in grey matter volume were observed between patient groups.

Table 4.3 Parameter estimates of local maxima within significant cluster of reduced grey matter volume in patients with CHI relative to controls (significant at p<0.008 cluster level FWE corrected, corrected for non-isotropic smoothness). $K_{e=}$ cluster size.

K_e	FWE-p	Location of local maximum	T	Local maximum co-ordinates
1697	0.002	Left frontal pole	4.42	-38 62 -10

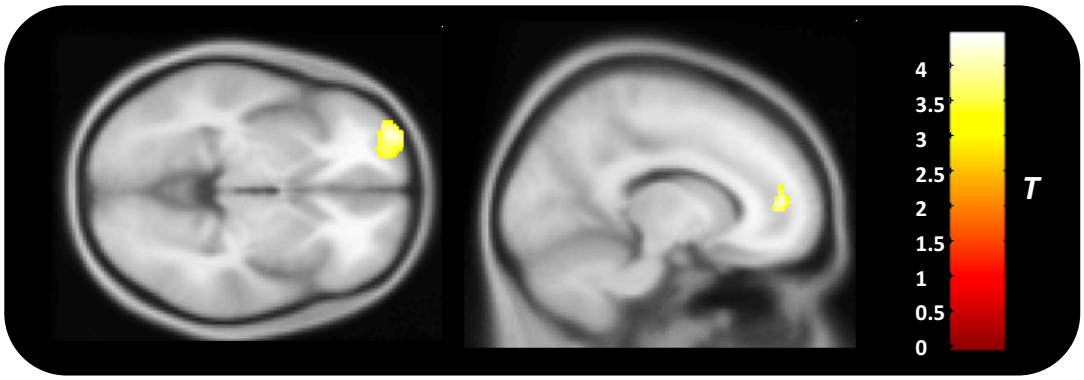

Figure 4.6 Cluster of reduced grey matter volume in left frontal pole extending into the left paracingulate gyrus in patients with CHI compared to healthy controls (-12, 58, -4). Results are colour-coded according to T-values.

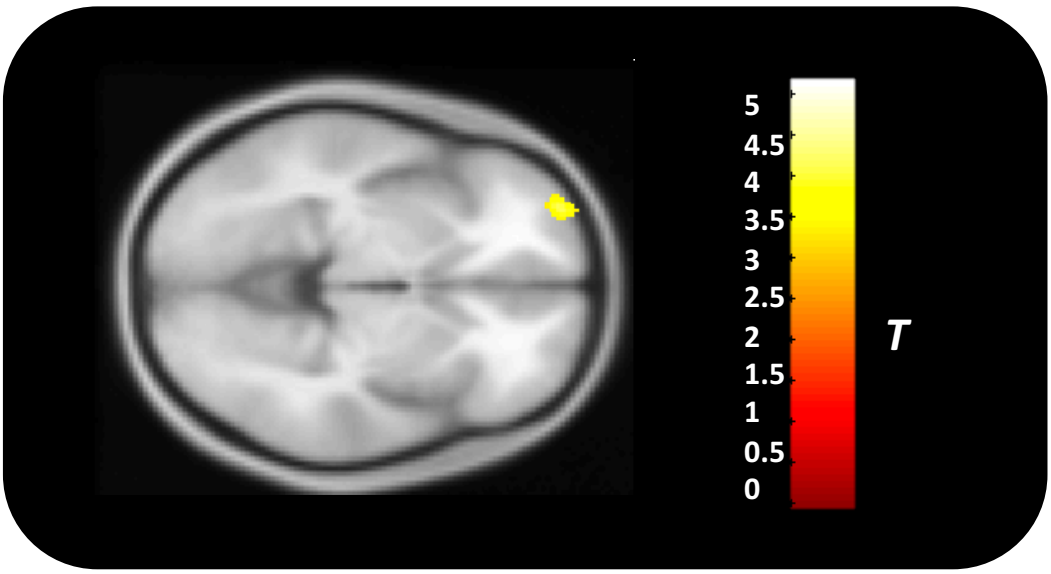

Figure 4.7 Replication of grey matter volume reduction in left frontal pole at uncorrected level in small cohort of patients with CHI and healthy controls (-12, 58, -4). Results are colour coded according to T-values.

4.3.2.2 White matter

An ANCOVA showed significant group differences in white matter volume in frontal and posterior regions. These were followed up with post-hoc t-tests, described below.

4.3.2.2.1 White matter volume reductions in patients with CHI relative to healthy controls

VBM analysis identified five large clusters of significantly reduced white matter volume in patients with CHI relative to healthy controls. These clusters extended over more than one structure. Table 4.4 describes peak voxel coordinates of all significant clusters. The first cluster covered regions of left frontal white matter, including the anterior corona radiata, anterior thalamic radiations, cingulum and genu of the corpus callosum. The second cluster covered posterior portions of the brain and included

bilateral occipital white matter, covering the forceps major, bilateral inferior fronto-occipital fascicules and left superior longitudinal fasciculus. This cluster also extended into the left hippocampal cingulum and left posterior thalamic radiations. The third cluster covered right occipital white matter (including the inferior fronto-occipital fasciculus and inferior longitudinal fasciculus), the body and splenium of the corpus callosum, the right cingulum, and extended into the thalamus. The fourth cluster covered left cerebellar white matter. The fifth cluster covered right frontal white matter, including the right inferior fronto-occipital fasciculus, right uncinate, genu of the corpus callosum, forceps minor and right anterior thalamic radiations. These results were replicated in the small cohort (Figure 4.9, Table 4.5).

Table 4.4 Significance and size of clusters of reduced white matter volume in patients with CHI relative to controls, with co-ordinates at T-values of local maxima Results are cluster-level corrected FWE-p<0.05, corrected for non-isotropic smoothness.

Cluster size	FWE-p	Location of local maxima	T	Local maxima co-ordinates
11755	<0.001	Left forceps minor	5.8	-15 62 8
5863	<0.001	Right forceps major	5.2	10 -86 6
5614	<0.001	Right cingulum	4.8	2 -27 28
1526	0.028	Left cerebellum	4.7	-22 -60 -51
2522	0.003	Right inferior fronto-occipital fasciculus	4.5	30 50 -6

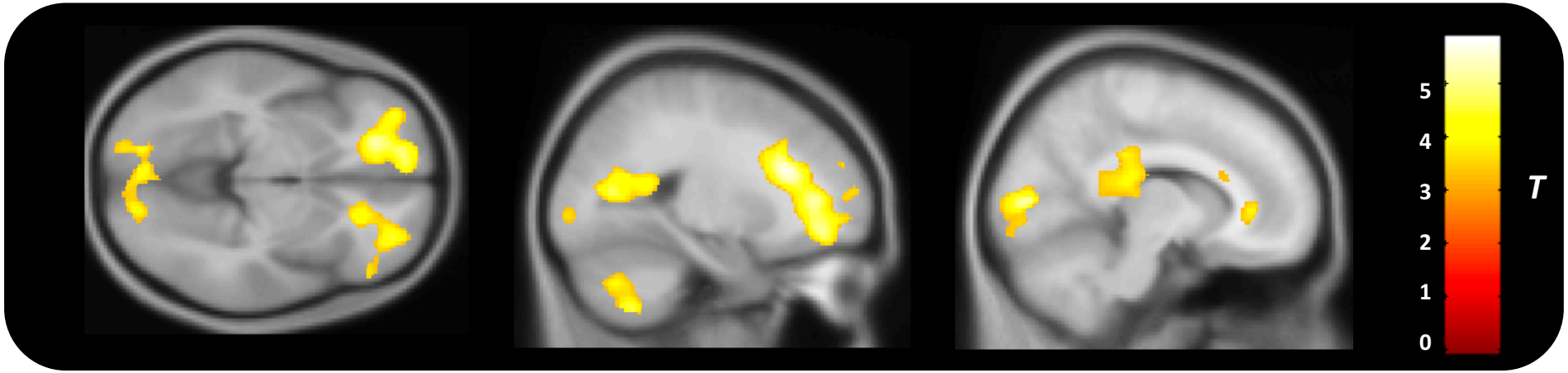

Figure 4.8 Significant clusters of reduced white matter volume across many regions in patients with CHI compared to healthy controls. Coordinates from left to right: 31 47 -6, -23 -60 -51, 11 -86 6. Results are colour coded according to T-values**.**

Table 4.5 Co-ordinates and peak voxel estimates of local maxima within significant clusters of reduced white matter volume in smaller sample of patents with CHI relative to controls. Where multiple representatives of the same regions were found the most significant peak voxel is reported. Results are cluster-level corrected FWE-p<0.05, corrected for non-isotropic smoothness.

Cluster size	FWE-p	Location of local maxima	T	Local maxima co-ordinates
7439	<0.001	Left anterior thalamic radiation	6.2	-33 44 3
1022	0.046	Left cerebellum	5.21	-21 -58 -51
719	0.042	Right cingulum	4.5	6 -26 34
513	0.01	Forceps major	5.1	-6 -86 20
1556	0.008	Right inferior fronto-occipital fasciculus	4.8	32 48 -4

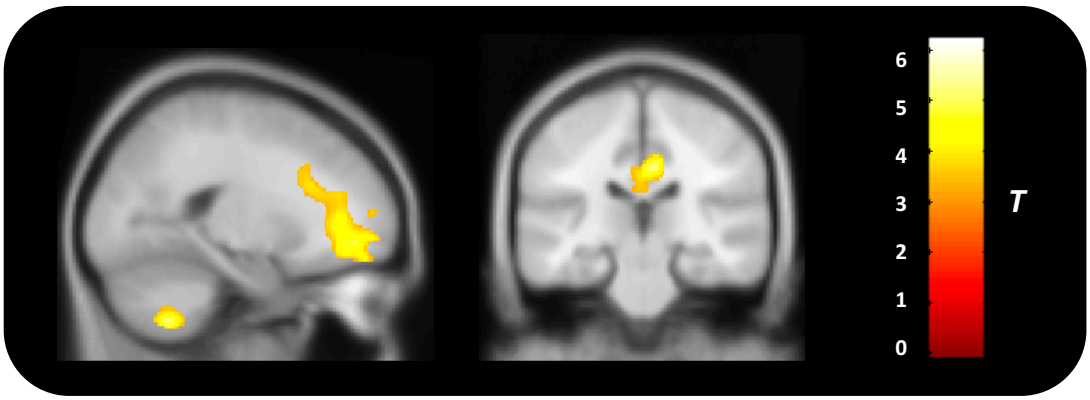

Figure 4.9 Significant clusters of white matter volume reduction across multiple regions in smaller cohort of patients with CHI compared to healthy controls. Coordinates: 6 -25 55. Results are colour coded according to T-values.

4.3.2.2.2 Differences in white matter volume between patients with KH and healthy controls

No clusters of significantly reduced white matter volume in the large cohort of patients were identified when comparing patients KH to healthy controls. In the smaller cohort, a cluster of significantly reduced white matter volume was observed in the left temporal pole extending into the parahippocampal gyrus (Table 4.6, Figure 4.10). This cluster was present as a trend in the large cohort at uncorrected level (-38, 3 -34, K_e=390, $p_{uncorrected}$=0.09). There were no significant increases in white matter volume in patients with KH compared to healthy controls in either group.

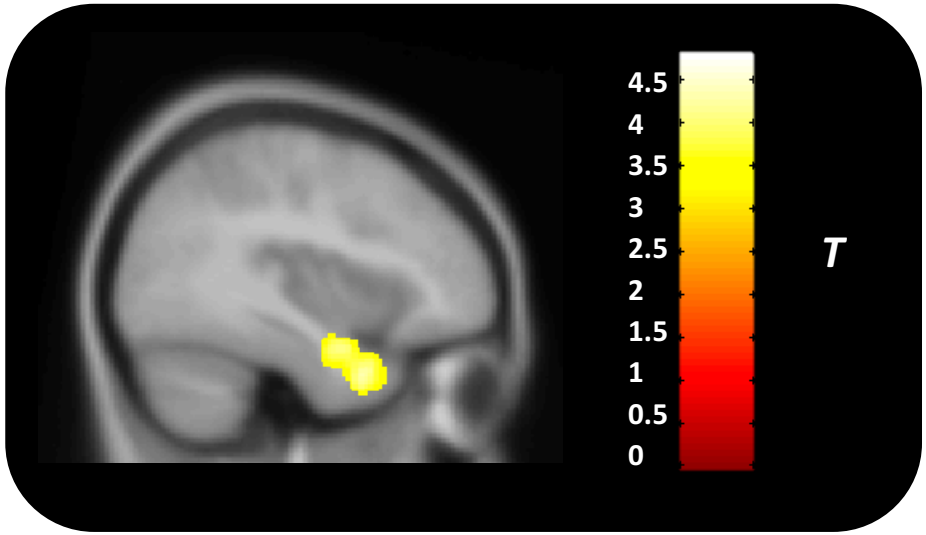

Figure 4.10 Significant cluster of reduced white matter volume in left temporal region in patients with KH compared to healthy controls in the small cohort. Coordinates: -38 8 -33. Results are colour coded according to T-values.

Table 4.6 Co-ordinates and peak voxel estimates of local maxima within significant clusters of reduced white matter volume in smaller cohort of patients with KH relative to controls.

K_e	FWE-p	Location of local maxima	T	Local maxima co-ordinates
1382	0.007	Left inferior longitudinal fasciculus	4.37	-38 8 -33

4.3.2.2.3 Differences in white matter volume between patients with CHI and patients with KH

A region of significantly reduced white matter volume in patients with CHI relative to patients with KH was identified in the left temporo-occipital junction (FWE p=0.013) covering the Inferior Longitudinal Fasciculus (Figure 4.11, Table 4.7). This difference was not identified in the small cohort (even at an uncorrected level).

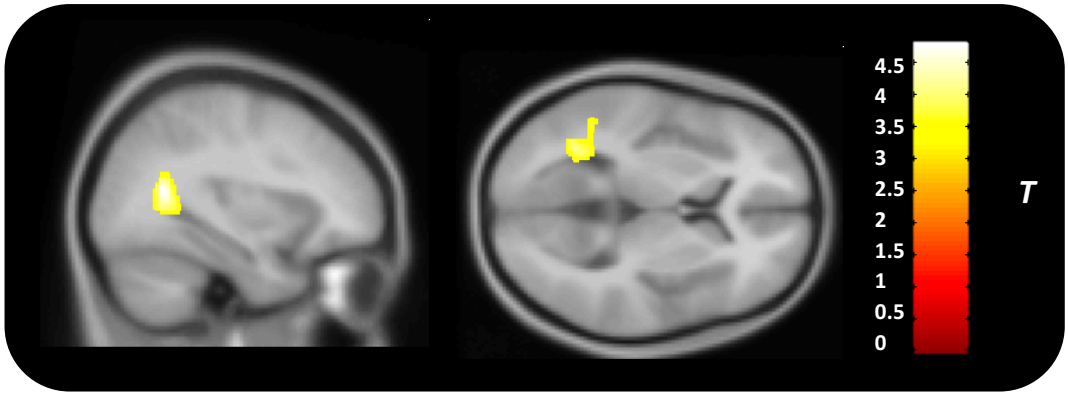

Figure 4.11 Significant cluster of white matter volume reduction in temporo-occipital region in large cohort of patients with CHI compared to patients with KH. Coordinates -32 51 5. Results are colour coded according to T-values.

Table 4.7 Co-ordinates and peak voxel estimates of local maxima within significant clusters of reduced white matter volume in large cohort of patients with CHI relative to patients with KH.

K_e	FWE-p	Location of local maxima	T	Local maxima co-ordinates
1309	0.013	Left Inferior Longitudinal Fasciculus	4.8	-34 -58 15

4.4 Discussion

This chapter has explored global brain volumes and regional differences in grey and white matter volume in patients with CHI and KH compared to healthy controls. These analyses reveal a marked effect of hypoglycaemia on white matter volume in patients with CHI relative to healthy controls, as well as focal reductions in frontal grey matter. Focal white matter volume loss in patients with KH compared to healthy controls is evident at an uncorrected level, but does not manifest as global atrophy. There were no focal or global matter grey volume differences between patient groups, but a white matter volume decrease in the left Inferior Longitudinal Fasciculus in patients with CHI relative to those with KH was observed.

4.4.1 Reduction of intracranial volume

Significantly reduced ICV in patients with CHI compared to both healthy controls and patients with KH in the face of preservation of total GM and CSF volume is indicative of the extent of white matter volume loss in this patient group. In this study it is the only global measure on which patients with CHI and patients with KH are significantly different and this difference is presumably driven by the profound atrophy of white matter in patients with CHI. Importantly, many studies have reported significantly smaller head circumference in those who experience early hypoglycaemia compared to the normal population (Burns et al., 2008; Cresto, Abdenur, Bergada, & Martino, 1998; Duvanel, Fawer, Cotting, Hohlfeld, & Matthieu, 1999). Indeed, Cresto et al. (1998) found that reduced head circumference was present in those with brain

atrophy and also correlated with adverse sequelae such as motor disability. These authors have argued that, in this context, reduced head circumference reflects underlying white matter pathology, although it could also be argued to reflect poor head growth. In the face of preserved grey matter volumes, the results presented in this chapter support the argument that white matter pathology underlies reduced head circumference.

4.4.2 White matter volume reduction

The white matter atrophy in patients with CHI presents as an overall reduction in white matter volume, accompanied by focal loss of white matter volume that is particularly evident in frontal regions but also extends to posterior regions. The severity of this white matter volume loss is corroborated by many reports in the neonatal literature of lesions and severe atrophy in widespread white matter regions, as reported in the introduction to this chapter.

Significant clusters of white matter volume reduction in patients with CHI compared to controls covered many regions. Frontal white matter was severely affected and this extended into the genu of the corpus callosum. The fibres of the genu of the corpus callosum curve outwards to form the forceps major (Catani & Thiebaut de Schotten, 2008). In patients with CHI regions assumed to contain these fibres had significantly less white matter volume than healthy controls. In addition, volume reduction was observed in the splenium of the corpus callosum, whose fibres extend outward to form the forceps minor (a region also affected in patients with CHI). The white matter

volume loss in patients with CHI covered regions containing association, projection and commissural fibres. These will be explored in detail in Chapter Six.

The corpus callosum has previously been implicated in hypoglycaemia. Kumaran (2012) found that patients with CHI had significantly reduced Fractional Anisotropy (a marker of white matter integrity available through analysis of Diffusion Weighted Imaging) of the corpus callosum compared to patients with KH. Furthermore, the volume of the splenium in an adult with diabetes was reduced following episodes of severe hypoglycaemia, as was the volume of the cingulate and cerebellum (Kirchhoff et al., 2013). The results presented in this chapter add to the evidence that hypoglycaemia damages these white matter regions, but also demonstrates a greater frontally-distributed pattern of volume loss than has been previously reported.

The implication of frontal regions is at odds with many reports in the literature which state volume loss and evidence of lesions primarily in parieto-occipital areas (Alkalay, Sarnat, et al., 2005; Caraballo et al., 2004; Filan et al., 2006; Gataullina et al., 2013; Hayasaka et al., 2004; Kinnala et al., 1999; Traill et al., 1998; Wang et al., 2012; Wong et al., 2013). Some studies have reported white matter volume loss and evidence of lesions in periventricular regions (Murakami et al., 1999; Yeon, Hyun, Goo, & Ho, 2006). Only a small number of studies indicate involvement of the frontal lobes (Barkovich et al., 1998; Burns et al., 2008; Gataullina et al., 2013). These results were surprising given existing reports in much of the literature. There could be a number of reasons for the difference reported in previous studies that cite mostly posterior tissue damage and the findings reported in this chapter. First, previous studies have often

included participants with differing aetiologies of hypoglycaemia, often with co-morbidities such as extreme prematurity, hypoxia or epilepsy, making it difficult to discern true hypoglycaemic brain pathology. It has been suggested that the posterior pattern of damage often reported in neonates with hypoglycaemia may result from the inclusion of neonates with hypoxic-ischaemic encephalopathy, and that this pattern of damage is not a marker of damage caused solely by hypoglycaemia (Burns et al., 2008). Second, the cohort of children included in this study is older than those who have been previously reported, and to my knowledge, is the first cohort to be compared against a healthy control group. For some of the children involved the disease course had not resolved and the protracted development of the frontal lobes might render them particularly vulnerable to hypoglycaemic insult occurring outside the neonatal period.

White matter volume loss in patients with KH compared to healthy controls was observed in the left anterior temporal pole and extended into the parahippocampal gyrus. This was present at uncorrected cluster-level in the large cohort and FWE-corrected level in the small cohort. Taken together with Kumaran's (2012) finding of white matter lesions in patients with KH, the results presented in this chapter add to the argument that patients with KH might be at risk of neuropathology as a result of the hypoglycaemic episodes that they experience, despite the presence of ketone bodies during these episodes.

The difference in white matter volume between patients with KH and patients with CHI is less clear. The volume decrease in the occipito-temporal region in patients with CHI

relative to patients with KH is topologically consistent with studies that cite a primarily posterior vulnerability to hypoglycaemia due to hyperinsulinism, however this effect was not as robust as the other effects identified throughout this chapter, which were all replicated in the small and large cohorts. Patients with KH have a similar range of total white matter volumes to those with CHI (Figure 3.4). The lack of difference in total white matter volume between patients with CHI and patients with KH suggests that those with KH may fall somewhere in between healthy controls and patients with CHI in terms of white matter pathology. It is possible that there is a spectrum of damage within the KH group that varies as a function of medical variables, such as age at presentation. While it's difficult to tease apart the role of ketone bodies from other factors, this might explain the focal volume difference observed in patients with CHI relative to patients with KH. Indeed, Gataullina and colleagues (2013) found that lesions in parieto-occipital white matter occurred only in children who experience hypoglycaemia before the age of six months, and studies on childhood diabetes have often associated early onset with early episodes of hypoglycaemia with positive neuroimaging findings (Ferguson, Blane, Wardlaw, et al., 2005; Wootton-gorges & Glaser, 2007). Children with KH tend to develop the condition at a later age than those with CHI (Randell, 2013) although some of the children included in this study developed the condition at birth (see Chapter Two). Thus, the variation in age at presentation might be an important factor in associated neuropathology.

This study adds to the evidence that white matter pathology, in the form of volume loss, characterises the imaging profile of patients with CHI, but also suggests that patients with KH may be at risk of white matter pathology. The difference in white

matter profiles of patients with CHI and patients with KH is less clear. Therefore, replication from another analysis with a different group of patients with KH would be required to verify results.

4.4.3 Pathophysiology

It is not known how many of these patients might have had subtle white matter lesions so it is difficult to interpret these findings in terms of pathophysiology. The white matter volume loss could be a result of Wallerian degeneration, secondary to lesions in grey and white matter (Baldeweg et al., 2006). It could also be due to loss of myelin. Murakami et al. (1999) suggest that hypoglycaemia causes abnormal myelination from damage to the glial cells around the lateral ventricles, causing periventricular changes. Loss of myelin can be observed as reduced volume of white matter in VBM-style analyses, such as the one conducted in this study (Barnea-Goraly et al., 2005).

4.4.4 Focal grey matter volume changes

The grey matter volume reduction in the CHI group is a focal effect and is not observed as a global reduction in grey matter volume. Again, this finding is broadly at odds with the majority of reports stating posterior grey matter changes as a neuroimaging marker for hypoglycaemia (Boardman et al., 2013; Caraballo et al., 2004; Wang et al., 2012). However, this effect is highly significant, surviving a very stringent correction for multiple comparisons. This finding is consistent with the significance of frontal involvement of brain tissue reduction seen in this cohort and with the observed

behavioural impairments in tasks tapping the executive domain. As this cluster was not significant as a main effect, replication of this finding in a different sample would be necessary to validate its reliability.

4.4.5 No involvement of subcortical structures

Previous reports have indicated involvement of subcortical structures in patients with hypoglycaemia, particularly of the hippocampus, putamen and thalamus (Barkovich et al., 1998; Kinnala et al., 1999). This study has not shown grey matter volume reduction of subcortical structures in either of the patient groups. However, VBM may not be optimised to detect such differences. The choice of smoothing kernel applied here may be too large to detect focal differences in comparatively small structures and the stringent correction for multiple comparisons that is applied may result in Type-II errors. Chapter Five will examine volumetric differences in the thalami and basal ganglia through automated segmentation and volumetric differences in the hippocampi using manual segmentation.

4.4.6 Limitations

While the large clusters of significantly reduced white matter volume in patients with CHI indicate the extent of atrophy, as they covered many anatomical regions it is difficult to accurately locate the regions of white damage. What is apparent is that extensive white matter pathology is observed in children with CHI. A Tract-Based Spatial Statistics (TBSS) analysis in Chapter Six aims to more accurately identify regions of white matter pathology using markers of diffusion changes to infer structural

integrity of underlying white matter tracts. Another possible limitation of the current study is that VBM relies on extremely accurate registration in order to reliably indicate where differences between groups lie. This is a limitation of the method and misclassification of structures and incorrect inference about differences between groups is common if registration has failed. All possible steps were taken to reduce the chance of this occurring through visually checking each segmented and smoothed modulated image. Finally, other studies examining the difference in head circumference (which is analogous to ICV) have controlled for height when comparisons are made between groups (Nosarti et al., 2008) to assess whether the effects are due to a globally reduced growth or specific to brain pathology. This measure would strengthen any conclusion made about the reduction in ICV observed in CHI.

4.4.7 Conclusion

In conclusion, this study has demonstrated that children with CHI show global and regional white matter volume loss. This is the first study to identify regional white matter volume loss in this patient group via quantitative neuroimaging analysis, and the first to include only children with no neurological diagnoses. In addition, as the first study to characterise global changes in patients with CHI of this age, these findings suggest a lasting pattern of brain abnormality in patients with CHI, characterised by significant involvement of white matter in anterior regions. The primarily frontal nature of white matter volume reduction in patients with CHI has important implications for cognition and is in accordance with their demonstrated impairment in

attention and motor skills (Chapter Three). Neural correlates of these observed impairments are explored in Chapter Seven.

This study also highlights that children with KH might be at risk of white matter pathology as a result of hypoglycaemia. This does not manifest as global volume loss, but can be seen as a focal volume decrease. As mentioned previously, patients with KH might show a spectrum of pathology that is mediated by medical variables not examined here, such as the age at which hypoglycaemia first occurs. There is a consensus that early episodes of hypoglycaemia are particularly detrimental to the developing brain (Arnoux et al., 2010; Hussain & Cosgrove, 2005; Kapoor, James, & Hussain, 2009); however, as can be seen from the literature on early onset diabetes, this probably extends outside of the neonatal period. The difference observed in occipito-temporal regions between patients with CHI and patients with KH warrants further investigation and would require replication to verify the findings. However, if patients with KH were truly protected from neuropathology because of the presence of ketone bodies, one would expect to see a similar pattern of volume loss when comparing those with CHI against those with KH as in comparisons of those with CHI against healthy controls, which would include significantly greater white matter volume in primarily anterior regions. That there were no differences between patient groups in frontal regions is consistent with their shared impairment in tasks of an executive nature. Indeed, this study suggests that the understanding of ketotic hypoglycaemia as a 'benign' disease with no associated adversities needs to be reconsidered.

Chapter 5

Subcortical Volumes

5 Subcortical volumes

This chapter focuses on the integrity of the hippocampus, thalamus and basal ganglia structures in patients with CHI and KH. A review of relevant studies examining the effect of such episodes in the context of transient hypoglycaemia, EODM and CHI is given. Here, measurements of the hippocampi (obtained through manual tracing) and measurements of the thalami and basal ganglia (obtained through semi-automatic segmentation) are compared between healthy controls, patients with KH and patients with CHI.

5.1 Introduction

The effects of hypoglycaemia on subcortical structures in the brain have been well documented in older diabetic patients who can experience severe hypoglycaemia because of over-administration of insulin, sometimes resulting in coma. These studies often find lesions to the basal ganglia, hippocampus and thalamus (Jung, Kim, Lee, Yoon, & Byun, 2005; Kalimo & Olsson, 1980; Kirchhoff et al., 2013). Animal research has suggested that the caudate, putamen and hippocampus are particularly sensitive to hypoglycaemic insult (Auer, Wieloch, Olsson, & Siesjö, 1984; Suh, Hamby, & Swanson, 2007). In the rat, the dentate gyrus of the hippocampus has been shown to be very susceptible to hypoglycaemia, with neuronal necrosis visible on histological stains, and some studies in human adults have replicated this finding, showing a similar vulnerability (Auer, 1986, Auer, Hugh, Cosgrove & Curry, 1989).

The effect of hypoglycaemia on subcortical structures in the immature brain is not as well documented, especially in the context of repetitive hypoglycaemia due to hyperinsulinism or ketotic hypoglycaemia. In the newborn, the thalamus and hippocampus are regions of particularly high glucose metabolism and at two to three months of age, glucose use increases in the basal ganglia. This increased demand for glucose is thought to render these regions particularly sensitive to hypoglycaemic insult at these time points (Chugani, 1998). Accordingly, the limited research into the effects of early hypoglycaemic episodes on subcortical structures has documented pathophysiology (in the form of neuron reduction) in the basal ganglia and thalami of infants (Anderson, Milner, & Strich, 1967; Banker, 1967).

5.1.1 Vulnerability of the hippocampus

Studies of imaging profiles in early onset type one diabetes mellitus (EODM) offer an insight into the effect of hypoglycaemia on the immature brain outside of the neonatal period. One report of EODM stated evidence of Mesial Temporal Sclerosis (MTS) in 16% of patients. However, no significant differences were observed in the rates of MTS between groups who had and had not experienced episodes of hypoglycaemia (Ho et al., 2008). The authors state that the classification of a hypoglycaemic event employed here (hypoglycaemia resulting in seizure or coma) might have distorted these results, as less severe hypoglycaemic events could go unnoticed. Ferguson and colleagues (2005) reported a significantly higher occurrence of small punctate white matter lesions in the hippocampi of children with early onset of T1DM compared to those with later onset, although a link between episodes of hypoglycaemia and this

pathology was not explicitly made (Ferguson, Blane, Wardlaw, et al., 2005). Animal research has offered further insight; in both diabetic and non-diabetic rat pups, extensive damage to the hippocampus has been observed after episodes of hypoglycaemia (Bree, Puente, Daphna-Iken, & Fisher, 2009).

In contrast to reports of hippocampal damage in EODM, Hershey et al. (2010) found that diabetic children with a history of hypoglycaemia had increased hippocampal volume. This was an unexpected finding and the authors speculated that this might be a pathological response to severe hypoglycaemia, in the form of gliosis, or a compensatory response to injured neurons. Thus, even in the field of early onset diabetes, which has been quite extensively researched, it is difficult to draw conclusions about the effects of early hypoglycaemia on subcortical brain structures. However, in all of the studies mentioned above, the hippocampus has been implicated following hypoglycaemia, suggesting that this region might be vulnerable to dysglycaemia.

Reports of hippocampal damage in patients with hypoglycaemia (not in the context of diabetes) are few. Gataullina and colleagues (2013) found that five patients showed lesions in the temporal cortex that extended into the hippocampus (Gataullina et al., 2013). Kumaran (2012) found that there was a relatively high proportion (29%) of patients with CHI who had reduced hippocampal volume identified on conventional neuroradiological assessment, and seven percent of patients with KH were also identified as having reduced hippocampal volume. The percentage of patients with hippocampal volume reduction was not significantly different between the two patient

groups and manually measured hippocampal volumes were not found to differ between patients with CHI and patients with KH. Given that ketone bodies are presumed to prevent hypoglycaemic brain damage through the provision of alternative fuels, these were unexpected findings. However, no healthy control group was available for comparison, limiting any conclusions that could be made regarding relative preservation of brain tissue in either patient group.

5.1.2 Vulnerability of the thalamus and basal ganglia

There are conflicting reports about the involvement of subcortical structures besides the hippocampus in children who experience hypoglycaemia without diabetes. This literature has been reviewed in the introduction, but a short summary of the studies that have reported involvement of the basal ganglia and thalamus will be given here.

Barkovitch et al. (1998) found that one in five hypoglycaemic neonates had lesions to the pallidum, while Kinnala et al. (1999) found the basal ganglia and thalamus to be affected in a neonate with hypoglycaemia (although this damage appeared to have disappeared when the CT scan was repeated at two months of age). Burns and colleagues (2008) went beyond these case studies to report on a large cohort of neonates with hypoglycaemia, where 40% were found to have lesions in the basal ganglia or thalamus.

Reports are fewer in older children, although Kara et al. (2006) found lesions in the putamen in a six-year-old with hypoglycaemic coma due to undiagnosed hyperinsulinism. Avatapalle et al. (2013) also observed basal ganglia changes in a

cohort of children both persistent and transient CHI, but did not state the incidence of this finding in their sample. Gataullina et al. (2013) examined children with Fatty Acid Oxygenation Disease (FAOD) and Glycogen Storage Disease type 1 (GSD1) (n=14), as well as children with CHI (n=33). All of these conditions result in an inability to produce alternative fuels during episodes of hypoglycaemia. MRI scans were performed between one month and five years of age. They found that basal ganglia lesions were present in those who experienced hypoglycaemia between six and 22 months, although notably these were present only in children with FAOD or GSD1. In a later study, the same author found that out of 21 hypoglycaemic patients (who developed epilepsy) three had lesions in the basal ganglia (Gataullina et al., 2014).

While some studies find normal appearing subcortical structures (Filan et al., 2006; Murakami et al., 1999; Yalnizoglu, Haliloglu, Turanli, Cila, & Topcu, 2007), the majority report involvement of the basal ganglia and thalamus, indicating their vulnerability to hypoglycaemic events (Avatapalle et al., 2013; Barkovich et al., 1998; Burns et al., 2008; Gataullina et al., 2013, 2014; Kara et al., 2007) and some studies have found that the hippocampus is vulnerable to hypoglycaemic events (Auer, 1986; Gataullina et al., 2013; Puente et al., 2009). However, these studies are limited by small sample sizes, inclusion of patients with comorbidities, heterogeneity of cohorts and, importantly, reliance on visual inspection of MR and CT images to assess damage. In addition to this, some studies lack specificity about the involvement of subcortical structures, such that it is not always clear which structures within the basal ganglia are affected (Avatapalle et al., 2013; Burns et al., 2008). Finally, there is little consensus as to what might be the lasting effects of lesions to these areas. Without a study examining

subcortical damage in school-aged children following hypoglycaemic events, it is not possible to say whether damage to these regions is a lasting or transient feature of hypoglycaemic brain damage.

5.1.3 Aims and hypotheses

Amid reports of damage to these structures secondary to hypoglycaemia, the aim of this study was to examine the integrity of the hippocampus, thalamus and basal ganglia in a homogenous cohort of school-aged patients with CHI and patients with KH relative to a healthy control group. This will allow the lasting effects of early hypoglycaemic insult to subcortical structures to be determined and to assess whether damage to these structures (in the form of volume loss) appears as a defining feature of any patient group. Previous VBM analyses failed to detect FWE-corrected volume reduction in subcortical structures. However manual and automatic segmentations may be more sensitive to volume reductions of these smaller structures (see Chapter Four for further comment).

Concordant with most reports in the literature, and the suspected vulnerability of these regions to hypoglycaemia, it was hypothesised that patients with CHI would show reduced hippocampal volumes, as well as reduced volume of the thalamus and basal ganglia structures compared to healthy controls. Owing to the lack of literature on outcomes after ketotic hypoglycaemia, specific hypotheses were not made about which structures (if any) might be compromised in this patient group when compared to healthy controls. If ketone bodies are protective to these structures, then it is expected that these structures will be preserved in patients with KH relative to

controls, and accordingly will mirror the results obtained by comparing healthy controls to those with CHI. However, given the results of the HBI study (Kumaran, 2012), some hippocampal volume reduction in patients with KH was anticipated.

This chapter comprises two studies; one comparing volumes of the hippocampi obtained through manual measurements, and the other comparing volumetric differences obtained through automatic segmentations of the basal ganglia and thalamus.

5.2 Methods

5.2.1 Participants

Participants have been described in detail in Chapter Two. Briefly, the study of hippocampal volumes compared all patients with CHI and KH against a large control group (n=82) for whom hippocampal volume measurements performed by the same researcher (Prof. David Gadian) were available. This control group was made up of 64 children assessed as part of an MRC study; *Hypoxia/ischaemia in children: Patterns of neuropathology and associated memory impairment*, and 18 typically developing children whose data were collected by the author ('small cohort' see Chapter Two). Groups did not differ significantly in age (ANOVA $p>0.05$) or gender (χ^2 test, $p>0.05$). Age and gender of the groups are outlined in Table 5.1. This analysis was repeated in the small cohort only. Age and gender of the small cohort were not significantly different. Group descriptives are outlined in Table 5.2.

Table 5.1 Age and gender of cohorts used in hippocampal volume analysis.

	Controls (n=82)	CHI (n=31)	KH (n=20)
Age; mean (SD)	135 (26)	131 (30)	122 (34)
Gender	37 male	20 male	11 male

Table 5.2 Age and gender of small cohort, hippocampal volume analysis

	Controls (n=18)	CHI (n=19)	KH (n=12)
Age; mean (SD)	131 (26)	131 (32)	140 (31)
Gender	8 male	13 male	6 male

The study comparing volumes of basal ganglia structures was conducted in both the large and small cohorts. One healthy control was excluded from the analysis, as segmentation of subcortical structures was deemed inaccurate. Age was comparable between groups in the large cohort (one-way ANOVA, $p>0.05$), but gender did differ significantly between groups ($\chi^2 =6.7$, $p=0.035$). This was due to significantly more females in the healthy control cohort. Age and gender in the small cohort were not significantly different. Age and gender of the groups are outlined in Tables 5.3 and 5.4.

Table 5.3 Age and gender of large cohort

	Controls (n=43)	CHI (n=31)	KH (n=20)
Age in months; mean (SD)	141 (31)	131 (30)	122 (34)
Sex	15 male	20 male	11 male

Table 5.4 Age and gender of small cohort

	Controls (n=17)	CHI (n=19)	KH (n=12)
Age; mean (SD)	133 (26)	131 (32)	140 (31)
Gender	8 male	13 male	6 male

5.2.2 Imaging

T1-weighted MRI scans were used in all analyses reported here. Scan parameters are outlined in detail in Chapter Four. All hippocampi were manually measured by Prof David Gadian, who was blinded to clinical diagnosis. The datasets were reformatted into 1mm thick contiguous slices in a tilted coronal plane perpendicular to the long axis of the hippocampus using MedX 3.43 (Medical Numerics, Inc.) Hippocampal cross-sectional areas were measured and defined by anatomical landmarks across the entire length of the hippocampus (as described in Cooper et al., 2013). Volumes of the hippocampi were obtained by summing the cross-sectional areas and multiplying by the distance between the slices. ICV was measured and all volumes were subsequently corrected for ICV; all analysis of hippocampal volumes reported here are on ICV-corrected volumes. Volumes of the basal ganglia and thalamus were obtained using FIRST (Patenaude, Smith, Kennedy, & Jenkinson, 2011), a software available as part of FMRIB Software Library (FSL) (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). FIRST is a model-based segmentation tool that segments the caudate nucleus, putamen, globus pallidus, nucleus accumbens, hippocampus, amygdala and brainstem. As previous reports in the literature have suggested damage only to the basal ganglia and thalamus secondary to hypoglycaemia, only the integrity of the caudate nucleus, putamen, globus pallidus and thalamus were examined. Segmentations of these structures were inspected for every participant and small manual corrections were made where necessary. Although FIRST does automatically segment the hippocampus, segmentation of this structure is poor (Guderian et al., 2015). Therefore, the 'gold standard' manual segmentations of the hippocampus were used here.

5.2.3 Statistical Analysis

To examine differences in hippocampal volumes between groups, an ANCOVA was performed on mean hippocampal volume (left plus right; already corrected for ICV), with age and gender entered as covariates. To examine unilateral differences, a MANCOVA was performed with age and gender entered as covariates. This was followed up by separate univariate ANCOVAs examining differences in left hippocampal volumes between groups and right hippocampal volumes between groups, corrected for multiple comparisons using the Bonferroni method. Post-hoc pairwise comparisons were conducted and corrected for multiple comparisons using the Bonferroni method.

To study differences between subcortical volumes obtained using FSL FIRST, a MANCOVA was run to examine group differences in the mean (left plus right) volumes of the caudate, putamen, globus pallidus and thalamic volumes. Age, gender and ICV were entered as covariates. A series of univariate ANCOVAs were run to examine differences in left volumes and right volumes of each structure, corrected for multiple comparisons using the Bonferroni method. Post-hoc pairwise comparisons were adjusted for multiple comparisons using the Bonferroni method.

5.3 Results

5.3.1 Comparison of hippocampal volumes

An ANCOVA on mean (left plus right) hippocampal volume showed a significant main effect of group ($F_{(2, 128)}$=5.08, p=0.008). Bonferroni-corrected post-hoc tests revealed that patients with CHI have significantly lower mean (left plus right) hippocampal volume than healthy controls (p=0.005; Figure 5.1). No other comparisons were significant. To examine differences in unilateral volumes between groups, a MANCOVA was conducted, and a significant main effect of group was found ($F_{(2, 128)}$=6.37, p=0.002). Separate univariate ANCOVAs were considered significant at 0.025 after Bonferroni correction for multiple comparisons. These revealed significant differences between groups in the left hippocampus ($F_{(2, 128)}$= 6.24, p=0.003) and a trend for group differences in the right hippocampus ($F_{(2, 128)}$=3.45, p=0.035). Pairwise Bonferroni-corrected post-hoc comparisons (t-tests on adjusted means) showed that patients with CHI have significantly lower left hippocampal volume compared to healthy controls (p=0.002, Figure 5.2). Although the ANCOVA on right hippocampal volume indicated only a trend, post hoc analyses revealed significantly lower right hippocampal volumes in patients with CHI relative to controls (p=0.03, Figure 5.2). No other comparisons were significant. Figure 5.1 shows mean (left plus right) hippocampal volumes in all three groups. These findings were not replicated in the small cohort (MANCOVA p=0.168).

A mixed design ANCOVA (with age and gender entered as covariates) showed that there was no within group main effect of side of hippocampus ($F_{(1,128)}$=0.13, p=0.72)

and no interaction between group and side of hippocampus ($F_{(2, 128)=}1.26$, p=0.287). Thus, across groups, hippocampal volume did not differ according to side, and this did not change depending on group membership.

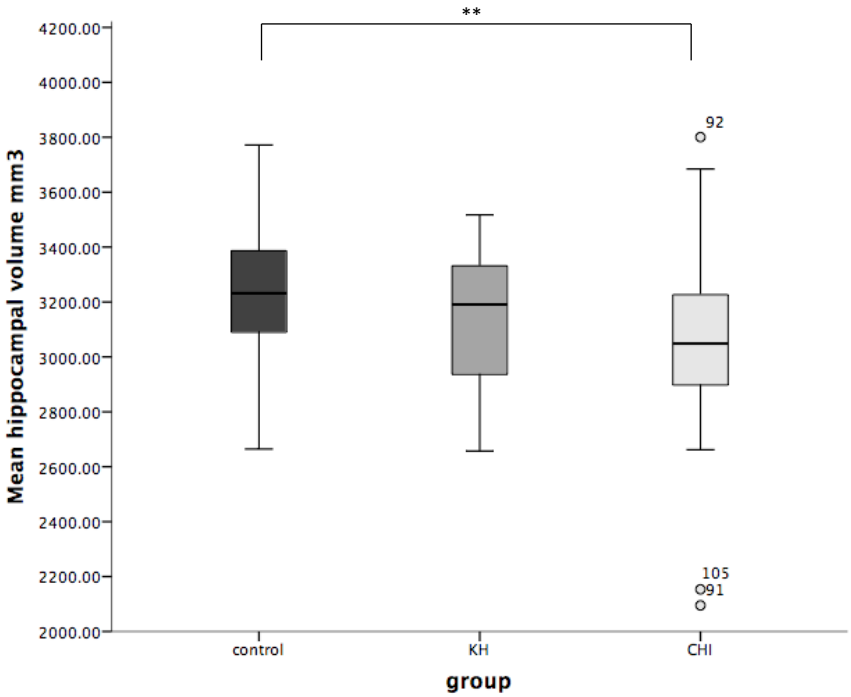

Figure 5.1 Box-plot showing median, interquartile range and range of manually measured hippocampal volumes (corrected for ICV). Patients with CHI have significantly smaller hippocampal volumes than healthy controls.

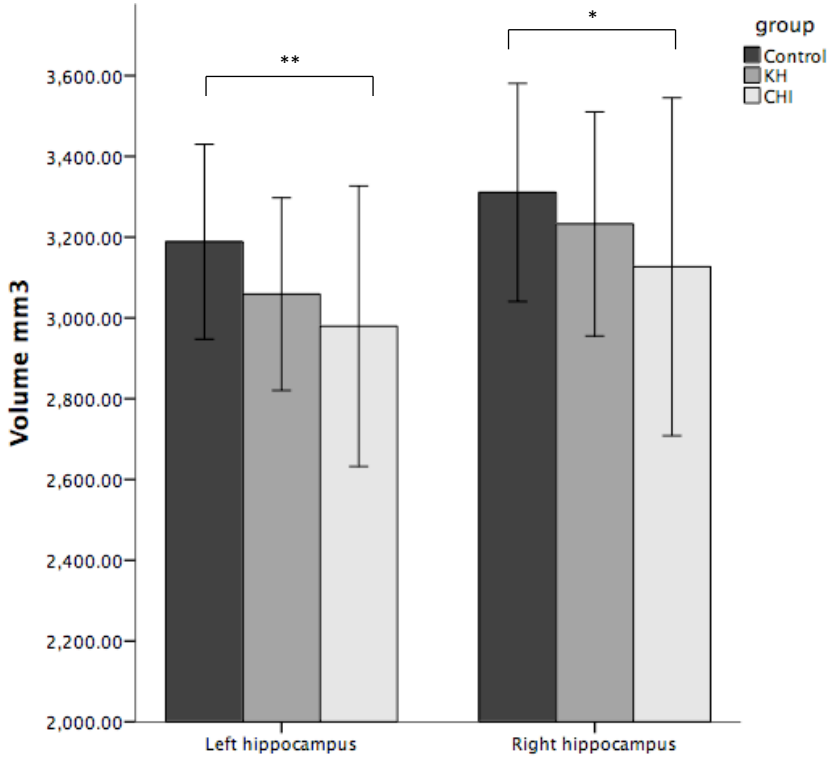

Figure 5.2 Left and right ICV-corrected hippocampal volumes. Error bars represent +/- one standard deviation from the mean. Patients with CHI have significantly smaller hippocampal volumes than healthy controls, which is a bilateral effect.

Table 5.5 Mean hippocampal volumes after correction for ICV.

	Controls (n=82) volume mm^3	CHI (n=31) volume mm^3	KH (n=20) volume mm^3	ANOVA F	ANOVA p	Sig Post-Hoc
Left Hippocampus (SD)	3189 (241)	2979 (347)	3058 (238)	6.24	**0.003	** CHI<Con
Right Hippocampus (SD)	3311 (270)	3127 (418)	3233 (277)	3.45	*0.035	* CHI<Con

5.3.2 Basal ganglia and thalamic volumes

A MANCOVA on mean (left plus right) thalamic and basal ganglia volumes (caudate nucleus, putamen and pallidum) showed a significant main effect of group ($F_{(4,86)}$=2.42, p=0.014). Follow up univariate ANCOVAs (considered significant at 0.0125 after Bonferroni correction for multiple comparisons) showed group differences in the volume of the thalamus ($F_{(2, 88)}$=6.58, p=0.002) and a trend for differences in the pallidum ($F_{(2, 88)}$=3.166, p=0.048). Post-hoc Bonferroni-corrected pairwise comparisons showed that patients with CHI had significantly lower thalamic volume than healthy controls (p=0.002) and patients with KH (p=0.033). A trend for lower volume of the pallidum in patients with CHI relative to controls was observed (p=0.071). No differences in volumes of the caudate and putamen were observed (p>0.1 for all). These results are shown in Figure 5.3. In the small cohort, a MANCOVA did not reveal a main effect of group (p=0.108), although there was a trend for group differences in mean (left plus right) thalamic volume ($F_{(2,42)}$=3.9, p=0.028), driven by trends for smaller volumes in patients with CHI compared to healthy controls (p=0.055) and patients with KH (p=0.076).

Next, analyses were conducted to examine whether the structures that differed significantly between groups had unilateral effects. A MANCOVA on left and right thalamic volumes revealed a statistically significant main effect of group ($F_{(2,88)}$=8.2, p=0.001.). Follow up univariate ANCOVAs (considered significant at p=0.025 after Bonferroni correction for multiple comparisons) revealed group differences in the left thalamus ($F_{(2, 88)}$=5.18, p=0.007) and in the right thalamus ($F_{(2, 88)}$=7.64, p=0.001).

Bonferroni-corrected post-hoc tests showed that volume of the right thalamus was significantly smaller in patients with CHI than healthy controls (p=0.001) and patients with KH (p=0.016). Volume of the left thalamus was also significantly smaller in patients with CHI relative to healthy controls (p=0.007) and there was a trend for smaller volume of the left thalamus in patients with CHI relative to those with KH (p=0.084). These results are shown in Table 5.6. In the small cohort, group differences were replicated in right thalamic volume ($F_{(2,42)=}$4.35, p=0.019). Patients with CHI had significantly lower volume of the right thalamus than controls (p=0.044) and patients KH (p=0.049). A trend for differences between groups in volume of the left thalamus was observed ($F_{(2,42)}$=3.23, p=0.05), and post hoc tests showed patients with CHI had lower left thalamus volume than healthy controls (p=0.085).

A MANCOVA on left and right volumes of the pallidum revealed a statistically significant main effect of group ($F_{(2,88)}$=3.17, p=0.047). Follow up univariate ANCOVAs (considered significant at p=0.025 after Bonferroni correction for multiple comparison) showed a trend for differences in volume of the right pallidum ($F_{(2,88)}$= 2.73, p=0.07) and left pallidum ($F_{(2,88)}$=2.5, p=0.087), with post-hoc pairwise comparisons indicating a trend for lower volume of the right pallidum in patients with CHI relative to controls. These results are shown in Table 5.6. In the small cohort a MANCOVA indicated a trend for significant differences between groups (p=0.057), with univariate analyses revealing a trend for differences in the volume of the left pallidum ($F_{(2,42}$)=3, p=0.06) but no significant differences between volumes in the right pallidum. Post-hoc pairwise comparisons did not indicate any significant differences between groups.

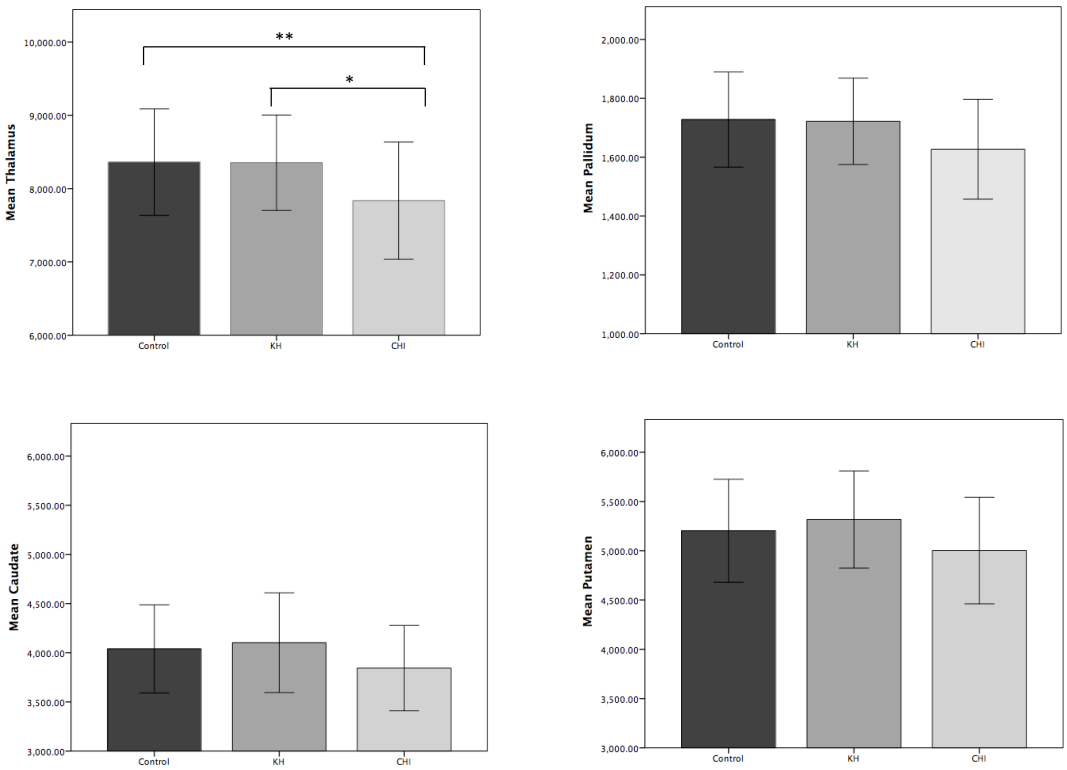

Figure 5.3 Thalamic and basal ganglia volumes in healthy controls, patients with KH and patients with CHI. Error bars represent +/- one standard deviation from the mean. Patients with CHI have significantly lower thalamic volume relative to patients with KH and healthy controls. Trends for lower pallidum volumes in patients with CHI relative to controls were also observed,

Table 5.6 Subcortical volumes obtained through automatic segmentation (mm^3) for healthy controls, CHI and KH. Significance values ** indicates $p<0.01$, * indicates $p<0.05$ $^{+}$ indicates a trend, $p<0.09$

Structure	Controls (n=43) mean mm^3	CHI (n=31) mean mm^3	KH (n=20) mean mm^3	ANOVA F	ANOVA P	Post-hoc
Left Thalamus	8470 (762)	7967 (824)	8443 (647)	5.18	0.007	**CHI<Con $^{+}$CHI<KH
Right Thalamus	8253 (706)	7707 (786)	8226 (663)	7.64	0.001	**CHI<Con *CHI<KH
Left Pallidum	1719 (173)	1621 (172)	1721 (148)	2.51	0.087	
Right Pallidum	1736 (166)	1633 (183)	1722 (157)	2.74	0.07	$^{+}$CHI<Con

5.4 Discussion

5.4.1 Effects of hypoglycaemia on hippocampal volume

Analysis of the large cohort showed a significant reduction in hippocampal volume in patients with CHI relative to controls. Although these results were not replicated in the small cohort, this finding is in line with reports of the hippocampus being selectively vulnerable to hypoglycaemic insult in children (see Case DA 12, Dzieciol et al., 2016) and adults, as well as animals (Auer et al., 1984; Auer, 1986). Furthermore, the findings presented in this chapter add to the reports of hippocampal vulnerability to hypoglycaemia in early onset diabetes (Bree et al., 2009; Ferguson, Blane, Wardlaw, et al., 2005). Importantly, this is the first study to show a significant effect on the volume of the hippocampi in older children with CHI, which manifests as a reduction of mean volume and is driven by bilateral volume reduction. These results extend the reports of individuals with hippocampal volume reduction identified in the HBI study (Kumaran, 2012) and provide a much needed comparison to a healthy control group to confirm that this volume reduction is not consistent with natural variation in hippocampal volume. Importantly, in the HBI study, one third of those with hippocampal volume reduction also had epilepsy. Here it is shown that hippocampal volume reduction is evident even in children without epilepsy.

Patients with KH do not have significantly lower hippocampal volumes than controls, or significantly greater hippocampal volume than CHI patients. However, although the

difference was not statistically significant, patients with KH do have lower hippocampal volume than controls and this appears to be a unilateral effect, being particularly evident on the left side (although a significant group-by-side interaction was not observed). Indeed, hippocampal volumes of those with KH appear to fall in between those with CHI and healthy controls (Figure 5.1). These data suggest that in some children with KH there may not be a complete preservation of the hippocampus and raise questions regarding the protective role of ketone bodies during hypoglycaemia.

The differences in hippocampal volume were not replicated when analysis was repeated in the small cohort. Replication of these findings in a different sample would be advantageous in allowing inferences about the effect of hypoglycaemia on hippocampal volume to be made with confidence. Nevertheless, the findings presented here are highly significant in the large cohort of patients and are bolstered by previous reports of selective vulnerability of the hippocampus to hypoglycaemic events. These data suggest that early episodes of hypoglycaemia are detrimental to the hippocampus, which may have implications for memory function in children with CHI (Cooper et al., 2013; Muñoz-López et al., 2017; Vargha-Khadem et al., 1997). Indeed, there have been reports of memory problems in children with early onset T1DM (Hershey et al., 2005), which may be related to the vulnerability of the hippocampus to hypoglycaemia. The relationship between memory functioning and hippocampal volume will be explored in Chapter Seven.

5.4.2 Reduced thalamic volumes

Patients with CHI have significantly lower mean (left plus right) thalamic volume than healthy controls and those with KH. This is a profound effect, replicated in both the large and small cohorts, and is due to bilateral volume decreases. These results show for the first time that vulnerability of the thalamus to hypoglycaemia is a lasting group-level feature in CHI and expand on the studies of neonates and infants where thalamic damage has been identified (Anderson et al., 1967; Banker, 1967; Burns et al., 2008; Kinnala et al., 1999). This lasting volume reduction is in contrast to reports from Kinnala et al. (1999), where damage to the thalamus was observed to disappear over time (although this could be due to pseudo-normalisation, as described in Chapter One). The volume of the thalamus is preserved in patients with KH, thus in these patients this structure appears to be protected from hypoglycaemic insult.

Most sensory inputs to the cortex pass through the thalamus, and it is therefore a key structure: some argue that it plays a pivotal role in cortical development. This has been observed if projections from the thalamus to the neocortex are altered in the first half of gestation (Johnson and de Haan, 2015; Rakic, 1988), but also as a function of early injury due to premature birth (Ball et al., 2012). In its role as a gateway for sensory inputs, the thalamus is essential for many functions including motor skill and memory. The anterior thalamic nuclei are part of the hippocampal-thalamic circuit, crucial for memory and learning (Aggleton et al., 2010), and acting as a relay centre between the cerebellum, basal ganglia and cortex, the thalamus is crucial for skilled movement (Evarts & Thach, 1969). The scans acquired as part of this thesis are not of high enough resolution to accurately delineate the anterior thalamic nuclei, but the association

between reduced thalamic volume and observed motor deficits in this cohort of patients with CHI warrants investigation. This will be addressed in Chapter Seven.

5.4.3 Preservation of the basal ganglia

A trend for volume reduction in the pallidum in children with CHI compared to healthy controls was observed, but it was unclear from the analyses in the large and small cohorts whether this was driven by a unilateral effect. Significant differences in other basal ganglia volumes between groups were not observed. The relative preservation of basal ganglia structures (excluding the pallidum) is an interesting finding, as most studies that report damage to subcortical structures report damage specifically to the basal ganglia (Barkovitch et al., 1998; Kinnala et al., 1999; Kara et al., 2006; Burns et al., 2008; Avatapelle et al., 2013; Gutaullina et al., 2013; Gataullina et al., 2014). Gataullina et al. (2013) found that basal ganglia lesions were common (in five out of 10 participants) in those who experience hypoglycaemia between the ages of six and 22 months. It is not clear which structures of the basal ganglia were affected in the latter study, so it is difficult to draw conclusions about the agreement of these findings with the findings presented in this chapter. If this age range were a particularly sensitive period for the basal ganglia, then it would be reasonable to expect damage to these structures in both patients with CHI and patients with KH, who were experiencing episodes of hypoglycaemia during this time frame (unless ketone bodies are playing a protective role in the case of children with KH). The data reported in this chapter do not suggest that profound basal ganglia damage is a lasting neuroimaging feature of hypoglycaemia, at least when quantitative analyses are performed, although there is

some implication of vulnerability of the pallidum to hypoglycaemic events occurring in the context of CHI. However, it is important to note that the caudate follows a U-shaped developmental trajectory (Lenroot & Giedd, 2006), which (given the wide age range of participants) may have distorted these results. The results presented in this chapter may also differ from those of previous studies as all previous reports have relied on visual inspection of MR scans, rather than quantitative analyses. In this cohort, there may be individuals with reduced volume of basal ganglia structures but a non-specific reduction of global basal ganglia volumes does not characterise those with CHI or those with KH.

5.4.4 Selective vulnerability

Chugani (1998) states that because of the particularly high demand for glucose the hippocampus and thalamus are especially vulnerable to hypoglycaemic insult in the first two months of life. Indeed, this timeframe is identical to that when almost the entire CHI cohort reported here had experienced severe and recurrent episodes of hypoglycaemia, which might explain the reduction in thalamic and hippocampal volumes observed in this patient group. Regarding the thalamus specifically, this theory may be supported by the significant difference in thalamic volume between those with KH and those with CHI; those with KH generally experience episodes of hypoglycaemia later in infancy and childhood (see Chapter Two), when the demand for glucose in the thalamus decreases, although some of the cohort reported in this study did present as neonates.

However, it is unlikely that vulnerability of certain structures to hypoglycaemia depends entirely on supply and demand of glucose (Barkovich et al., 1998; Vannucci & Vannucci, 2000), which is consistent with the finding that hippocampal volumes between the two patient groups are not significantly different, and with other reports of hippocampal vulnerability to hypoglycaemia outside of this critical time period. Other mechanisms of damage such as excitotoxicity due to energy failure will render regions that are dense with NMDA receptors (such as the striatum, thalamus and globus pallidus) vulnerable; the density of NMDA receptors changes over development and may explain some of the variability observed in different cohorts (Auer, 2004; Auer, 1986; Barkovich et al., 1998). If age at insult is to be considered as a factor, in accordance with studies citing damage to the hippocampus in children and adults with diabetes, it is likely that the vulnerability of the hippocampus to hypoglycaemic events extends beyond the first few months of life (Furguson et al., 2005; Chalmers et al., 1991; Kirchhoff et al., 2013), which might explain the lack of statistical difference between those with KH and those with CHI.

The availability of ketone bodies might also play a role in the preservation of the thalamus in patients with KH, given the selective preservation of the thalami in these patients. However, in this study the role of ketone bodies as a neuroprotective factor remains unclear, primarily because of the results of the hippocampal volumetry. It cannot be definitively argued that the hippocampus is completely preserved against hypoglycaemic damage in the presence of ketone bodies because those with KH do show lower hippocampal volumes than healthy controls, and patients with KH do not have significantly larger hippocampi than patients with CHI. From these data it is

difficult to tell whether the presence of ketone bodies or other factors, such as the age at which hypoglycaemic events occur, is important for determining integrity of these subcortical structures.

5.4.5 Limitations

There are limitations of this study pertaining to the automatic segmentation of the basal ganglia and thalamus. In many participants, the segmentation of the caudate was very poor and had to be corrected manually. This may have interfered with the results obtained. Ideally, all segmentations of the thalamus and basal ganglia analysed here would have been obtained automatically, with no requirement for manual corrections, to avoid introducing error and bias. Another limitation exists in the study of hippocampal volumes, where comparing smaller numbers of participants (i.e. analyses of the small cohort) did not yield significant differences between groups, which raises questions concerning the robustness of this effect. Furthermore, the VBM study in Chapter Four failed to identify any differences in subcortical structures between groups. This may be due, at least in part, to the choice of smoothing kernel and the stringent correction for multiple comparisons applied. Indeed, at a lower threshold, uncorrected for multiple comparisons, thalamic volume reduction can be seen in patients with CHI relative to controls. It seems unlikely that the effects of hypoglycaemia on thalamic volume reported here are false positives as they survive correction for multiple comparisons and are replicated in the small cohort. Future studies could focus on segmentation of the nuclei of the thalamus to discern if degree of damage differs between nuclei and relate this to functional outcome.

Finally, all previous reports of damage to subcortical structures following early hypoglycaemia (in the form of lesions of volume loss) have done so through visual inspection of MR images, performed by radiologists. MR images of the participants included in this study were not reviewed in this way. While strengths lie in being able to determine group level effects through quantitative neuroimaging analysis, a weakness of this study is that it is not possible to state what effects may be at an individual level.

5.4.6 Conclusion

To conclude, these data indicate that patients with CHI who experienced early and repeated episodes of hypoglycaemia show selective, group level damage to the thalamus and hippocampi, alongside a comparable sparing of most basal ganglia structures. The volume reductions in patients with CHI relative to healthy controls in the thalamus and hippocampus are bilateral effects and are in accordance with previous reports (in neonates and infants) of the vulnerability of these structures to the deleterious effects of hypoglycaemia. Children with KH have preserved thalamic and basal ganglia volumes, but there is some suggestion of reduced hippocampal volume. It is unclear what the role of ketone bodies is here - whether they play a protective role for some structures only (e.g. the thalamus) or if other factors such as 'sensitive' periods are important for determining vulnerability to hypoglycaemic insult. Regional vulnerability of these structures to hypoglycaemic insult is likely to depend on a complex interaction of factors, including age-dependent glucose demand, variations

in the density of excitatory amino acid receptors (which vary as a function of age) and may also be influenced by the availability of alternative fuels. From these data it is not possible to say whether the presence of ketone bodies is neuroprotective. The volume reductions observed in children with CHI are likely to have implications for behavioural outcome, and the relationship between these structures and their associated outcomes will be explored in Chapter Seven.

Chapter 6

Tract-Based Spatial Statistics

6 Tract-Based Spatial Statistics

Chapter Four showed that widespread white matter abnormalities are evident throughout much of the brain in children with CHI. The microstructural properties of white matter can also be investigated using Diffusion Tensor Imaging (DTI), which allows the examination of indices of diffusion throughout the brain. This chapter explores white matter abnormalities, analysing diffusion-weighted images using a voxel-wise approach to compare the structural integrity of white matter tracts between patients with CHI, patients with KH and healthy controls. These results are presented after an introduction to diffusion-weighted imaging and a brief review of the literature. Finally, indices of diffusion are compared between groups.

6.1 Introduction

Diffusion Weighted Imaging (DWI) and DTI measure the movement of water in the human brain and analyses the three-dimensional shape of diffusion, respectively (Huisman, 2010). This allows the examination of tissue integrity beyond the usual image resolution (Le Bihan et al., 2001), by allowing the detection of microstructural properties.

6.1.1 Principles of diffusion MRI

6.1.1.1 Diffusion-weighted Imaging and Image Contrast

Diffusion-Weighted Imaging (DWI) was introduced in the 1990s as a means of investigating changes in water diffusion following stroke (Moseley, Mintorovitch, & Kucharczyk, 1990), before finding application in the study of fibre tracts (Basser, Mattiello, & LeBihan, 1994). Diffusion arises from random thermal translational motion of molecules (also termed Brownian motion). Because the displacement distances are small, and equivalent to cellular dimensions, studying water diffusion in human tissue allows us to investigate cellular integrity (Gadian, 1995). In DWI, image contrast is generated from differing rates of diffusion of water molecules. Brownian motion varies between different tissue classes in the brain, and the MR signal can be sensitised to this diffusion.

In order to detect diffusion, a pair of gradient pulses is applied within a spin echo sequence. This renders images sensitive to the small displacement of water molecules (Gadian, 1995). The first pulse causes a change in the precession rate of protons, as described in Chapter Three. When this pulse is turned off, the protons return to their original precession rate, but are out of phase. In order to refocus the phases of the spins, a second gradient pulse, with opposing effect to the first, must be applied. If there is no movement of molecules (diffusion) between the application of the two pulses, perfect re-phasing occurs. If diffusion is present, a signal loss will occur (Mori & Zhang, 2006). Diffusion-weighted images can be displayed in the form of Apparent Diffusion Coefficient (ADC) maps that result from this signal loss.

A comparison between a non-diffusion image and a diffusion image needs to be made in order to obtain ADC values. Typically, imaging protocols use so-called b-values of 0 (non-diffusion weighted) and 1000 s/mm^2 (diffusion-weighted) to detect an ADC (McRobbie, Moore, Graves & Prince, 2006). B-values are determined by the gradient pulse strength, duration and spacing, and a higher b-value leads to a greater signal loss. A non-diffusion-weighted image with a b value of 0 will look like a T2-weighted image; in contrast, a diffusion-weighted image acquired with a b value of 1000 s/mm^2 will have low signal in areas of fast water movement (e.g. cerebrospinal fluid; CSF) and high signal in areas of more restricted diffusion (e.g. white matter). In this way, adjusting the b-values allows the creation of an image that is sensitive to the freedom of water molecules to diffuse in different regions of the brain.

6.1.1.2 Anisotropy and the diffusion tensor

Anisotropy refers to the directional dependence of the restriction experienced by water molecules. Isotropic diffusion is diffusion that is equal in all directions. In CSF, the diffusion rate is high and water molecules move around relatively unrestricted. In grey matter the diffusion rate is lower than in CSF, as there are barriers to diffusion, but they do not systematically direct the movement of water in any particular direction (Jellison et al., 2004). In white matter, diffusion is anisotropic as it is restricted by axonal membranes, the myelin sheath and other biological particles that act as a hindrance to diffusion, systematically preventing diffusion in different directions (Mori & Zhang, 2006). Molecules move more easily parallel to the orientation of tracts, whereas movement perpendicular to tracts is restricted (Jellison

et al., 2004). This means the diffusion is more extensive along the tracts than perpendicular to them. It is this anisotropy that we measure in Diffusion Tensor Imaging (DTI), and that we use to infer the integrity of white matter. If the integrity of white matter is damaged, such as through loss of myelin, or through axonal widening, diffusion will be less restricted compared to a healthy, undamaged system. The pulsed magnetic field gradients described above highlight this anisotropy by comparing signal intensities obtained in differing directions. There is less diffusion-weighted signal when the pulsed field gradients are applied along the direction of the tract than when they are in the transverse direction (Gadian, 1995).

The diffusion tensor contains information about the diffusion of water molecules in any given voxel and infers the magnitude and directionality of water movement. Six values are contained within the tensor; three eigenvectors and three eigenvalues. Eigenvectors correspond to the main directions of diffusion, while eigenvalues represent apparent diffusivities along these eigenvectors. In anisotropic matter, the tensor can be thought of as an ellipsoid, with the principal eigenvector oriented in the direction of maximum diffusion (i.e. diffusion parallel to the direction of the white matter tract). This is the dominant direction of water motion in a given voxel, and it aligns with the orientation of fibres in a tract. The two perpendicular eigenvectors represent the medium and minor principal axes of the ellipsoid (Jellison et al., 2004). Here the principal eigenvector has a greater magnitude than the perpendicular eigenvectors, and this results in a 'stretching' of the tensor so that it resembles an ellipsoid (Figure 6.1). In isotropic environments, the tensor can be thought of as a perfect sphere, where the three eigenvectors are equal in magnitude. Therefore, the

values of these eigenvectors can be used to infer the integrity of fibre tracts in each voxel across the brain.

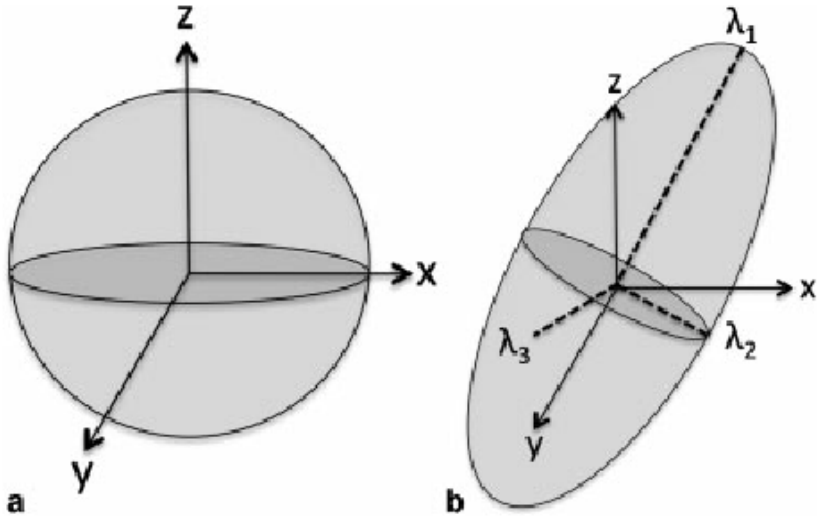

Figure 6.1 The diffusion tensor in an isotropic environment (a) and an anisotropic environment (b). Adapted from Naragni, Awdeh, Vibhor, Andreisek, & Chhabra, 2015

6.1.1.3 Indices of Diffusion

Many indices of diffusion can be calculated from the diffusion tensor. They include Fractional Anisotropy, Mean Diffusivity, Axial Diffusivity and Radial Diffusivity. Fractional Anisotropy (FA) is one of the most widely used. FA ranges between 0 and 1, where 0 reflects completely isotropic diffusion and 1 completely anisotropic diffusion. Mean diffusivity (MD) is the mean of the three eigenvalues and is an indication of average diffusivity. High mean diffusivity is considered to be indicative of a loss of cell structure (for example, a loss of cellular membranes that would act as a hindrance to water diffusion). Other measures of tract integrity that can be extracted from the

tensor are Axial Diffusivity (AD) and Radial Diffusivity (RD). AD is the principal eigenvalue of the tensor (the direction of maximum diffusion), and is thought to indicate more coherent cell structure and intact axons. RD is the average of the second and third eigenvalues, and indicates the diffusion of water parallel to the principal eigenvector (Ciccarelli, Catani, Johansen-Berg, & Clark, 2008). RD is commonly believed to reflect degree of myelination (Song et al., 2002). Thus, each of these indices provide a measure of axonal integrity derived from the DWI, and can be analysed using tractography, where apriori regions of interest must be defined, or using whole brain approaches that analyse only the core white matter skeleton. The latter method will be described in the methods section below.

The distinction between reductions or increases in diffusion indices is an important one as it may indicate mechanisms of damage. FA is not specific to either axonal damage or demyelination. Myelin loss and axonal damage can both be present in white matter changes. Increased RD, in combination with unchanged AD, is widely considered to reflect loss of myelin (Song et al., 2002, 2005). Demyelination is associated with an increase in RD because the loss of myelin permits freer movement of water perpendicular to the myelin layers. In contrast, axonal damage is associated with a decrease in axial diffusivity. It has been suggested that this might result from loss of coherent organisation of the axon (Budde et al., 2008).

6.1.2 White matter damage after hypoglycaemia

As has been reviewed previously, many studies have reported white matter damage secondary to neonatal hypoglycaemia. Most of these report MR or CT findings during the neonatal period. Of these neonatal studies, a majority cite white matter damage (lesions, volume loss or restricted diffusion) in the occipital and parietal lobes as being the most common biomarker of neonatal hypoglycaemia (Alkalay, Flores-Sarnat, et al., 2005; Barkovich et al., 1998; Caraballo et al., 2004; Filan et al., 2006; Kinnala et al., 1999; Traill et al., 1998). One study examined the medical history of infants who had posterior white matter damage (in the form of reduced white matter density or presence of lesions) and found that neonatal hypoglycaemia had occurred in the majority of subjects (Wang et al., 2012). Another study used CT imaging and found reduced white matter density in the occipital lobes in 73% of a sample of children and young adults who had a history of neonatal hypoglycaemia (Caraballo et al., 2004). In contrast to these reports of posterior white matter involvement, one group studied infants with neonatal hypoglycaemia and found a much more varied pattern of damage, with global involvement of white matter that correlated with later cognitive impairment (Burns et al., 2008). Here, 80% of infants with moderate to severe white matter damage demonstrated moderate to severe impairment at follow-up.

Few studies have specifically examined indices of diffusion following neonatal hypoglycaemia. One study reported that in a group of four patients with transient neonatal hypoglycaemia, all showed restricted diffusion in the occipital lobes, identified through inspection of DWI (Filan et al., 2006). Another showed that eight of

25 neonates with hypoglycaemia showed restricted diffusion in the occipital lobes, which was identifiable just one week after the hypoglycaemic event (Tam et al., 2012). Therefore, across these two papers there appears to be a consistent pattern that hypoglycaemic events in infancy can lead to damage to white matter in the occipital lobe, which can be measured with DWI.

It is obvious from these studies that damage to white matter is implicated following early hypoglycaemia. What is unclear from these studies is how specific this damage is to hypoglycaemia alone and whether this manifests as a truly group level pattern of damage, as opposed to damage only identifiable on an individual level. All of the studies mentioned above have reported abnormalities in neonates and infants with comorbidities, such as hypoxia, preterm birth and epilepsy, and have relied on visual, qualitative analysis of T2, FLAIR or DWI scans to identify regions of damage. Very few studies have focused on outcome in children with CHI as a stand-alone condition and none have examined potential brain injury in KH in relation to typically developing children.

Instead of using typically developing children as a control group against which to compare patients with CHI, Kumaran's HBI study (2012) used children with KH as a control group because they are considered to be neuroprotected. A Tract-Based Spatial Statistics (TBSS) analysis, which is a whole brain analysis of the integrity of core white matter tracts, showed that relative to children with KH, children with CHI have focally-reduced FA across the corpus callosum. An extraction of mean FA across the skeleton showed that patients with CHI have reduced FA relative to patients with KH.

However, as described in Chapter One, there were similar instances of white matter lesions in the two groups, occurring in roughly one-third of patients. Therefore, although those with KH had a preserved profile on diffusion imaging compared to those with CHI, the HBI study also showed that white matter lesions were present in those with KH, a group traditionally thought to be neuroprotected. The direct comparison of these two patient groups, in the absence of a normal control group, make conclusions about the damage incurred by hypoglycaemic events difficult to assess. In addition, the inclusion of children with known neurological disorders makes it impossible to ascertain the damage to white matter caused by hypoglycaemia alone.

6.1.3 Aims and hypotheses

The objective of the current study was to use DTI to evaluate the effect of recurrent episodes of hypoglycaemia on white matter tract integrity in patients with CHI and patients with KH relative to a healthy control group.

In accordance with the findings of the Voxel-based Morphometry (VBM) study presented in Chapter Four, it was hypothesised that patients with CHI would show widespread structural damage to white matter compared to controls. Given the results of the VBM study and previous reports of white matter compromise in KH, it was hypothesised that as a group, patients with KH would also show evidence of structural damage to white matter. Finally, this study aimed to verify if the structural differences that have been suggested to exist between patients with CHI and those with KH are maintained when comparing only neurologically normal patients to each other. If patients with KH are protected from the adverse effects of hypoglycaemia, then the

comparison of patients with CHI to healthy controls should yield the same results as the comparison of patients with CHI to patients with KH.

6.2 Methods

6.2.1 Participants

Analyses were conducted in both the large and small cohorts (see Chapter Two for a description of the cohorts).

Forty-two typically developing children, 24 patients with CHI and 17 patients with KH were included in the large cohort (Table 6.1). One typically developing child was removed from the analysis (because of movement artefact), while one had no DTI data available. Six patients with CHI were removed from the analysis (five because of excessive movement, one because of gross structural abnormality), and data was unavailable for one patient. Two patients with KH were removed because of their young age and lack of suitable controls (<7 years), while two had no DTI data available.

Table 6.1 Age and gender of subjects in large cohort

Group	N	Age in months (sd)	Gender
Controls	42	142 (30)	15 male
CHI	24	133 (32)	14 male
KH	17	129 (31)	10 male

As the first analysis was run with patients and controls whose data were collected across different studies, a second analysis was conducted to verify that results were similar for the small cohort, solely collected by the author of this thesis, in preparation for the analyses in Chapter Seven. Age and gender are shown in Table 6.2.

Table 6.2 Age and gender of small cohort

Group	N	Age in months (sd)	Gender
Controls	17	132 (27)	8 male
CHI	13	131 (37)	7 male
KH	12	140 (30)	6 male

6.2.2 Image acquisition and DTI data analysis

Neuroimaging acquisition parameters are described in detail in Chapter Four. For DTI, gradients were applied using 60 non-collinear directions, with a b-value of 1000 s/mm^2. Voxel-wise statistical analysis of the DTI data acquired in this study was carried out using Tract-Based Spatial Statistics (TBSS; Smith et al., 2006), which is part of FSL (Smith, 2004). This whole-brain technique allows examination of white matter integrity without the need for determining a-priori tracts of interest. Each dataset was visually inspected for data quality, and datasets with severe artefact induced by head motion were discarded from further analysis. An FA image was created for each participant using the Oxford Centre for Functional MRI of the Brain (FMRIB) Diffusion Toolbox (FDT). This involves referencing DTI data to a non-diffusion weighted volume to correct for eddy currents and subject head motion, and applying the Brain Extraction Tool

(BET) to remove signal produced by skull and surrounding tissue [Smith 2002]). Finally, FDT involves fitting a tensor model to each voxel of raw diffusion data. This allows calculation of the tensor eigenvalues, from which maps of FA, MD, AD and RD can be calculated. All images are then aligned to a standard template provided by FSL, and these nonlinear transforms are applied to bring all FA images into standard space. All images are merged to create a mean FA image, which is thinned to produce the mean FA skeleton containing voxels with FA above 0.2. Finally, each participant's data is projected onto the skeleton by filling it with diffusion values from the nearest relevant tract centre. The same registration and projection parameters were applied to non-FA data (AD, MD and RD).

To explore group differences, voxel-wise statistics were performed on the data, with age and gender added as covariates to the general linear model. An ANCOVA was performed first and main effects were followed up with post-hoc comparisons. All analyses were corrected for multiple comparisons across the white matter skeleton using Threshold Free Cluster Enhancement (TFCE). Mean FA, MD and RD values were extracted from the skeleton of each subject to allow for comparisons of global white matter damage.

6.3 Results

In this section, the outcome of between-group analyses of FA, MD, RD and AD revealing regional differences in white matter integrity across the brain will be

presented. This will be followed by an ANCOVA comparing average global diffusion values (derived from the above indices) across groups.

6.3.1 Fractional Anisotropy: Differences between groups

A main effect of group was observed ($p<0.05$), with differences in FA observed in the genu of the corpus callosum and anterior thalamic radiations. Follow-up t-tests showed that compared to typically developing controls, patients with CHI show reductions of FA across multiple regions of the white matter skeleton (Figure 6.2A). These included associative fibres (inferior fronto-occipital fasciculus, superior longitudinal fasciculus), the cingulum (both the hippocampal cingulum and the cingulate gyrus), commissural fibres (genu, body and splenium of the corpus callosum, forceps minor, left forceps major) and projection fibres (anterior and superior corona radiata, corticospinal tracts, internal and external capsule, cerebellar peduncles, as well the fornix). No differences were observed between patients with KH and healthy controls, and there were no significant differences between patient groups.

This analysis was repeated in the small cohort, and differences in FA between patients with CHI and healthy controls were maintained (Figure 6.2B). When comparing patients with KH to controls, significant differences were observed in the left genu of the corpus callosum (Figure 6.3) while trends (set at $p=0.08$) were seen for reduction of FA in the body and splenium of the corpus callosum (bilateral), the anterior corona radiata (bilateral) and the left external capsule. Again, no significant differences between patient groups were observed.

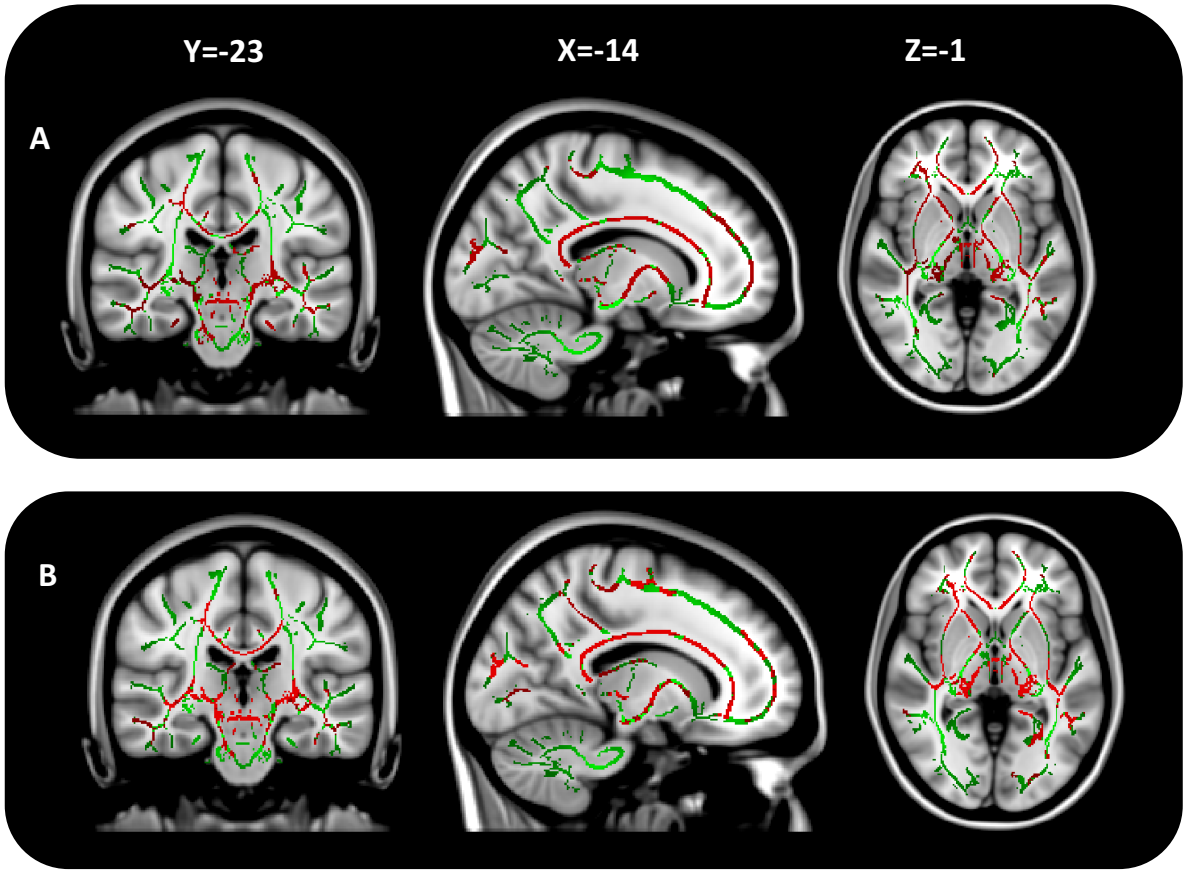

Figure 6.2A: Analysis of the large cohort revealed significantly lower FA in patients with CHI relative to healthy controls across much of the white matter skeleton. B: Analysis of the small cohort of patients showed the same reduction of FA in patents with CHI relative to controls. The white matter skeleton is shown in green. Regions of reduced FA (at $p<0.05$) are shown in red.

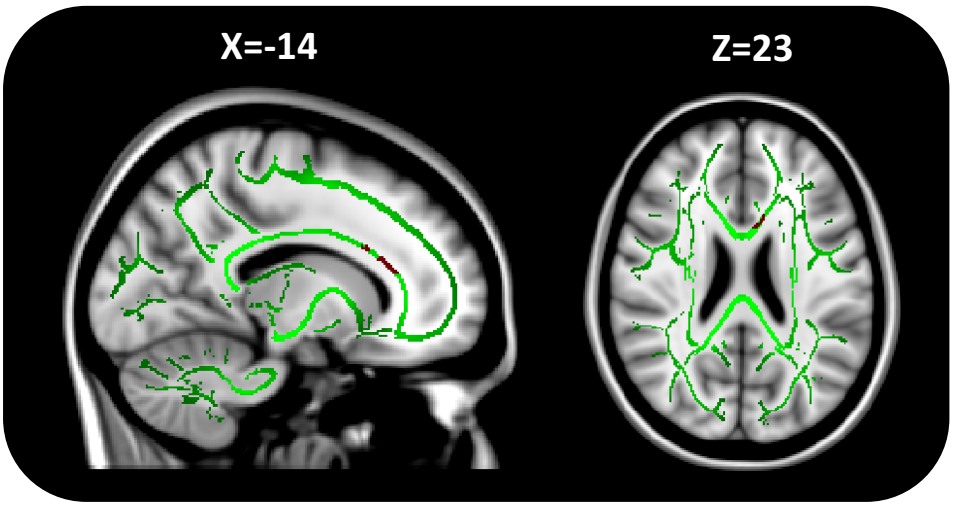

Figure 6.3 Reduced FA in the corpus callosum identified in the small cohort of patients with KH compared to controls. The white matter skeleton is shown in green. Regions of reduced FA (at $p<0.05$) are shown in red.

6.3.2 Radial diffusivity: differences between groups

A significant main effect of group was observed. Post-hoc analyses showed that relative to healthy controls, patients in the large cohort with CHI showed increases in RD across the same regions that showed reduced FA (Figure 6.4A). No other comparisons were significant. These results were replicated in the small cohort (Figure 6.4B).

6.3.3 Mean Diffusivity: Differences between groups

No main effect of group was observed, but given the clear effect on white matter outlined in Chapter Four; post-hoc analyses were conducted to explore differences

between groups. Relative to healthy controls, patients with CHI showed increases in MD, which were most prominent across the entire corpus callosum (Figure 6.5A). Regions in the posterior corona radiata, posterior thalamic radiations (bilateral) and retrolenticular part of the internal capsule (left) and left sagittal striatum (inferior longitudinal fasciculus and inferior fronto-occipital fasciculus) were also affected. No other comparisons were significant. These results were replicated in the small cohort (Figure 6.5 B).

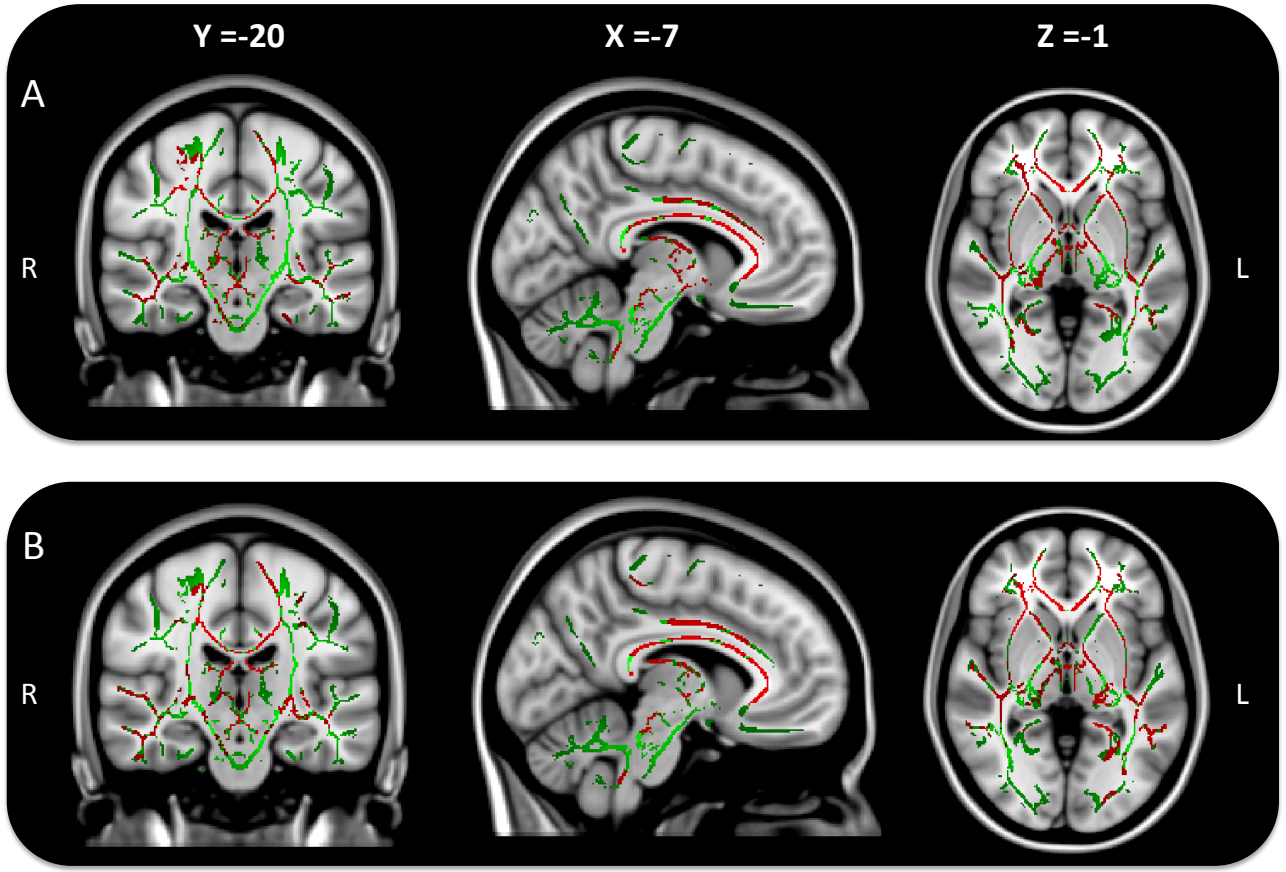

Figure 6.4A: Analysis of the large cohort showed higher radial diffusivity in patients with CHI relative to controls across much of the white matter skeleton. B: replication of findings in the small cohort. The white matter skeleton is shown in green. Regions of increased RD are shown in red

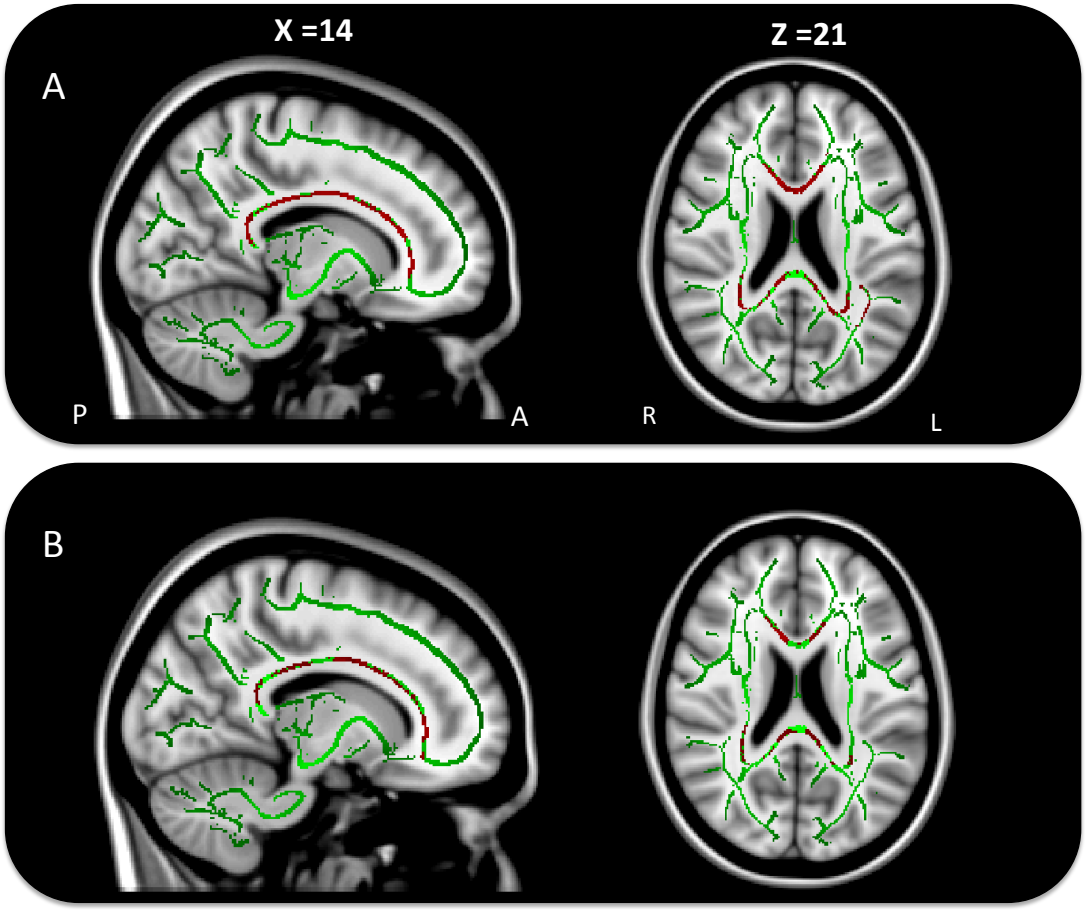

Figure 6.5A: Analysis of large cohort showed regions of higher MD in patients with CHI relative to controls, which were most prominent across the corpus callosum. B: replication of these results in analysis of small cohort. The white matter skeleton is shown in green. Regions of increased MD are shown in red.

6.3.4 Axial Diffusivity

No significant difference in axial diffusivity between groups was observed (in main effects or follow-up analyses).

6.3.5 Global FA, MD and RD differences between patients and controls.

Global FA, MD and RD values were extracted from the skeleton for each participant and compared using ANCOVA, controlling for age and gender. Following on from the

identification of focal differences between groups as described above, these extracted values allow the investigation of global group level differences. Global AD values were not extracted as no indication of differences between groups was observed from the TBSS analysis. There was a significant difference between groups in global FA values, $F_{(2,78)}$ =3.91, p=0.024. Bonferroni-corrected post-hoc tests showed that there was a significant difference between typically developing children and patients with CHI (p=0.02), but not between controls and patients with KH, or between patients with CHI and patients with KH (Figure 6.6) There was a trend for significant differences between groups on mean RD values, after controlling for age and gender, $F_{(2,78)}$=2.86, p=0.075. Pairwise comparisons revealed that this was driven by trend level differences between controls and patients with CHI (p=0.068 [Figure 6.6]). MD did not differ between groups (Figure 6.6). These results were replicated in the small cohort.

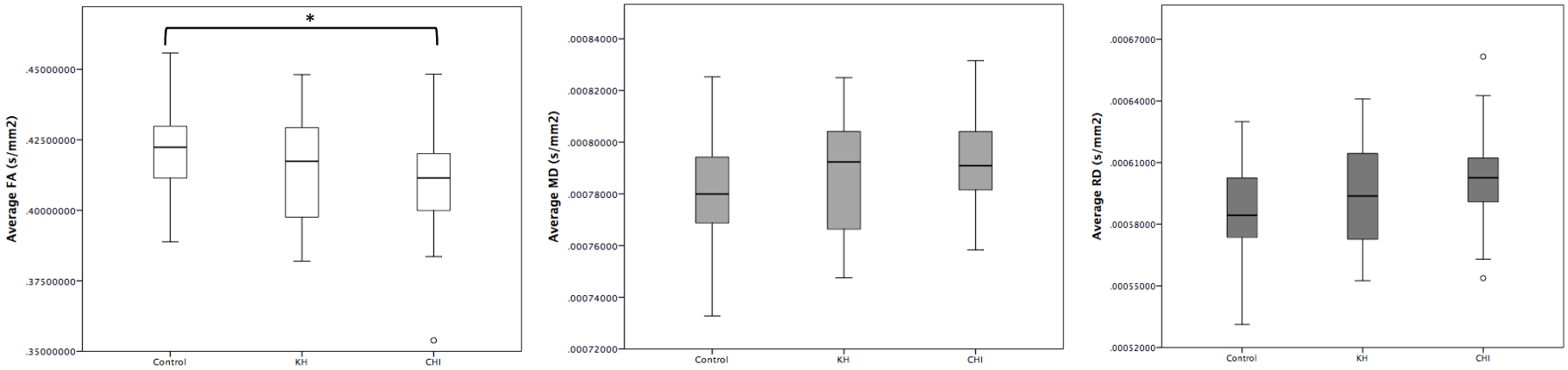

Figure 6.6 Box-and-whisker plot showing the median, interquartile range and range of average FA, MD, and RD values (s/mm^2) across the entire white matter skeleton in healthy controls, patients with KH and patients with CHI. Patients with CHI have significantly lower average FA than controls, while trends for increased mean global RD in patients with CHI relative to healthy controls were identified.

6.4 Discussion

This study has examined the integrity of white matter tracts throughout the brain, and has shown that patients with CHI show widespread diffusion changes in comparison to healthy controls. Importantly, the regions of altered diffusion in patients with CHI correspond with the regions of reduced white matter volume identified in Chapter Four. These findings are discussed in detail below.

6.4.1 Differences in white matter microstructure

This study has shown that children with CHI have diffuse structural alterations across much of the white matter skeleton when compared to healthy controls. Lower global FA and higher mean RD values were observed in patients with CHI compared to controls. TBSS analysis revealed that FA is reduced in many regions, both cortical and subcortical. This was accompanied by increases in RD in the same regions. Increases in mean diffusivity were mainly limited to subcortical regions (the corpus callosum, internal capsule and anterior thalamic radiations). This pattern of abnormality is broadly at odds with what has been reported in the literature in neonates, infants, and infrequently, children, where studies have reported a primarily posterior pattern of damage (Alkalay, Flores-Sarnat, et al., 2005; Barkovich et al., 1998; Filan et al., 2006; Kinnala et al., 1999; Murakami et al., 1999; Tam et al., 2012; Traill et al., 1998; Wong et al., 2013; Yalnizoglu et al., 2007). This study has shown that changes to white matter in a group of school-aged children with hyperinsulinism are far more widespread than has been previously reported. These results are in accordance with the diffuse nature

of white matter damage documented by Burns et al. (2008), and the results of white matter VBM analysis presented in Chapter Four. As the first study to include a homogenous group of only neurologically normal children with CHI, these changes are likely to be a result of being exposed to early, severe and recurrent episodes of hypoglycaemia.

The picture of potential white matter involvement in children with KH is less clear. At a first glance, in this report there are few significant effects that distinguish patients with KH from healthy controls. However, the finding that FA is reduced in a subset of these children (i.e., in the small cohort) is not one that should go unnoticed, as it suggests that children with KH could be at risk of compromised white matter integrity following episodes of hypoglycaemia, despite the supposed neuroprotective role of ketone bodies. There could be a number of reasons for this finding. It is possible that, as this group included a higher proportion of older children than the large cohort, they experienced more episodes of hypoglycaemia, contributing to the reduction of FA observed in the corpus callosum. The protracted development of the corpus callosum that continues into the third decade (Giedd et al., 1996) might render this region especially vulnerable to episodes of hypoglycaemia occurring later in life. However, as these results were identified in the small cohort they should be interpreted with caution and replication in a different group would be necessary to validate these findings.

The lack of statistical difference between patients with CHI and patients with KH in any of the indices of diffusion investigated here is in direct contrast to a similar TBSS

analysis that showed differences in the corpus callosum between patients with KH and patients with CHI (*HBI study;* Kumaran, 2012). However, the results presented here are in keeping with the findings of the same study where white matter lesions and atrophy were as common in children with KH as they were in children CHI. The findings presented in this chapter suggest that other factors, such as structural differences due to epilepsy, might have contributed to the differences between patients with KH and patients with CHI identified in the HBI TBSS study (Kumaran, 2012). Taken together, the results presented in this chapter and that of Kumaran 2012 suggest that children with KH may be at some risk of neuropathology. More work is needed to establish whether there are clinical variables (e.g. at presentation) that determine the integrity of white matter in the context of hypoglycaemia, especially in patients with KH.

6.4.2 Mechanisms of damage

The globally increased RD identified in patients with CHI relative to controls combined with the white matter volume loss observed in Chapter Four is strongly suggestive of disruption to myelin after severe and recurrent hypoglycaemia in patients with CHI. The findings of this study suggest that there may be a vulnerability of myelin to hypoglycaemic events. Possible mechanisms of this damage will be discussed in Chapter Eight.

As neither MD nor RD appeared to be increased in the KH patients relative to controls, it would appear that the mechanisms of possible white matter damage in those with KH and those with CHI do differ. However, it is not possible to say whether this is due

to the availability of ketone bodies during episodes of hypoglycaemia, or because of the differing ages at which hypoglycaemia occurs, with some arguing that topography of damage is determined by age at insult (Gataullina et al., 2013). This effect of age on the topography of brain damage is an important consideration. The differences seen in these cohorts could reflect the differing ages at presentation between these two patient cohorts; ketotic hypoglycaemia usually presents between 18 months and five years of age, and resolves by the age of nine to 10 years (Hussain, 2005b). In this sample, presentation ranged from one day to five years and in over half of the patients the disease was on-going (see Chapter Two). Repeated episodes of hypoglycaemia during different sensitive periods may, therefore, determine damage to underlying brain structures.

6.4.3 Limitations

Diffusion Tensor Imaging (DTI) has limited capability to detect real changes in tissue microstructure in regions of crossing fibres that include more than one coherently oriented fibre population (Tournier, Calamante, & Connelly, 2012). Such regions include the corticospinal tract (CST) so positive results in this area should be interpreted with caution. DTI can assess tissue microstructure in pathways that consist of isolated and well-defined tract bundles much more reliably, for example in regions such as the internal capsule, cerebellar peduncles and corpus callosum (Pierpaoli et al., 2001). Therefore, despite limitations in the method, a large proportion of the results achieved in this cohort can be considered to be reliable, particularly with regard to the involvement of the descending motor tracts in patients with CHI. The diffusion

characteristics of white matter tracts in patients with KH is unclear and requires further research for verification.

6.4.4 Implications for behavioural outcome

The pattern of damage observed in patients with CHI is likely to have implications for cognitive and motor functioning. Within white mater tracts, the amount of myelination and axonal thickness play a role in conduction speed, providing faster information transfer and allowing precise timing in communication between two cortical areas (Nagy, Westerberg, & Klingberg, 2004). In patients with CHI, the diffuse nature of the damage to projection pathways that are crucial to skilled movement is likely to have implications for motor functioning. The marked changes in FA and RD in descending motor pathways including the internal capsule and cerebellar peduncles (which can be reliably measured with DTI), as well as changes in the anterior thalamic radiations and corpus callosum, suggests that motor skills in this group of patients could be particularly affected. Damage to regions important for motor skills has been previously reported in relation to hypoglycaemia; in a study that examined infants at risk of neonatal encephalopathy, Tam et al. (2012) found that infants with hypoglycaemia had increased CST damage, and that the amount of damage was predictive of later motor outcome. Burns et al. (2008) found that hypoglycaemic infants with damage to the posterior limb of the internal capsule had poor motor outcomes at two-year follow up. The white matter abnormalities in key motor tracts in patients with CHI are in accordance with the motor profile of this group. Similarly, the structural alterations to white matter in association and commissural fibres may explain the impairments in

executive functions that have been presented in Chapter Three. The integrity of these fibre bundles has been associated with performance on tasks assessing function tasks in many studies. The association between neuroimaging indices of damage, in particular white matter microstructure, and neuropsychological outcome will be explored in Chapter Seven.

6.4.5 Conclusion

In summary, in the context of CHI severe and recurrent hypoglycaemia leads to widespread changes to white matter microstructure. These results strongly suggest that myelin loss is evident in children with CHI. In children with KH, alterations to white matter microstructure are suggested despite the presence of ketone bodies. It might be that the age at which hypoglycaemia occurs and the continuation of hypoglycaemic episodes are greater determinants of outcome than the presence of ketone bodies. A follow-up study, investigating the relationship between status of disease course, age at presentation and indices of diffusion would help to elucidate the role that age at injury plays in determining white matter damage after hypoglycaemia.

Chapter 7

Structure-Function Relationships

7 Structure-Function relationships

This chapter examines the relationship between observed behavioural deficits in patients with CHI and patients with KH relative to the integrity of subcortical structures, global and regional grey and white matter volumes, and indices of diffusion. A brief review of the behavioural profile is given followed by a review of imaging findings. Finally, relationships between these structures and the relevant cognitive and motor abilities are presented.

7.1 Introduction

The findings presented in this thesis show that patients who experience early and severe episodes of hypoglycaemia are impaired on tasks tapping executive functioning and motor skills. In both patient groups, a deficit was observed in executive functions (working memory, processing speed, sustained and selective attention and task switching/attentional control), as well as fine and gross motor skills. Table 7.1 shows the functions that are preserved or impaired in patients with KH and those with CHI. Performance measures on tests tapping these functions correlated with one another and loaded onto the same components in the principal component analysis conducted in chapter three, revealing latent variables composed of cognitive and motor variables that cluster together (Figure 7.1). The clustering of the tests under each component implies that they are likely to rely on similar processes and have similar neural underpinnings.

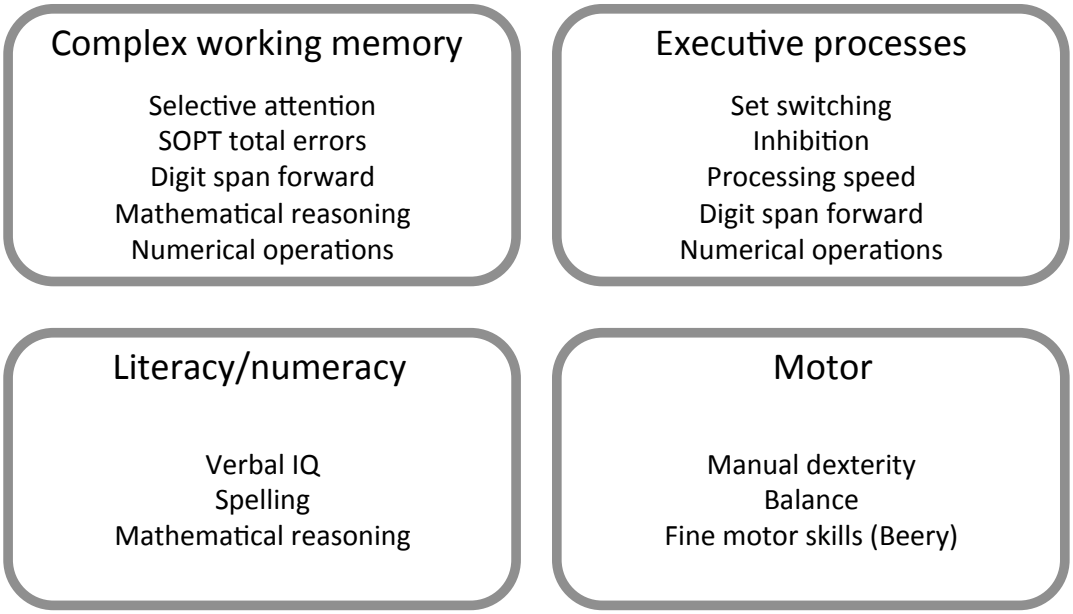

Figure 7.1 Components produced from Principal Components Analysis. Tests that fall under each component are shown.

Volumetric analyses of T1-weighted MRI scans have shown volume reduction of the thalamus and hippocampus in patients with CHI, as well as marked effects on white matter volume and microstructure that are primarily evident in (although not restricted to) anterior and subcortical regions (Table 7.2). Associative fibres that were found to have reduced integrity in patients with CHI relative to controls (and are related to cognitive functions assessed in this thesis) included the cingulum and superior longitudinal fasciculus. The cingulum is a medial associative bundle that runs within the cingulate gyrus, curving around the corpus callosum and connecting frontal and temporal cortices, and it is crucial for memory, attention and learning (Catani & Thiebaut de Schotten, 2008; Catani & de Schotten, 2012). The superior longitudinal fasciculus runs between the parietal lobe and frontal regions

Table 7.1 Domains of function affected in both patient groups ✓ Differences between patients and standard means ✓* Trend for differences between patients and standard means ✗ No differences between patients and standard means

	Intelligence	Academic attainment	Working memory and processing speed	Attention	Memory	Motor Skills
KH	✗	✗	✓ *	✓	✗	✓
CHI	✓ (NVIQ)	✓ (spelling)	✓	✓	✗	✓

Table 7.2 Neuroimaging profile of both patient groups. ✓ Differences between patients and controls ✓* Trend for differences between patients and controls ✗ No differences between patients and controls

	GM volume	WM volume	White matter microstructure	Hippocampal volume	Thalamic volume	Basal ganglia volume
KH	✗	✓*	✓*	✗	✗	✗
CHI	✓	✓	✓	✓	✓	✓*

(Schmahmann et al., 2007) and was also observed to be reduced in integrity in patients with CHI. Projection fibres that were found to be affected in patients with CHI relative to controls included the internal capsule and corona radiata, whose fibres ascend from the thalamus to the cortex, and descend from fronto-parietal cortices to reach the basal ganglia (Catani & Thiebaut de Schotten, 2008). The cerebral peduncles and corticospinal tracts were also reduced in integrity. These are the main sensory and motor pathways of the brain, responsible for all voluntary movement (Catani & Thiebaut de Schotten, 2008), and given their reduced integrity in patients with CHI, may underlie motor impairments observed in this group.

The corpus callosum (the largest bundle of white matter fibres in the human brain) consists of commissural fibres that transfer information between the two hemispheres. It can be divided into three sections; the genu (anterior), body and splenium (posterior; Catani & Thiebaut de Schotten, 2008), all of which were found to be reduced in integrity in patients with CHI relative to controls. Some evidence for reduced integrity in the genu was also observed in patients with KH, although these results should be interpreted with caution, as they were only identifiable in the small cohort of patients. The integrity of these fibres is essential for many cognitive functions, particularly those that demand executive control, and for motor functions (Murray et al., 2016; Nosarti et al., 2004; Catani and Thiebaut de Schotten, 2008).

In light of the cognitive and neuroimaging findings, the aim of the current chapter is to determine the relationship between neuropsychological outcome and (a) volume changes in subcortical structures (b) reductions in grey and white matter volume, and (c) relevant white matter tracts. A review of anatomical systems subserving memory, executive functions and motor skills will be given, followed by analyses attempting to link neuropsychological outcome to these systems.

7.1.1 Neuroanatomical correlates of memory

The hippocampal-diencephalic system includes the hippocampus, fornix, mammillary bodies, and the thalamus. The hippocampus projects directly to the anterior thalamic nuclei and mammillary bodies via the fornix, and indirectly via the mammillo-thalamic tract (Aggleton, Vann, & Saunders, 2005; Dzieciol et al., 2017). This system is crucial for the correct encoding and recall of episodic information (Aggleton & Brown, 1999; Aggleton et al., 2010), and damage to any part of it can produce memory impairments. While memory function was not reduced at a group level in either patient group, hippocampal volumes were significantly reduced in patients with CHI. It is possible that the degree of hippocampal volume reduction is not sufficient to produce memory impairment at a group level, but is still related to memory performance. This has been reported in children of very low birth weight, where despite an overall reduction in hippocampal volume, no group level deficits on verbal and non-verbal recall were observed (Isaacs et al., 2000). Despite the fact that the KH group did not show a significant reduction in hippocampal volumes, rates of verbal memory deficits (IQ-corrected discrepancies between predicted versus actual MQ scores) were similar in

the two patient groups. The relationship between hippocampal volume and memory functioning is well established (Cooper et al., 2013; Isaacs et al., 2000; Muñoz-López et al., 2017; Vargha-Khadem et al., 1997) and will be explored further here. Although damage to the anterior thalamic nuclei is known to produce memory impairments (Aggleton & Brown, 1999), the resolution of the MRI scans acquired in this thesis was too low to delineate these nuclei. Therefore, the relationship between the integrity of the thalamus and memory functioning will not be explored here.

7.1.2 Neuroanatomical correlates of executive functions

The abilities that fall under the umbrella term of executive function (working memory, information processing, and attention) are closely interlinked, and various networks (in particular the fronto-parietal and fronto-striatal networks) are purported to support these functions.

The fronto-parietal network includes the dorsolateral and ventral prefrontal cortices, and is a cortico-cortical network of association fibres (see above) between the prefrontal cortex and the posterior parietal cortex. These regions are thought to be crucial for executive functioning, with studies repeatedly demonstrating associations between the integrity of the fronto-parietal fibres and performance on tasks that are dependent of executive functioning in children and adolescents (Murray et al., 2016; Nagy et al., 2004; Otsby et al., 2011). The long association fibres of the superior longitudinal fasciculus (SLF) and the cingulum are the main pathways connecting the prefrontal cortices and the parietal cortex. Integrity of the SLF and cingulum as

indicated by DTI has been found to be related to working memory (Otsby et al., 2011; Vestergaard et al., 2011; Winston et al., 2013), as well as to sustained attention tasks (Klarborg et al., 2013) in typically developing children and adolescents. Functional MRI studies in children and adults have shown activation of this fronto-parietal network during working memory tasks which correlates with working memory performance (Darki & Klingberg, 2014; Kalpakidou et al., 2014). In addition, over typical development the increasing activation of these regions is associated with increasing FA of these fibres, in turn leading to better working (Olesen, Nagy, Westerberg, & Klingberg, 2003). Therefore, both structural and functional imaging studies support the notion that these fibres are important for executive functioning.

In addition to this fronto-parietal network, the fronto-striatal network is crucial to executive functions. This cortical-subcortical network has connections between the prefrontal cortices and the striatum, globus pallidus and mediodorsal nucleus of the thalamus. Neurons in the prefrontal cortex project to these subcortical regions, allowing interaction and influencing motor actions through timing, initiation and inhibition, thus supporting working memory and executive functioning (D'Esposito & Grossman, 1996). The integrity of tracts within this network (such as the internal capsule and corona radiata) has been related to executive function performance in children and adults (Brinkman et al., 2012; Darki & Klingberg, 2014; Niogi et al., 2008; Niogi, Mukherjee, Ghajar, & McCandliss, 2010). The volumes of subcortical structures involved in this network (namely the thalamus) have also been implicated in tasks assessing the working memory component of executive functions (Little et al., 2010; Omizzolo et al., 2014; Ullman, Almeida, & Klingberg, 2014). Some studies have also

found associations between the integrity of commissural fibres (e.g. the genu of the corpus callosum) and performance in executive function tasks in adults (Niogi et al., 2010), developmental populations (Murray et al., 2016; Nosarti et al., 2004) and children as young as 12 months old (Short et al., 2013).

Although its role in working memory is under dispute, some reports have shown that the hippocampus plays a role in working memory in adolescents (Finn, Sheridan, Kam, Hinshaw, & D'Esposito, 2010), which hippocampal input diminishing as a function of age. Here, the hippocampus and prefrontal cortex were found to co-activate during working memory tasks in early adolescence, but only co-activated under high working memory load in late adolescence. This is considered to reflect the immaturity of the prefrontal cortex, which continues to develop until late adolescence (Lenroot & Giedd, 2006), with recruitment of the hippocampus necessary for task success until this time point, even for low working memory conditions. Further evidence for the recruitment of the hippocampus under high working memory load conditions has been provided in adults, where a relationship between hippocampal volume and working memory performance has been demonstrated in the high-load conditions of the SOPT (Geva et al., 2016).

The demonstrated atrophy in white matter (particularly in prefrontal regions) and the integrity of tracts identified as compromised will be investigated in relation to executive functioning. Although these effects were primarily evident in patients with CHI, the relationship between structural integrity of these regions and executive functioning will be explored in both patient groups. Given the presence of

hippocampal volume loss observed in patients with CHI, the relationship between hippocampal volume and performance on the SOPT will be examined, as will relationships between thalamic volume and components of executive functioning.

7.1.3 Neuroanatomical correlates of motor function

The striatum and the thalamus play a crucial role in motor functioning. The thalamus is a relay centre between subcortical areas and the prefrontal cortex; therefore motor control and motor learning is likely to be directly mediated by the thalamus through basal ganglia-thalamocortical circuits as well as cerebellar-thalamocortical pathways (Haber & Calzavara, 2009; Middleton & Strick, 2000). The volume of the thalamus has been implicated in the motor proficiency of at risk developmental populations previously; in a sample of preterm children with Developmental Coordination Disorder, thalamic volumes were found to correlate with motor proficiency (De Kieviet et al., 2014). The integrity of the white matter tracts within these loops have been shown to be related to motor proficiency in children; the corticospinal tract (which descends into the corona radiata, and then subcortically forms the internal capsule) is a key motor pathway (Diamond, Scheibel & Elson, 1985), and fractional anisotropy of the corticospinal tract has been found to be related to motor functioning in premature children (De Kieviet et al., 2014) and in children with growth hormone deficiency (Webb et al., 2012).

The commissural fibres of the corpus callosum are also extremely important for motor co-ordination, with the genu projecting to prefrontal motor areas and the body

projecting to supplementary motor areas as well as subcortical nuclei (Huang et al., 2005; Paul et al., 2007). Microstructure of the corpus callosum and the forceps major has been related to motor impairment in preterm children (De Kieviet et al., 2014; Estep et al., 2014). This chapter will explore whether volumes of the striatum and thalamus, alongside white matter integrity of the key motor tracts, are related to the restricted motor profile observed in both patient groups.

The imaging results presented within this thesis have shown that hippocampal-diencephalic, fronto-stratial, fronto-parietal and thalamo-cortico networks are compromised after hypoglycaemia, particularly in the context of CHI. These could produce impairments in memory, executive function and motor co-ordination, which might be mediated by the degree of damage observed within these networks. The following analyses attempt to elucidate the relationship between the relationship between these compromised systems and the observed impairment in cognition and motor skills.

7.2 Methods

7.2.1 Subcortical structures, grey and white matter volume

For tests that were administered to the large cohort (e.g. CMS, Total composite score of the MABC-2) regressions were conducted to examine the association between relevant structures and neuropsychological test scores. To control for effects of age

and ICV, these were also entered as predictors[21]. Bootstrapped coefficient values (based on 1000 samples) are reported here. For tests that were administered to the small cohort only, and for components produced by the PCA, semi-partial correlations were performed on residual volumes (corrected for age and ICV)[22] and the neuropsychological tests scores. Here, Kendall's tau coefficient ($_{t}b$) is reported owing to the small number of participants included (Field, 2013).

Full participant demographics are available in chapter Two. Here, 24 children with CHI, and 14 children with KH had completed memory assessments. Measurements of the hippocampi were available for all of these children. Twenty-eight patients with CHI and 19 children with KH had completed the MABC-2 and for these children measurements of the thalami were available.

To examine the association between motor and attention scores and thalamic volumes further, correlations between volumes and component scores produced by the PCA were performed. Measurements of thalamic volume and component scores were available for 19 children with CHI and 11 children with KH.

7.2.2 Voxel-Based Morphometry

Voxel-based morphometry was used to examine the association between regional grey and white matter volume and component scores produced by the PCA. Data were

[21] Except in the case of hippocampal volumes, which were already corrected for ICV
[22] residuals from linear regressions where age and ICV were entered as the predictor and the structure was entered as the dependent variable

available for 16 children with CHI and 11 children with KH (except for the complex working memory component, where data for 10 patients with KH were available). Relationships between white and grey matter volume with factor-based component scores were conducted using linear regression in SPM12. Age, gender and ICV were entered as covariates of no interest. Results were corrected for non-stationarity and all results reported here are significant at whole-brain cluster level corrected FWE $p<0.05$.

7.2.3 Indices of diffusion

Indices of diffusion (FA and RD) from tracts of interest were extracted from the TBSS skeleton using masks created from John Hopkins University (JHU) white matter labels (1mm), available as part of FSL. Mean FA or RD of each tract was calculated by averaging right and left values.

For tests that were administered to the large cohort, but were not in components produced by the PCA (e.g. letter-number sequencing from the WISC-IV working memory test, attention/concentration from the CMS), regression analyses were conducted to examine the association between relevant structures and neuropsychological test scores, controlling for the effect of age. Bootstrapped coefficient values (based on 1000 samples) are reported here. For tests not included in the PCA, but specific to the small cohort (e.g. digit span backwards), semi-partial

correlations were performed on age-corrected FA or RD values[23] and the neuropsychological test scores.

For relationships between component-based scores and diffusion metrics, data from 13 children with CHI and 11 children with KH were available (except for the complex working memory component, where data for 10 patients with KH were available).

Diffusion metrics obtained from the genu and body of the corpus callosum, corticospinal tract, internal and external capsule, anterior and superior corona radiata and cerebral peduncles were used to examine their association with motor scores. These regions were chosen based on literature citing dependence of motor functioning on these tracts. Results were considered significant at 0.00625 after Bonferroni correction for multiple comparisons.

To examine the relationship between attention and executive function and white matter tracts, FA and RD of the whole corpus callosum, anterior, superior and posterior corona radiata, superior longitudinal fasciculus and cingulum were extracted. Results were considered significant at 0.006 after Bonferroni correction for multiple comparisons.

[23] residuals from linear regressions where age was entered as the predictor and the tract was entered as the dependent variable

7.3 Results

7.3.1 Subcortical volumes

7.3.1.1 Hippocampal volume and memory functioning

Mean hippocampal volume (corrected for ICV) and age were entered as predictor variables for memory scores. Five regression analyses were conducted to examine associations between hippocampal volume and verbal immediate/delayed, and visual immediate/delayed memory, as well as the total memory quotient score. Results were considered significant at 0.01 after Bonferroni correction for multiple comparisons. In patients with CHI, mean hippocampal volume was a significant predictor of memory quotient scores, explaining 35% of the variance in scores (R^2=0.35, p=0.01) as well as verbal delayed memory (R^2=0.37, p=0.01; Table 7.3. fig 7.2). Mean hippocampal volume did not significantly predict visual memory, or verbal immediate memory scores. Mean hippocampal volume was not a significant predictor of memory scores in healthy controls or patients with KH (p>0.05 for all).

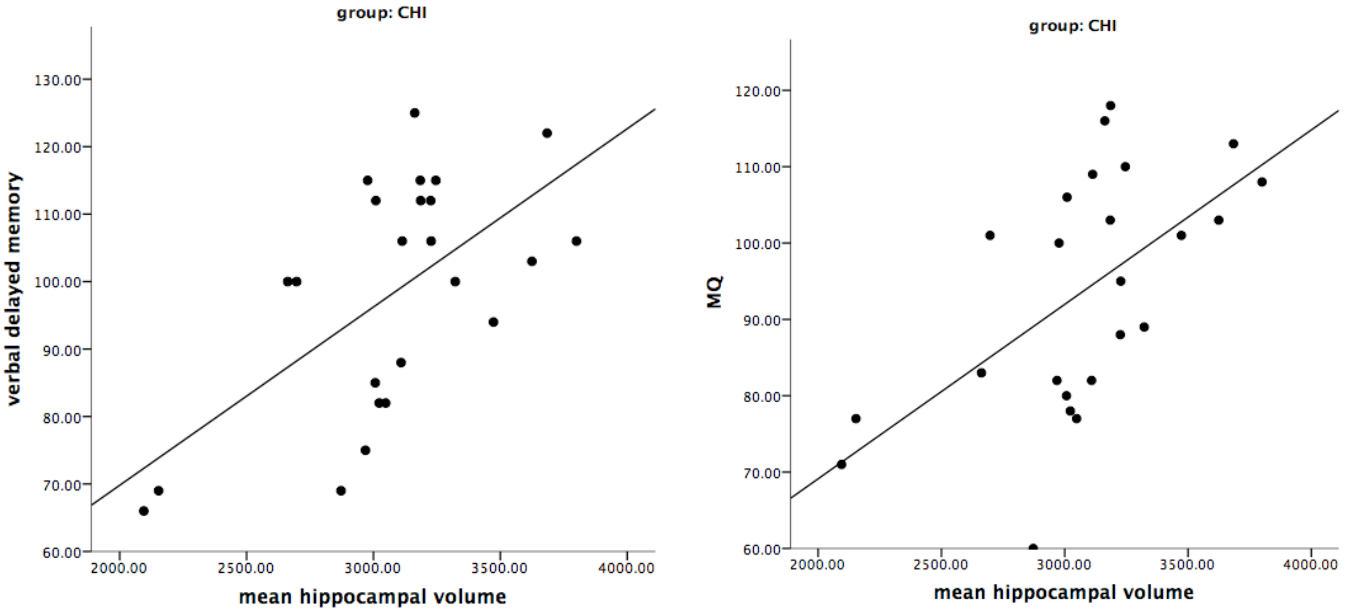

Figure 7.2 Significant relationship between mean hippocampal volume and verbal delayed memory (left, R^2=0.37) and memory quotient (MQ; right, R^2=0.35) scores in patients with CHI.

Table 7.3 Linear model of predictors of memory scores in patients with CHI, with 95% Confidence Intervals (CI) reported in parentheses. CI and standard errors (SE) based on 1000 bootstrap samples. b=unstandardized coefficient, β=standardized coefficient

	b	SE B	β	P
Verbal delayed memory				
Constant	18.86 (-27.78-61.80)	21.20		0.32
Age	-0.03 (-0.23-0.2)	0.12	-0.05	0.79
Mean hippocampal volume	0.02	0.11	0.62	0.001
Memory quotient (MQ)				
Constant	27.48 (-12.69-58.14)	17.17		0.106
Age	-0.06	0.097	-0.12	0.51
Mean hippocampal volume	0.02	0.05	0.62	0.001

To further explore the relationship between impaired memory functioning and reduced hippocampal volume, a comparison was made between patients with significantly lower MQ than predicted from their IQ and their peers whose MQ was in line with their IQ. This analysis was conducted across a collapsed patient group. Independent t-tests comparing patients with an impaired MQ (n=9) against those with intact MQ (n=22) showed that patients with impaired memory had significantly lower hippocampal volumes than patients with intact memory (t=3.7, p=0.001). A similar result was observed when comparing patients with impaired verbal delayed memory (N=10) to those with intact verbal delayed memory (n=20), where patients with impaired memory had significantly lower hippocampal volumes than patients with intact memory (t=3.2, p=0.003). A trend was observed for smaller hippocampal volume in patients with impaired verbal immediate memory (N=11) compared to those with

intact verbal immediate memory (N=21; t=1.7, p=0.09). Figure 7.3 shows the comparison between those with impaired or intact verbal memory/MQ.

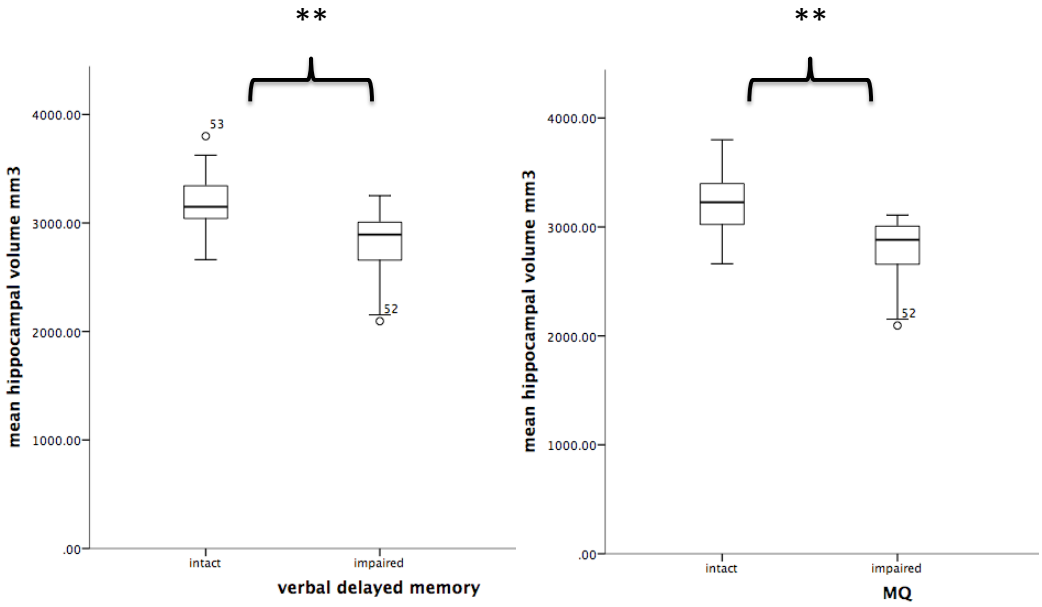

Figure 7.3 Box-and-whisker plot showing median, interquartile range and range of mean hippocampal volume in patients with intact memory relative to patients with preserved memory. Verbal delayed memory and memory quotient are shown here. Patients with intact memory had significantly greater hippocampal volume than patients with impaired memory. A significant difference in mean hippocampal volume between patients with impaired memory and patients with preserved memory.

SOPT

Semi-partial correlations (controlling for age) showed no significant relationship between hippocampal volume and any trial of the SOPT, in either of the patient groups.

7.3.1.2 Basal ganglia and thalamus; relationship to motor function

To examine the contribution of the thalamus and basal ganglia structures to motor ability, separate regressions were run with mean structure volumes (ICV-corrected)

and age as the predictor variables, and the 'total' motor score from the M-ABC as the outcome variables. Volumes of the thalamus, putamen, caudate nucleus and pallidum did not predict motor scores in either patient group. Similarly, no significant relationship was observed from semi-partial correlations on these subcortical structures with the motor component produced from the PCA (t<0.1 for all).

7.3.2 Grey and white matter volumes

7.3.2.1 Whole brain volumes

Motor skills

The contribution of whole brain volumes to motor proficiency (total motor scores from the MABC-2) was explored. Whole brain data and motor scores were available for 25 participants with CHI and 18 participants with KH. White matter volume (corrected for ICV) significantly predicted total motor scores in patients with CHI when age was held constant (R^2=0.34, p=0.01, Table 7.4, but not in patients with KH (R^2=0.18, p=0.22). Grey matter volume (corrected for ICV) was not a significant predictor of total motor scores in patients with CHI or in patients with KH.

Table 7.4 Predictors of motor ability in patients with CHI. 95% Confidence Intervals (CI) reported in parentheses. CI and standard errors (SE) based on 1000 bootstrap samples. b=unstandardized coefficient, β=standardized coefficient

	b	SE B	β	P
Constant	66.44 (38.83-100.51)	15.68		0.001
Age	0.14 (-0.12-0.33)	0.12	0.27	0.24
White matter volume	199.02 (14.29-433.64)	106.54	0.39	0.054

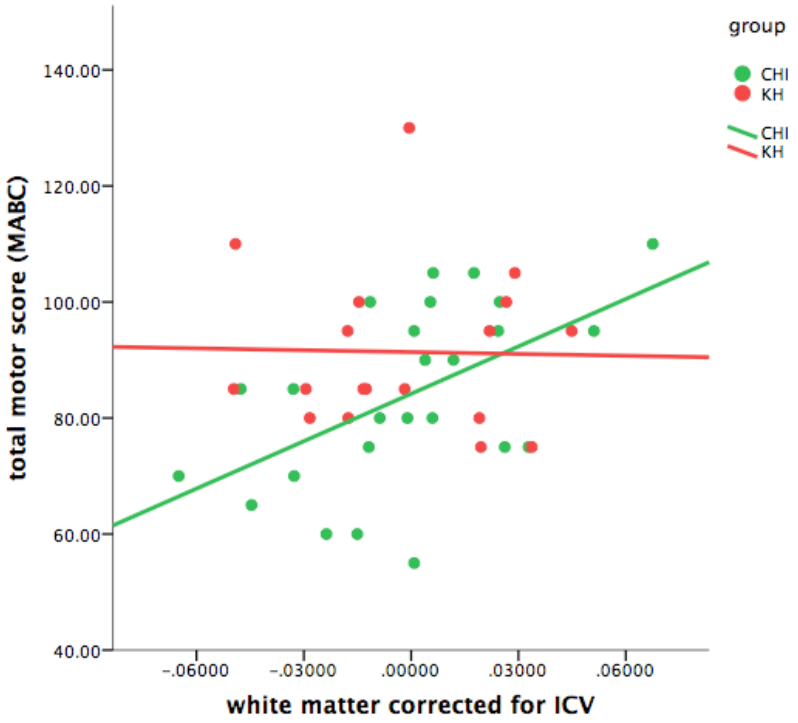

Figure 7.4 Relationship between motor scores and white matter volume. White matter volume significantly predicted motor scores in patients with CHI (shown in green; R^2=0.34) but not in patients with KH (shown in red; R^2=0.18)

To further explore the relationship between white matter volume and motor skills, patients across the two patient groups were categorised as having preserved motor ability or impaired motor ability (combining those who were classed as 'at risk' or

significantly impaired' by the MABC-2 classification system [Chapter Three]). This analysis was conducted across a collapsed patient group. White matter volumes (corrected for age and ICV) were compared between these two groups using independent t-tests. Here it was found that patients with preserved motor skills had significantly greater white matter volume than those who had impaired motor skills ($t=2.1$, $p=0.039$; Figure 7.5).

In order to ensure that the characteristics of any one patient group were not driving the results, this analysis was conducted again in the two patient groups separately. Significant differences were not seen between groups in patients with KH ($t=1.4$, $p=0.19$) or patients with CHI ($t=1.6$, $p=0.13$), although in both cases patients with motor impairment had lower white matter volume than those with preserved motor ability (Figure 7.6).

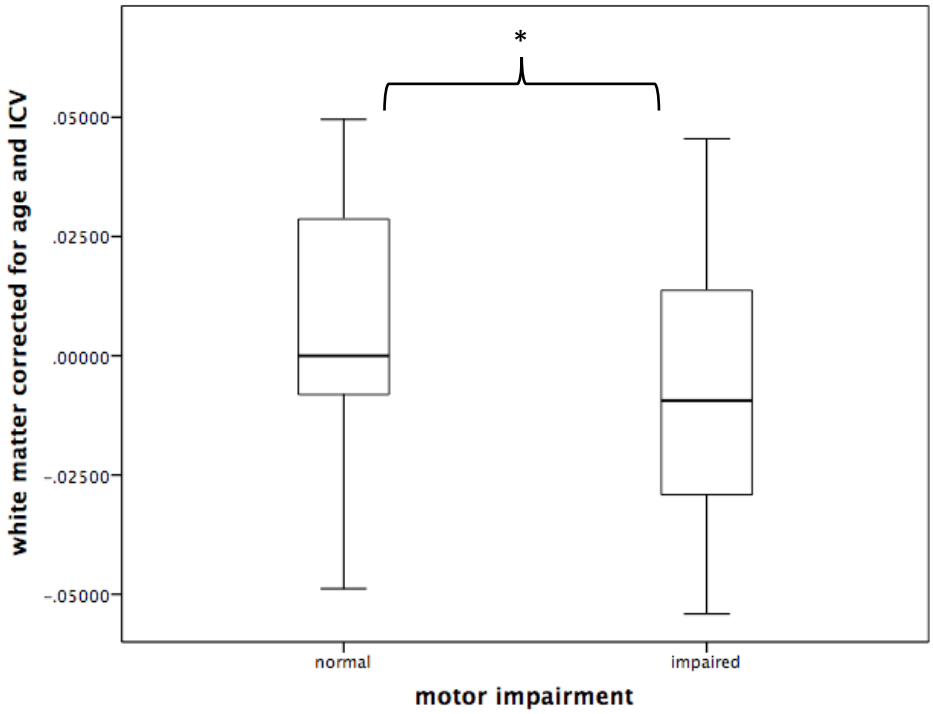

Figure 7.5 Box-and-whisker plot showing median, interquartile range and range of total white matter volume in patients with a normal motor profile relative to patients with impaired motor ability. Patients with impaired motor ability have significantly lower white matter volume relative to patients with preserved motor ability.

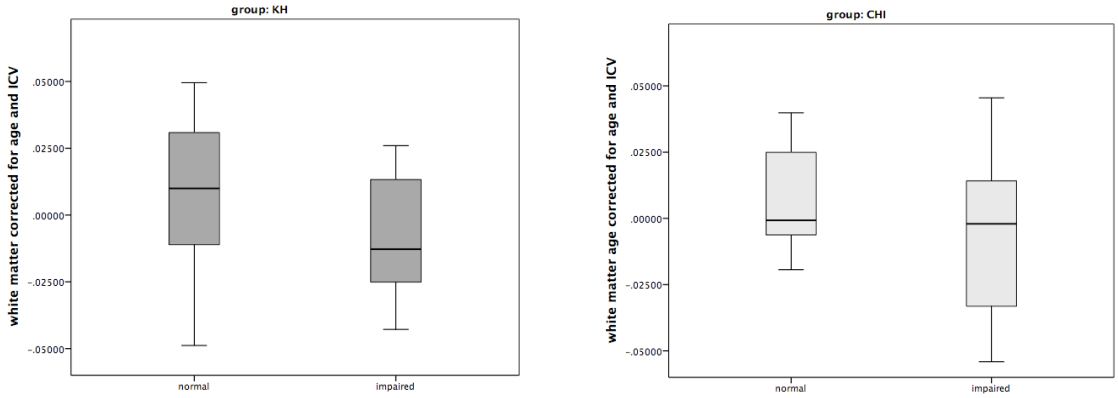

Figure 7.6 Box-and-whisker plots showing the median, interquartile range and range of total white matter volume in patients with a normal motor profile compared to patients with impaired motor ability. Patient groups are shown separately.

Total brain volume and component scores

No significant correlation was observed between total white matter volume or total grey matter volume (corrected for age and ICV) and any component score in either patient group.

7.3.2.2 Voxel-based Morphometry

A whole brain VBM study was conducted to observe association between white and grey matter volume and component scores measuring complex working memory, executive processes, motor skills and literacy and numeracy. For these regressions only component-based scores created in chapter three were entered as regressors.

In patients with CHI, scores on the motor component correlated with white matter volume in a cluster covering the anterior corona radiata, and with grey matter volume in the right insula, pallidum and right postcentral gyrus. No correlations between brain regions and scores on the 'executive' component were observed. Scores on the complex working memory and literacy and numeracy components correlated with white matter volume in bilateral regions covering the forceps major (Figure 7.7).

In patients with KH, scores on the motor component correlated with grey matter volume in the left lingual gyrus and postcentral gyri. No correlations with white matter were observed. Scores on the executive component correlated with white matter volume in a region of the right anterior corona radiata and temporal part of the superior longitudinal fasciculus (right), as well as in a region covering the left CST. Scores on the literacy and numeracy component also correlated with white matter

volume in a region covering the left CST, and with grey matter volume in the middle frontal gyrus. Scores on the complex working memory component correlated with grey matter volume in the right inferior frontal gyrus and left thalamus, but not with white matter. These results are shown in Figure 7.7.

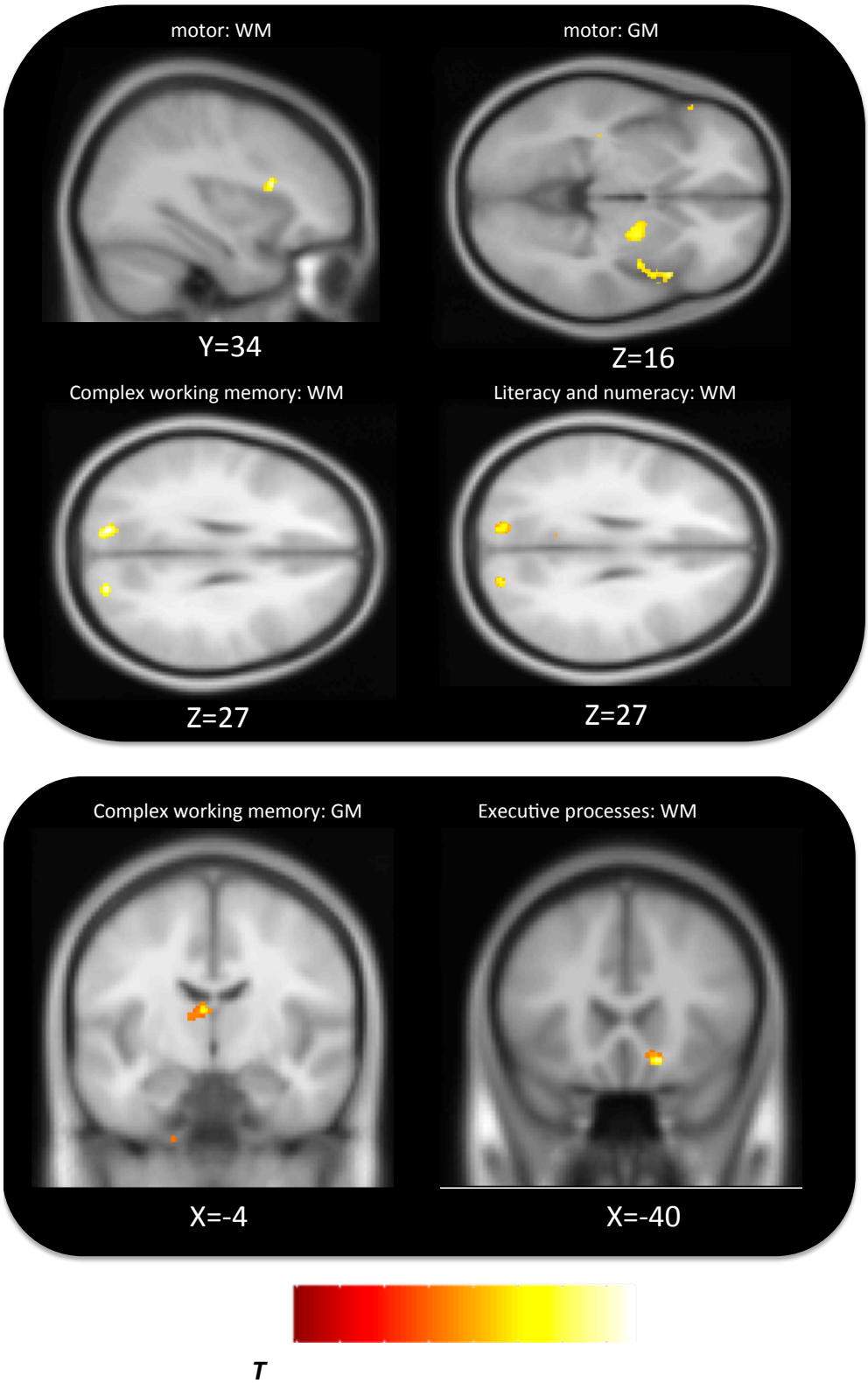

Figure 7.7 VBM regression analyses. Top box: In patients with CHI, motor component correlates with white matter volume in right ACR and grey matter volume in the right insula and putamen. Complex working memory and literacy and numeracy components both correlate with forceps major (bilateral). Bottom box: In patients with KH, complex working memory correlates with GM volume in the thalamus, while executive component scores correlate with white matter in right ACR.

7.3.3 Relationship between fractional anisotropy, radial diffusivity and behavioural data

7.3.3.1 Regressions in the large cohort

Executive functions (attention, working memory and switching scores)

Radial diffusivity in the cingulum significantly predicted attention/concentration scores from the CMS in patients with CHI ([n=18] R^2=0.45, B=-0.72, p=0.002; Figure 7.8). No other correlations survived correction for multiple comparisons.

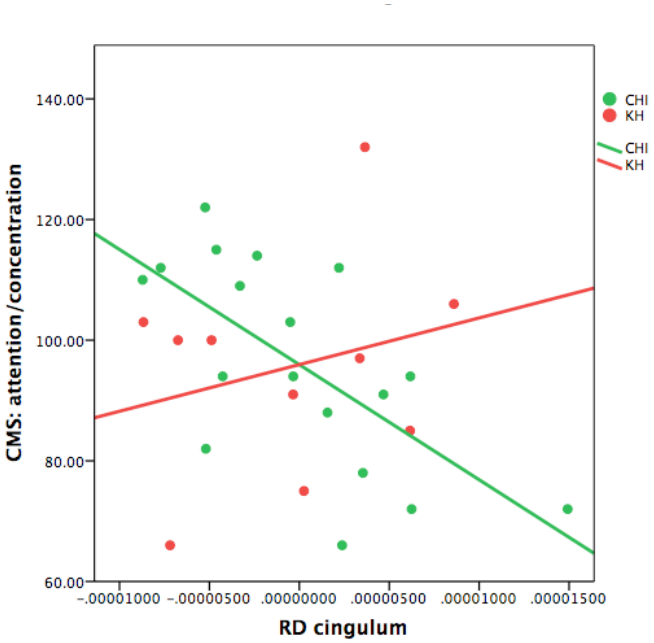

Figure 7.8 Relationship between radial diffusivity (RD) in the cingulum and CMS attention/concentration test scores. RD in the cingulum significantly predicted attention/concentration scores in patients with CHI (R^2=0.45) but not in patients with KH.

7.3.3.2 Factor-based scores and relationship with indices of diffusion

Motor component

No significant correlation between relevant motor tracts and the motor component was observed in either patient group.

Attention and executive function components

No correlations between the tracts of interest and component scores survived correction for multiple comparisons.

7.4 Discussion

7.4.1 Memory and the hippocampus

This study has found that hippocampal volume is significantly predictive of memory function in patients with CHI, in the absence of a group level memory deficit. Furthermore, across patient groups, patients with impaired memory had significantly smaller hippocampal volume than their memory-intact counterparts. This adds further evidence to the importance of the hippocampus to memory functioning, (Aggleton et al., 2010; Cooper et al., 2013; Muñoz-López et al., 2017; Vargha-Khadem et al., 1997), showing that even within a patient group that has hippocampal damage, those with a selective memory impairment have smaller hippocampal volumes than those with intact memory.

Results of this study did not show a relationship between hippocampal volume and performance on the SOPT. This might be due to the fact that these patients (specifically patients with CHI) have a group level working memory deficit that is apparent at low as well as high memory loads. In any case, there are conflicting reports about the role of the hippocampus in working memory tasks (Nosarti & Froudist-Walsh, 2016), with some stating that hippocampal involvement in working memory performance is negatively correlated with age and that after early adolescence, the hippocampus supports working memory for only high mnemonic loads (Finn et al., 2010). Performance on the SOPT in these patients, who are impaired at all loads but the elementary four-item load, might be mediated more by the disrupted white matter within the prefrontal cortex which is essential for working memory (Goldman-Rakic, 1995), particularly in monitoring responses that are self-generated (Petrides, Alivisatos, Meyer, & Evans, 1993) rather than hippocampal volume.

7.1.1 Associations between motor skills, subcortical volumes, and grey/white matter

In both patient groups, VBM identified that grey matter volume in the postcentral gyrus was positively related to scores on motor component. The postcentral gyrus is the somatosensory centre of the brain, and is integral to motor functioning as it guides motor output through somatosensory feedback (Kaas, 2004). Although differences in grey matter volume between patients and controls were not observed in this region, these results suggest that the observed motor abilities in each patient group could in part be related to the integrity of grey matter in this somatosensory region, which might mediate observed deficits.

Contrary to expectations, this study found no relationship between the volume of the entire thalami and motor functioning. Given the thalamic volume reduction in patients with CHI and impairment on motor tasks, it is surprising that no relationship between thalamic volume and motor functioning was observed, especially in light of previously identified relationships between this structure and motor outcome in similar developmental populations (De Kieviet, Piek, Aarnoudse-Moens, & Oosterlaan, 2009). There could be a number of reasons for this, the most likely being that the thalamus is composed of multiple distinct nuclei. The measure of thalamic volume produced by the automatic segmentation method used in this thesis may be too crude to detect such specific structure-function relationships.

In keeping with the lack of relationship between thalamic volumes and motor functioning, no association between whole volumes of the basal ganglia and motor functioning were observed. However, VBM analyses in patients with CHI showed a positive correlation between motor component scores and grey matter volume in the pallidum. In light of the trend towards reduction of the pallidum in patients with CHI (Chapter Five) these results could suggest that patients with smaller volumes of the pallidum have poorer motor outcome. In addition, in patients with CHI, motor component scores were significantly correlated with white matter volume in a region covering the anterior corona radiata, and total white matter volume predicted total motor scores from the MABC-2. Despite the fact that indices of diffusion were not significantly associated with motor outcome, these results suggest that, at least in patients with CHI, white matter and grey matter volume within the neo-striatal circuit

is important in determining motor outcome, a finding that is bolstered by volume reductions and reduced FA identified in these regions, which might result in disrupted communication between the cortex, basal ganglia and the thalami.

Another interesting finding regarding white matter volume and restriction of motor skills was observed in the comparison of white matter volume in patients (across patient groups) who had significantly impaired or borderline motor proficiency relative to those who had a normal motor profile. This analysis yielded analogous findings to hippocampal volume in memory-impaired compared to unimpaired patients; those with impaired motor skills had significantly lower white matter volumes than their unimpaired peers, even against a backdrop of a patient group with identified white matter volume reduction.

7.1.2 Neuroanatomical correlates of executive functions

The only association between indices of diffusion in the fronto-parietal network and executive function performance was between the cingulum and the attention/concentration measure of the CMS, which was found exclusively in children with CHI. This was surprising, particularly with the CHI group, where white matter disruption was pervasive. There could be a number of reasons for this. First, voxel-wise methods as employed here may not be optimal for detecting relationships between structure and function. It is possible that the method used lacks sensitivity for delineating specific tracts and extracted diffusion values may reflect the characteristics of surrounding tracts, rather than a specific fibre bundle (Vestergaard et al., 2011).

This is particularly pertinent in regions of crossing fibres (such as the SLF) where the measure of diffusion extracted could very feasibly contain information about the fibres surrounding the tract of interest (Froudist-Walsh et al., 2015; Groeschel et al., 2014). In addition to this, the method of analysis employed here did not allow the differentiation of relevant tracts into their functional subparts. For example, the cingulum was not divided into its ventral and dorsal parts, which are reported to have different functional attributes (Froudist-Walsh et al., 2015). In short, the method used in this DTI study attempting to link structure to function may have been too crude. It is also possible that patients with early damage to the systems that support working memory may have reorganised function to different regions, making relationships between structure and function difficult to identify (Froudist-Walsh et al., 2015); however, if this were the case then a degree of preservation of function would be expected. Such preservation is not seen in either patient group. Therefore, it is likely that limitations in the methods used, rather than a true absence of association between these regions and executive function ability, have resulted in the lack of association observed in this study.

Scores on the working memory and literacy and numeracy component were both correlated with white matter volume in occipital regions in patients with CHI; not the frontally mediated result that was expected. However, the forceps major are the occipital radiations of the splenium of the corpus callosum, the volume of which has been found to be predictive of letter fluency in preterm populations(Nosarti et al., 2004). In patients with KH, scores on the executive component were significantly correlated with frontal regions, with areas of white matter that covered the anterior

corona radiata and superior longitudinal fasciculus, as well as frontal grey matter. Complex working memory was also found to correlate with grey matter volume in the thalamus, the volume of which has previously been related to current and future working memory capacity in children (Little et al., 2010; Ullman et al., 2014). Therefore, although the expected relationship between complex working memory and frontal white matter was not replicated across the two patient groups, as a whole these findings are broadly similar with previous results.

7.1.3 Limitations

Many of the limitations of this study have been discussed above. In addition to these, averaging of FA over hemispheres may have resulted in reduced sensitivity to detect significant correlations. Furthermore, the results of the VBM may be lacking in specificity and unable to detect changes in a specific tract; the width of one cluster could feasibly cover several tracts.

7.1.4 Conclusion

To conclude, this study has found that hippocampal volume predicts memory scores in patients with CHI, and that across patient groups, those with impaired memory have significantly lower hippocampal volume than those with intact memory. White matter volume is predictive of degree of motor functioning in patients with CHI, and in an analogous pattern to the observed inter-patient difference in hippocampal volume, patients with a significant motor impairment had significantly lower white matter

volume than their typically-functioning counterparts. Associations between the integrity of the cingulum and a measure of executive functioning was observed in patients with CHI, while scores on the components of executive function and working memory produced by the principal components analysis correlated with different regions in the two patient groups. Further work employing different methods of analysis is required to establish the neural underpinnings of these components.

These analyses have demonstrated that white matter is particularly compromising to executive and motor skills, even in the absence of robust group level changes to white matter in patients compared to controls (as in patients with KH). These are indications that the observed deficits in executive functioning and motor skills are related to compromised integrity of brain tissue. Furthermore, there is likely a spectrum of tissue damage in both patient groups that may be distinguishable by separating those who are significantly cognitively/motorically impaired from those who are not. Applying this technique has provided further evidence that it is not easy to distinguish patients with KH from patients with CHI. Further work is required to establish what the limiting events leading these volume compromises might be.

Chapter 8

General Discussion

8 General Discussion

The aims of this thesis were to document the long term effects of early and repetitive hypoglycaemia on cognitive and motor outcome in children with CHI and KH, and to determine the lasting effects of hypoglycaemia on grey and white matter in these children. This thesis also aimed to determine the relationship between brain structures and cognitive and motor outcome. An additional aim was to establish the extent to which children with CHI are distinguishable from children with KH, who are thought to benefit from availability of ketone bodies during episodes of hypoglycaemia.

These aims were achieved through a comprehensive assessment of cognitive, motor and behavioural functioning (Chapter Three), exploring various factions of cognition with tests specifically assessing executive functioning and motor skills. Neuroimaging investigations included analyses of T1-weighted structural scans to assess global and regional differences in tissue volume (Chapter Four), plus volumetric analyses of the hippocampus, thalamus and basal ganglia (Chapter Five). Analysis of diffusion-weighted images assessed the integrity of white matter tracts, and different indices of diffusion gave an indication of the type of white matter damage incurred following hypoglycaemia (Chapter Six). Finally, relationships between the observed cognitive and motor deficits and the brain measures were explored (Chapter Seven). This section will summarise the findings of each chapter, and will discuss the implications of those findings. The limitations of this thesis work and proposals for future work will also be discussed.

8.1 Review of findings

8.1.1 Cognitive and motor profile

Relative to standard population means, patients with CHI show preserved verbal intelligence and have a general ability index within the normal range. However, non-verbal intelligence scores were selectively affected and fell significantly below the standard population mean. In contrast to this profile of mostly preserved intellectual ability, patients with CHI show significant impairments in executive functions, namely those that fall under the categories of attentional control, cognitive flexibility and speed of information processing. Motor abilities were significantly compromised in patients with CHI, with fine motor skills and visual-motor integration specifically affected. Indeed, it is likely that the observed deficit in motor skills and visual perception contributed to a lowered non-verbal IQ, with demands placed on these skills during this test. Although more than one quarter of patients with CHI were or had been in receipt of educational support, group level differences in measures of scholastic attainment were only identified in spelling. As a group, patients with CHI have a normal memory profile, despite a number of children having significantly impaired memory abilities. Therefore, the group-level profile of patients with CHI is one of largely preserved intelligence, memory and academic attainment, with specific deficits in executive functions and motor skills.

Relative to standard population means, patients with KH have an entirely preserved group-level verbal and non-verbal intelligence. However, an assessment of executive functions showed selective weaknesses, particularly on those tasks assessing attentional control. Other executive abilities in patents with KH were impaired to a

lesser degree than those with CHI; deficits in working memory and processing speed existed at trend level. Motor skills were also affected; gross motor coordination abilities were impaired and there was some evidence to suggest that fine motor abilities were lowered. Although over one-third of children were receiving (or had been in receipt of) educational support, group-level scholastic attainment was normal as were memory and learning abilities (although, as in CHI, a number of children had significantly impaired memory relative to their intellectual ability). Therefore, while comparable to standard population means on measures of intelligence, academic attainment and memory, patients with KH show selective impairments in executive functions and motor skills. These findings have confirmed that patients with KH are not protected from adverse cognitive effects following hypoglycaemia.

Patients with CHI and KH therefore share a similar cognitive and motor profile, with assessments highlighting specific deficits in executive functions and motor skills. No significant differences in scores between patient groups were observed, indicating similarities in strengths and weaknesses. Importantly, parental reports corroborated the profiles outlined above, with concerns about inattentive behaviour or issues relating to working memory repeatedly raised and replicated across the three parental questionnaires that were used.

The findings presented in this thesis point to two specifically compromised systems subsequent to early hypoglycaemic episodes in the context of CHI and KH; the supervisory control system, responsible for executive functions, and the motor control

system. Through this study a behavioural phenotype that is specific to children with CHI and children with KH has been established.

8.1.2 Global and regional brain volumes

The neuroimaging studies presented within this thesis are the first reports of school-aged children with CHI and KH to use quantitative neuroimaging methods to explore damage after hypoglycaemia relative to a healthy control group. Importantly, these children were free from epilepsy and had no neurological diagnoses. Therefore, assessment of these children provides an opportunity for assessing the neural consequences of hypoglycaemia in the absence of confounding medical problems.

Analysis of total tissue volumes showed that intracranial volume (ICV) was significantly reduced in patients with CHI relative to healthy controls and to patients with KH. Poor head growth and smaller head circumference has been noted previously in infants and young children with neonatal hypoglycaemia (Burns et al., 2008; Duvanel et al., 1999) and with CHI (Cresto et al., 1998) and is considered to be a proxy for underlying neuropathology (Duvanel et al., 1999). The data presented in this thesis go beyond a proxy measure to show that, compared to healthy controls, white matter volume is significantly compromised in patients with CHI. This manifests as a total reduction in white matter volume and VBM analyses identified this volume loss as widespread, occurring in prefrontal, subcortical and posterior regions of the brain. This loss of white matter was observed alongside a relative sparing of the grey matter of the cortex (although a focal volume reduction in the left frontal pole was observed).

In patients with KH, global white matter volume was lower than in controls, but this difference did not reach significance. Some evidence for focal white matter volume loss in the KH group was provided in the VBM analyses, with regional volume loss observed in the left temporal lobe. Despite the clear difference in ICV measurements between patients with CHI and patients with KH, differences in regional white matter volume between the two patient groups were unclear. Indeed, if children with KH were entirely protected from the adverse effects of hypoglycaemia, then (given the extensive volume loss in CHI relative to controls) one would expect to see a similar pattern of volume loss in CHI relative to KH. This suggests that any neuroprotective benefit of ketone bodies is not complete and may be mediated by other clinical factors.

In conclusion, this study demonstrates that white matter is particularly vulnerable in patients with CHI, and unlike the reports of white matter atrophy in neonates and infants following hypoglycaemia, is not isolated to parieto-occipital regions (Alkalay, Flores-Sarnat, et al., 2005; Barkovich et al., 1998; Caraballo et al., 2004; Filan et al., 2006; Gataullina et al., 2013; Kinnala et al., 1999; Traill et al., 1998). Widespread white matter volume loss is shown to be to be a lasting defining feature of early, severe and recurrent hypoglycaemia in the context of hyperinsulinism.

8.1.3 Diffusion characteristics of white matter

No significant differences in white matter microstructure were observed when comparing patients with CHI to patients with KH, in contrast to the findings of the HBI

study (where decreased FA) in the corpus callosum was found when comparing patients with CHI to patients with KH (Kumaran, 2012).

Compared to healthy controls, patients with CHI show widespread diffusion changes across the brain. Affected fibres include the long association fibres connecting distal cortical regions (the inferior fronto-occipital fasciculus, the superior longitudinal fasciculus and the cingulum), commissural fibres facilitating transfer of information across hemispheres (corpus callosum) and projection fibres responsible for motor control (corona radiata, corticospinal tracts and internal capsule), which were found to have reduced FA and increased RD in patients with CHI compared to controls. Importantly, these regions correspond to those identified in the VBM study. These areas of altered diffusion are far more widespread than the regions identified in the HBI study (Kumaran, 2012).

Some evidence for decreased FA in the corpus callosum of patients with KH was observed. The results of the studies presented in this thesis suggest that patients with KH may have compromised white matter integrity. If this is the case, it would explain why a more widespread pattern of white matter damage was not observed in the analyses of diffusion data presented in the HBI study.

8.1.4 Subcortical volumes

Hypoglycaemia due to hyperinsulinism was associated with reduced volumes in several subcortical structures. The thalamus was significantly reduced in volume in patients with CHI relative to controls and relative to patients with KH. The thalamus is a major

relay centre in the brain, with multiple connections to most cortical and subcortical regions (Johnson & de Haan, 2015; Aggleton, Vann, & Saunders, 2005). Compared to controls, patients with CHI had reduced hippocampal volumes, although this effect was not observed in the small cohort of patients, so some degree of caution should be used when interpreting these findings. No difference in hippocampal volumes was observed between patient groups, and although patients with KH had lower hippocampal volumes than controls, this effect was not statistically significant. The basal ganglia appear to be relatively preserved in both patient groups, although there was a trend toward volume reduction in the pallidum in patients with CHI.

This study shows that volume reduction in the thalamus is a lasting feature of hypoglycaemia due to hyperinsulinism, present beyond the previously identified infancy period (Burns et al., 2008; Kinnala et al., 1999). Furthermore, contrary to previous reports, (Avatapalle et al., 2013; Barkovich et al., 1998; Gataullina et al., 2013; Kara et al., 2007; Kinnala et al., 1999) global basal ganglia damage is not a robust feature of early hypoglycaemia. Alongside extensive white matter volume loss, subcortical structures crucial to motor and memory functioning are compromised in patients with CHI.

8.1.5 Relationships between brain and behaviour

The structural differences described above were associated with performance on assessments of cognitive and motor ability. In the absence of a group-level memory impairment, hippocampal volume significantly predicted memory ability in patients

with CHI. Furthermore, across patient groups volume of the hippocampus was significantly smaller in those who had a memory impairment relative to those who did not. Similarly, white matter volume was significantly reduced in those who had a motor impairment relative to those who did not. In patients with CHI, white matter volume was a significant predictor of motor ability, and white matter volume in regions covering fronto-striatal projections were found to be associated with motor abilities. In patients with KH, the volume of white matter covering these same fronto-striatal projections were associated with the component reflecting executive abilities. In both patient groups total white matter volume appears to be a determinant of motor impairment, while hippocampal volume is a determinant of memory impairment. The predictive capacity of white matter volume for motor impairment chimes with the findings of Burns et al. (2008) who found that infants with severe white matter damage had poor neurological outcome.

8.2 Factors that might influence neuropathology

Medical factors, including the presence of hypoglycaemic seizures, may selectively affect certain structures such as the hippocampus (Wolf, Bast, & Surtees, 2005). Seizures increase cellular energy demand and thus may be particularly deleterious to the brain in the context of hypoglycaemia (Boardman & Hawdon, 2015; Uria-Avellanal, Marlow, & Rennie, 2013). However, this thesis did not find any differences in whole brain or subcortical volumes in those with and without seizures in either patient group, although this may warrant further investigation. Some researchers have found hypoglycaemic seizures to be a risk factor for general cognitive ability, memory, and

executive functions (Kaufman et al., 1999; Rovet & Ehrlich, 1999), which implies that the presence of seizures due to hypoglycaemia could result in structural changes to the brain.

Other factors that may influence vulnerability of the brain to hypoglycaemic events include gestational age. Although none of the children included in this study were very preterm, moderate and late preterm as well as early term children were recruited to this study. It is possible that there is an interaction between degree of brain damage and vulnerability of the brain due to adversities associated with prematurity (although no statistically significant relationships were observed between these variables). This could apply specifically to those children who presented with hypoglycaemia in the neonatal period, when the immaturity of the brain renders it more susceptible to damage. This may have implications for cognitive and motor outcome, with studies finding associations between the number of weeks of gestation and cognitive and motor performance (De Kieviet et al., 2009; Marlow, Wolke, Bracewell, & Samara, 2005).

8.3 Parallels with other populations

8.3.1 Mechanism of damage

Hypoglycaemia results in energy failure across the brain and there are other cohorts who experience similar such energy failure, such as in those with hypoxia-ischaemia. Much like in hypoxia, some brain structures are more vulnerable to hypoglycaemic insult (Dzieciol et al., 2017; B. Y. Huang & Castillo, 2008; Muñoz-López et al., 2017). Huang and Costillo (2008) argue that brain regions most sensitive to hypoxia-ischaemia

are (1) those with the highest NMDA receptors (e.g. glutamate receptors) and consequently high susceptibility to excitotoxic injury, and (2) those areas with highest energy demand. In addition, they add that vulnerability is likely to be determined by the maturity of the brain. It is therefore likely that the sensitivity of brain regions to the effects of hypoglycaemia are determined via a similar mechanism, as episodes of energy failure underlies both of these conditions. These mechanisms will be discussed here.

The neuroimaging profile of patients with CHI is similar to that of patients who experience early, acute hypoxic-ischaemic events. These have been associated with hippocampal and thalamic volume reduction (Cooper et al., 2013; Dzieciol, 2015; Dzieciol et al., 2017). In both hypoglycaemia and hypoxia-ischaemia the brain experiences a systemic energy failure, which results in a release of excitotoxins. When excitotoxins (namely glutamate and aspartate) are released, as they are during hypoxia and hypoglycaemia, they bind primarily to NMDA receptor-mediated calcium channels, causing a significant influx of calcium into the postsynaptic neurons and triggering a cascade of processes which are cytotoxic, and may lead to apoptosis (Huang & Castillo, 2008). Regions that are highly populated with NMDA receptors may, therefore, be particularly sensitive to such damage (Auer, 1986; Barkovich et al., 1998; Huang & Castillo, 2008; Suh, Hamby, & Swanson, 2007). Subcortical structures such as the hippocampus, striatum and thalamus are regions with high populations of NMDA receptors (Barkovich et al., 1998; Johnston, 2005; Su & Wang, 2012). This sequence of events may render these regions particularly vulnerable to damage and could explain some of the similarities between the neural phenotype of these two patient groups.

In addition to being densely populated with NMDA receptors, the thalamus is a region with an extremely high energy demand during the first two months of life (Chugani, 1998; Chugani & Phelps, 1986). Damage to the thalamus in patients who experience episodes of hypoxia has been noted not just in the form of volume loss, but as restricted diffusion (Ward et al., 2006). Active myelination is an energy intensive process and the regions that have the most advanced myelination needs usually correspond to regions with the greatest energy demand (Huang & Castillo, 2008). It has been suggested that the thalamus might be particularly vulnerable to damage early in life (i.e. when those with CHI are experiencing hypoglycaemia), because during this period it is actively myelinating (Ward et al., 2006). Therefore, neonates and infants who experience compromising events, such as hypoxia or hypoglycaemia, may be at particular risk of thalamic damage.

Vulnerability of the thalamus has also been identified in premature (<33 weeks' gestation) cohorts (Boardman et al., 2010; Boardman et al., 2006; Nosarti et al., 2014) and this has often been coupled with diffuse white matter injury and focal volume loss in the corona radiata and centrum semiovale (Ball et al., 2012; Boardman et al., 2010). It has been suggested that these regions are particularly vulnerable in preterm individuals because they are among the first to myelinate (Ball et al., 2012; Nosarti et al., 2014), which is likely to be related to the developmental vulnerability of oligodendrocyte precursor cells causing a disruption to the maturation of myelin-forming oligodendrocytes (Back, Riddle, & McClure, 2007), and can cause chronic myelination disturbances (Kendall et al., 2014). Many studies find lasting widespread

white matter abnormalities in children born prematurely (Giménez et al., 2006; Nosarti et al., 2008, 2014; Soria-Pastor et al., 2008). Indeed, the degree of white matter pathology has been related to gestational age (Giménez et al., 2006), and the greatest risk for oligodendrocyte precursor cells coincides exactly with premature birth (between 24-32 post conceptual age; Back et al., 2001). Although the time windows at which damage through energy failure and stress might occur is different in patients with early hypoglycaemia and patients who are premature, the coupling of thalamic and white matter vulnerability observed in preterms is very similar to the profile of patients with CHI presented throughout this thesis.

The injury to oligodendrocyte precursor cells can be caused by hypoxic-ischaemic events leading to excitotoxicity, as well as oxidative stress (Back et al., 2001; Counsell et al., 2003). In accordance with the requirements of a stable energy substrate for myelination, widespread white matter diffusion changes have been identified using Diffusion Weighted Imaging (DWI) in infants after episodes of hypoxia (Tusor et al., 2012; Ward et al., 2006). However, the vulnerability of oligodendrocyte precursor cells has also been suggested in the context of hypoglycaemia. Thus, although occurring outside a critical window for oligodendrocyte precursor cells, hypoglycaemic events may have a direct effect on oligodendrocyte precursor cells. Studies have shown that hypoglycaemia can inhibit oligodendrocyte precursor cell proliferation, migration and differentiation, ultimately inhibiting oligodendrocyte formation and disrupting myelination (Yan & Rivkees, 2006). Taken together, the neuroimaging profiles of infants with early hypoxia, particularly premature infants, and the findings presented in this thesis (VBM white matter volume loss and widespread increases in RD) could

suggest that oligodendrocyte precursor cells are vulnerable to insult even outside of this critical time window, disrupting the formation of mature oligodendrocytes and limiting myelin production.

Some have argued that in premature infants, the integrity of the thalamus is key to the normal development of other cortical regions (Ball et al., 2012). A neuroimaging phenotype of reduced thalamic integrity (in the form of reduced thalamic volume and increased mean diffusivity) and widespread white matter disruption has been taken as evidence for disruption of the thalamocortical system subsequent to preterm birth, with increasing severity of this phenotype being related to poorer developmental quotients at two years of age (Ball et al., 2012; Boardman et al., 2010). Significant relationships between thalamic volume and white matter microstructure in subcortical regions, as well as relationships between thalamic volume and fronto-temporal regions and the hippocampus have provided further evidence for a widespread impact of early thalamic damage (Ball et al., 2012). The data presented in this thesis do not allow a corroboration of this theory; it is possible, however, that thalamic volume reduction is a predictor of more widespread white matter disruption and the compromise of other subcortical structures such as the hippocampus. It is also possible that damage to any region is propagated along the neuronal circuit via Wallerian degeneration (Baldeweg et al., 2006) or diaschisis (Feeny & Baron, 1986), which could also explain the widespread nature of the damage observed in patients with CHI.

8.3.2 Neuroimaging and cognitive profile

As mentioned previously, there are many interesting parallels that can be drawn between the neuroimaging profiles of children born prematurely and patients with CHI, including the aforementioned white matter disruption and thalamic involvement. Other similarities include a reduction in whole brain volume (Nosarti et al., 2002) and reduced head circumference (Nosarti et al., 2008). The volume of the hippocampi, caudate, cerebellum and corpus callosum have also been found to be reduced in children born preterm (Ball et al., 2012; Nosarti et al., 2002; Nosarti et al., 2008, 2014). Therefore, it could be said that these populations appear to have similar neuroimaging profiles.

Similarities in cognitive profiles are also evident between these populations. Patients with CHI and KH show a similar pattern of strengths and weaknesses to children who were born preterm. Studies of cognitive outcome following very preterm birth often report impairments in the domains of motor skills (De Kieviet et al., 2009; Marlow, Hennessy, Bracewell, & Wolke, 2007) and executive functioning (Johnson et al., 2016; Johnson & Marlow, 2017; Marlow et al., 2007; Mulder, Pitchford, Hagger, & Marlow, 2009; Mulder, Pitchford, & Marlow, 2011a, 2011b; Nosarti et al., 2007). Preterm children have a three to four fold increased risk of developing psychiatric disorders, the most common of which is ADHD (Johnson et al., 2016), with studies arguing that a core deficit in inattentiveness (rather than hyperactivity) leads to this diagnosis (Johnson et al., 2016; Mulder et al., 2011a). This core deficit in inattentiveness has been linked to a generally compromised executive network in other populations (Holmes et al., 2014) as well as being linked to impairments in specific executive

functions (e.g. working memory and processing speed; Mulder et al., 2011a) in preterm populations. Furthermore, the deficits in executive functions in preterm populations are noted over and above cognitive ability, mirroring the findings presented in this thesis (Johnson et al., 2016; Marlow et al., 2007; Nosarti et al., 2007). It has been suggested that owing to the developmental trajectory of executive functions, which are immature through childhood, they may be particularly sensitive to early cerebral insult (Anderson, 1998). The similarity between these patient populations supports this.

Premature birth has repeatedly been associated with poor academic attainment (Johnson et al., 2009; Johnson, Wolke, Hennessy, & Marlow, 2011; Simms et al., 2013)), with these effects being evident even in late preterm and early term births (Chan & Quigley, 2014; Johnson et al., 2015) and particularly in relation to mathematical abilities (Simms et al., 2013, 2015). The behavioural studies reported in this thesis did not find an effect on measures of mathematical ability in patients with hypoglycaemia, although a significant number of children in the study were SEN or receiving special educational support. Furthermore, this study only assessed mathematical and spelling to dictation ability. It is possible that other measures of academic attainment (such as single word reading, or reading comprehension) may have shown significant differences between patient groups relative to healthy controls.

The evidence reviewed in this section suggests that early insult to the brain may result in similar patterns of neuropathology that could be dependent on similar mechanisms,

resulting in an overlapping neuroimaging profile, and subsequently an overlapping cognitive profile that is particularly similar with regard to motor skill and executive functioning. Furthermore, these impairments are unlikely to be transient in nature; it has been shown that in ex-preterm adults, impairments in executive functioning persist into young adulthood (Nosarti et al., 2007).

8.4 Limitations

This study has a number of limitations and these are largely associated with studies on brain development in children with rare conditions. First, it is hard to recruit large numbers of patients with such conditions, particularly when stringent exclusion criteria are applied. Second, children with rare conditions often have comorbidities as a feature of the disorder, such as genetic mutations, which can complicate the interpretation of the results. Finally, as briefly discussed above, there are certain medical factors that might determine outcome, and which were not controlled for in this thesis. These limitations will be discussed in turn.

The studies presented throughout this thesis report on what is to date (to the best of the author's knowledge) the largest cohort of school-aged children with these conditions. Importantly, these children are free from epilepsy and have no known neurological diagnoses. However, the sample size of both patient groups is relatively small and might have limited power to detect significant effects. This might be particularly pertinent to patients with KH, who appear to be quite heterogeneous in

terms of outcome. Such heterogeneity would require large numbers of participants in order to establish significant group-level outcome.

Another limitation refers to the effect of genetic mutations on brain growth and development. Within the cohort of children with CHI, 39% had known genetic mutations causing hyperinsulinism. While the link between most of these genes and brain development has not been established, there is an association between the GLUD1 mutation and delayed development as well as propensity for epilepsy (Bahi-Buisson et al., 2008). Only one patient was identified as having a GLUD1 mutation and did not have epilepsy; the inclusion of this patient is unlikely to affect whole brain analyses. However, it is possible that the genes identified in congenital hyperinsulinism are implicated in brain development, and may have contributed to the differences observed. As the genetic mutations relevant to hyperinsulinism are broad, these genes would all have to be encoding for the same events e.g. myelination, in order to produce such a robust group-level profile. There is also a possibility that prenatal hyperinsulinism might have occurred, which could have a significant effect on prenatal brain development (Meissner et al., 2003; Warncke et al., 2016), particularly given the known vulnerability of OPC between 24-32 weeks as described above. Another limiting factor is that children who were born moderately prematurely were included in this sample. Although most research citing adversity following preterm birth focuses on those born very preterm (Marlow, 2014), there is evidence to suggest that even moderate prematurity, or early term birth, results in increased propensity for cognitive adversities (Chan & Quigley, 2014; Johnson et al., 2015).

Finally, the retrospective nature of this study makes it difficult to quantify what the level of insult (in terms of low blood glucose) in these patients is. As a result, it becomes challenging to establish a causal link between low blood sugar and the observed outcome, and it could be considered controversial to assume that the neuroimaging findings and cognitive profiles presented in this thesis are related to experiencing severe and recurrent episodes of hypoglycaemia. Without the availability of continuous and strict blood sugar monitoring and documentation of each recording, it is hard to differentiate which patients may have experienced protracted and extreme episodes of low blood sugar, and those who may have experienced milder episodes. The retrospective collection of this data (for example, lowest recorded blood glucose level) is challenging, particularly as some children can be asymptomatic when blood sugar levels are low. While it is possible to approximate extremely low levels of blood sugar (for example, through division of the groups in to those who had seizures as presentation symptoms versus those who did not), attempts to do so within the remit of this thesis did not yield significant findings. This approximation is crude however, and rests on the assumption that the blood glucose threshold for seizure onset is universal between individuals. Future studies could mitigate this limitation through longitudinal designs with strict monitoring of blood glucose levels from the outset.

8.5 Future work

8.5.1 Microstructural properties of white matter

This thesis has shown that early hypoglycaemia represents a risk to the integrity of white matter. The application of probabilistic fibre-tracking techniques would overcome the problem of lack of specificity in whole-brain approaches such as TBSS (Groeschel et al., 2014) by allowing a greater level of sensitivity in localising the regions affected by hypoglycaemia. This would allow further investigation into the potential involvement of the corpus callosum in patients with KH. Furthermore, the increased sensitivity to detecting tracts (such as the SLF) that are largely surrounded by crossing fibres might allow firmer conclusions to be drawn regarding the relationship between fibre integrity and cognitive/motor outcome. The structural connectivity of the thalamus will also be of particular interest here, given the volume reduction identified in CHI, and the relationship between executive functions, fronto-striatal white matter and thalamic grey matter identified in patients with KH (presented in Chapter Seven). The structural connectivity of specific tracts within the thalamocortical network have been related previously to executive functions and motor skills, and diffusion tractography allows the identification of these functionally distinct and important regions within the thalamus (Philp, Korgaonkar, & Grieve, 2014).

8.5.2 Thalamic Nuclei

The finding that the thalamus is reduced in volume in patients with CHI warrants further investigation that could be carried out by subdividing the thalamus into specific nuclei, although this would require T1 weighted scans with a higher field strength (e.g.

3T). Through this method, it would be possible to ascertain whether motor or memory specific nuclei are affected in patients with CHI and to relate the integrity of these nuclei to motor or memory functioning. It may also be possible to analyse any structural covariance between the integrity of the thalamus and more widespread regions, as in the study conducted by Ball et al. (2012).

8.5.3 Medical variables determining outcome

There are a number of medical variables that may be of importance in determining neuropathology and subsequent cognitive/motor dysfunction following hypoglycaemia. These include lowest recorded blood glucose level, the duration of hypoglycaemic episodes, and the frequency and timing of such episodes. Given the vulnerability of the immature brain to these events, it is likely that very early presentation (i.e. in the neonatal period) will have significant consequences for certain brain structures, and in turn, brain development. Factors such as the time lag between presentation of symptoms and treatment of disease will be important, as delays between presentation and diagnosis are likely to heighten the propensity for hypoglycaemic brain damage to occur (Rozance & Hay, 2006). A follow-up study on the outcome of patients with CHI and KH in relation to these medical variables (particularly the age at which symptoms first developed) would allow the investigation of these theories. This may be best achieved using longitudinal design, because (as mentioned above) this data can be difficult to collect retrospectively. In particular, patients with KH appear to be heterogeneous in term of their outcome, with some overlapping with healthy controls and others overlapping with patients with CHI. It is important to establish what might predict white matter damage and cognitive

outcomes in this patient group, and this may help further determine the neuroprotective role of ketone bodies which are unclear from the studies presented in this thesis.

8.5.4 Interventions

The study on cognitive and motor outcome in patients with CHI and KH found a marked impact on executive functioning in both patient groups, even in the absence of any group level neuropathology in patients with KH. An interesting avenue to explore here would be in interventions for the improvement of executive functioning, which are argued to be malleable and trainable (Amso & Scerif, 2015). Computerised programmes delivering executive function training have been found to be effective in improving working memory skills in both typically developing children and clinical populations (Astle, Barnes, Baker, Colclough, & Woolrich, 2015; Holmes, Gathercole, & Dunning, 2009; Klingberg, Forssberg, & Westerberg, 2002) that can have a broader impact on functioning; for example, on academic attainment in mathematics and reading (Holmes et al., 2009; Pascoe et al., 2013). In adults, performance gains after working memory training have been related to increased activation of frontal and parietal regions(Olesen, Westerberg, & Klingberg, 2004), and in typically developing children working memory improvements following working memory training corresponded to increased strength of the resting-state connectivity (Astle et al., 2015). This could therefore be a promising route to explore, with the hope of improving outcomes in these two patient groups.

8.5.5 Functional networks that underlie impairments

Given the evidence of a compromised top down supervisory control system in both patients with KH and patients with CHI, analyses of resting state functional MRI data would allow an investigation into whether this is related to altered functional connectivity in the appropriate networks (namely the default mode network, fronto-parietal control network and the salience network). Alterations in resting state networks have been suggested to be related to early structural damage in children born premature (Damaraju et al., 2010). Altered resting state networks have also been identified in adults who were born preterm, with the default mode network and salience network most altered in ex-preterm adults compared to controls. It has been suggested that this disconnection may provide a physiological basis for the persistent cognitive deficits observed in ex-preterm individuals (White et al., 2014).

8.6 Conclusion

This thesis has defined a behavioural phenotype specific to patients with CHI and KH, both of whom experience early and recurrent episodes of low blood sugar, which is characterised by an impairment in executive functions (the supervisory control system) as well as compromised motor abilities. Importantly, this phenotype is shared across patients with CHI and those with KH, although children with KH appear to have a less severe phenotype than those with CHI. This is echoed in the results of the neuroimaging analyses. The most striking neuroimaging abnormalities are the reduction in thalamic volumes, specific to patients with CHI, and the findings of extensive white matter abnormalities, which are profound in patients with CHI and

detectable to some degree in patients with KH. The neuroimaging profile of patients with KH indicates that hypoglycaemic events in this patient group do not have as profound an affect as when experienced because of hyperinsulinism. The damage may be globally less severe in this patient group, or the spectrum of damage to preservation may be highly heterogeneous. In both cases, this could be due to a variety of factors, varying as a function of age at insult, status of disease course and the availability of alternative fuels.

This study confirms that abnormal cognition and motor skills are common in these patient populations, even in the absence of confirmed neurological diagnoses. The finding of impaired executive functioning is likely to have significant implications for their progression throughout the schooling system and this might result in a failure to keep up with peers and to reach individual targets. Evidence suggests that executive dysfunction has the propensity to continue into adulthood. Therefore, aside from leaving a legacy through influencing achievement in the formal schooling years, executive dysfunction could have an impact on daily functioning past this time point. It is essential that these children are followed up, monitored and provided with the appropriate support in order to ensure that they are able to reach their fullest potential. This is especially pertinent to those with KH, who, as a group traditionally thought to be neuroprotected, are not currently characterised as being at risk and may have subtle but impactful deficits that go unnoticed throughout their childhood. Many of the children who participated in these studies have already faced early surgeries and multiple hospitalisations, and should be given the best possible chance to thrive in the face of these early adversities.

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