Type 1 Diabetes Mellitus and the Brain: Influence of Clinical Complications and Genetic Factors on Brain Structure and Cognitive Function

Stewart C. Ferguson

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To Lesley

(a) This Thesis was composed by Dr. Stewart C. Ferguson.

(b) The studies contained within this thesis were performed, analysed and written by myself.

Studies 1 and 2

- Inga Grant assisted in the Neuropsychological Assessment of participants.
- The Magnetic Resonance Neuroimaging protocol and post-hoc volumetric MRI analysis was executed by Mrs. Annette Blain, under the supervision of Professor Jonathan JK Best at the Department of Radiology, Royal Infirmary of Edinburgh.
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- The genetic analysis of specimens for APOE Polymorphisms was performed by Dr. Sian Ellard, under the supervision of Professor Andrew D Hattersley at the University of Exeter.

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ABSTRACT OF THESIS

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Name of Candidate: ***Dr Stewart C. Ferguson***

Address :

Postal Code:

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Type 1 diabetes mellitus is characterised by absolute insulin deficiency, chronic hyperglycaemia and intermittent hypoglycaemia consequent upon treatment with insulin. Severe hypoglycaemia, defined as hypoglycaemia sufficient to necessitate third party intervention for recovery, commonly complicates insulin therapy and repeated exposure may be detrimental to the brain. Microvascular disease, manifest as retinopathy, neuropathy or nephropathy, frequently complicates diabetes, the risk being related to long-term glucose control and increasing disease duration. Microvascular disease may also affect the cerebral circulation and could potentially compromise brain structure and intellectual performance. Type 1 diabetes commonly develops in childhood before full maturation of the central nervous system and the developing brain may exhibit relative vulnerability to damage as a consequence of exposure to severe hypoglycaemia, or the development of Diabetic Keto-Acidosis, in early childhood. Genetic factors influence the vulnerability of an individual to develop cognitive impairment following pathological processes known to disadvantage the central nervous system. Polymorphism of the Apolipoprotein-E gene has been identified as one such factor and is known to influence the prognosis and cognitive outcomes following a wide variety of cerebral insults.

The studies contained within this Thesis explore the long-term consequences the clinical factors described above on brain structure and the cognitive performance of young adults with Type 1 diabetes mellitus of long duration. The effects of polymorphism of the Apolipoprotein-E gene on the cognitive performance of young adults who have Type 1 diabetes mellitus are also evaluated.

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SUMMARIES OF STUDIES

SUMMARY OF STUDY 1

Objective

The childhood onset of type 1 diabetes (T1DM) may compromise intellectual development, and ability; children who develop T1DM aged ≤7yrs more frequently exhibit cognitive impairment, of which the pathogenesis and structural brain associations are unknown. Whether the pathogenesis relates to psychosocial and educational factors, or to diabetes and its complications, remains unresolved. We hypothesised that if neuroradiological abnormalities were present in association with differences in cognitive ability then an organic aetiology to the "early onset" effect was likely.

Methods

The effects of diabetes onset age on cognitive ability (neuropsychological test battery) and brain structure (magnetic resonance imaging) were examined in a cross-sectional evaluation of 71 young adults with long-duration T1DM diagnosed during childhood or adolescence. Severe hypoglycaemia exposure, retinopathy status and diabetes duration were also examined as possible correlates of cognitive and brain structure differences. No participants had previous neuropsychological pathology.

Results

In those who developed T1DM aged ≤ 7yrs current intellectual ability (WAIS-R performance IQ, $p=0.03$, $Eta^2=0.09$) and information processing ability (Choice Reaction time, $p=0.006$) were comparatively poorer than in those whose diabetes onset was later. Furthermore, lateral ventricular volumes were greater ($p=0.002$, $Eta^2=0.16$, MANCOVA) and ventricular atrophy (60% vs. 20%, $p=0.01$, log regression) was more prevalent in comparison with those diagnosed with T1DM aged >7yrs.

Conclusions

The onset of T1DM in early childhood was associated with modest differences in intellectual performance in adulthood and mild central brain atrophy. The pathogenesis of the differences observed in those with early onset T1DM remains unknown but is likely to have an organic aetiology.

SUMMARY OF STUDY 2

Objectives

Type 1 diabetes (T1DM) is characterised by intermittent severe hypoglycaemia, chronic hyperglycaemia and the development of microangiopathy, and commonly develops before neurodevelopment is fully completed. The diabetes-specific factors may impact upon brain structure. We aimed to determine the effects of these factors on the brain structure of young adults with T1DM.

Methods

133 normotensive people with T1DM [mean (range): age 32.3yrs (20-45), 51.9% male, diabetes duration 16.1yrs (9-31), onset age 16.1yrs (1-36; 19.8% diagnosed <8yrs), 36.1% background retinopathy] participated in a cross-sectional study. Participants had no neuropsychological pathology and varied by their severe hypoglycaemia exposure and microangiopathy status. Correlation identified associations between diabetes-related factors (severe hypoglycaemia, retinopathy, diabetes duration, diabetes onset age) and brain structure (volumetric MRI analysis). Linear Modelling (brain volumes) and Log Regression (atrophy, white-matter abnormalities) techniques identified independent predictors of outcomes.

Results

Mild ventricular atrophy was common (30.8%) but Sulcal atrophy (10.5%) was infrequent. An early diabetes onset age (< 8yrs) independently predicted ventricular atrophy (p=0.01, Log Regression) and greater Lateral Ventricular volumes (p=0.01, MANCOVA). Associations between diabetes-specific factors and volumetric measures were not observed. Peri-ventricular white-matter lesions were common (60%), mild and unrelated to diabetes-specific factors. Small Punctate White-Matter Lesions (SPWML) were common (45.1%), located in distinct brain areas [Hippocampus (20.3%), Basal Ganglia (39.3%), Centrum Semi-Ovale (31.1%)] and correlated with several diabetes-specific factors (retinopathy, onset age, duration). Logistic regression identified diabetes onset age as an independent predictor of Hippocampal (p=0.004) and Centrum Semi-

Ovale (p=0.01) SPWML and the overall presence/absence of SPWML (p=0.02): SPWML were more frequent in those with later onset diabetes. Retinopathy status independently predicted Hippocampal SPWML (p=0.03, Log Regression). No independent associations between diabetes duration, severe hypoglycaemia and SPWML were identified.

Conclusions

Subtle brain structural abnormalities are common in young adults with T1DM. These are characterised by mild brain atrophy, mild peri-ventricular white-matter abnormalities and a specific type of white-matter lesion, the Small Punctate White-Matter Lesion. Early diabetes onset appears to be the most important diabetes-specific determinant of the differences observed, predisposing to central brain atrophy yet conferring relative resistance to the development of white-matter abnormalities, particularly SPWML. Intracranial microangiopathy, as background retinopathy, may also predispose to SPWML in specific brain regions, whereas diabetes duration and severe hypoglycaemia exposure do not appear to confer an obvious disadvantage to brain structure in a young adult population with T1DM, within the sensitivity of the neuroimaging methodologies utilised.

SUMMARY OF STUDY 3

Objective

The ε4 allele of the Apolipoprotein-E (APOE) gene is associated with poor outcome following various cerebral insults. The relationship between APOE genotype and cognitive function in patients with type 1 diabetes is unknown. We aimed to determine whether the presence of the APOE ε4 allele was associated with cognitive disadvantage in people with Type 1 diabetes.

Methods

In a cross-sectional study of 96 people with Type 1 diabetes, subjects were APOE genotyped, previous exposure to severe hypoglycaemia was estimated by questionnaire and cognition assessed by neuropsychological testing. Cognitive abilities were compared using multivariate general linear modeling (MANCOVA) in those with (n=21), and without (n=75), the APOE ε4 allele.

Results

APOE ε4 selectively influenced cognitive ability in a gender-specific manner ($F=2.3$, $p=0.044$, $Eta^2=0.15$); women with APOE ε4 performed less well on tests of current, non-verbal intellectual ability (Wechsler Adult Intelligence Scale–Revised performance test score, $p=0.001$, Eta^2 0.26) and frontal lobe and executive function (Borkowski Verbal Fluency, $p=0.016$, $Eta^2=0.15$). Previous exposure to severe hypoglycaemia did not interact with APOE ε4 to produce cognitive disadvantage.

Conclusion

The APOE ε4 genotype is associated with specific cognitive disadvantage in young women with type 1 diabetes. APOE ε4 is unlikely to mediate susceptibility to hypoglycaemia-induced cognitive disadvantage

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Since the introduction of insulin to clinical use in 1921 it has been readily apparent that an excessive dose of insulin can lower the plasma glucose concentration to a sub-normal concentration resulting in hypoglycaemia, potentially to a sufficient depth of hypoglycaemia such that the supply of glucose to the brain becomes limited and temporary impairment of normal neurological functions can develop. The clinically obvious presentation of neurological compromise accompanying intermittent episodes of hypoglycaemia in patients with Type 1 diabetes has encouraged many researchers to explore the long-term consequences of these easily apparent and clinically dramatic episodes, under the hypothesis that such episodes of hypoglycaemia may have long-term detrimental sequelae for the central nervous system. A substantial body of literature now exists which details the consequences of hypoglycaemia upon the function of the brain, as evaluated by neuropsychological and neurophysiological tests, the structure of the brain as determined by neuroimaging techniques and the neuropathological sequelae as determined by histopathological techniques.

Traditionally, the clinical complications of Type 1 diabetes, which are manifest as microvascular disease, have been thought to spare the microvasculature of the central nervous system, such that the brain has not been considered as a primary target organ for the development of diabetes-related microvascular damage (Plum F, 1960). Certainly, when compared to the detrimental effects that Type 1 diabetes can inflict upon other organs, in particular the microvasculature of the retina, peripheral nervous system and the kidneys, the brain does at first inspection appear relatively resistant to clinically apparent disadvantage. However, as the retinal circulation is derived from the same network of blood vessels as the remainder of the intra-cranial vasculature this is perhaps an illogical assumption and subtle abnormalities of cerebral microvessels, similar to those observed in the retinal circulation, have been observed in the brains of those with Type 1 diabetes (Johnson et al., 1982). An increasing body of work, ranging from pre-clinical cell

culture and animal work, through to human research has examined the impact of Type 1 diabetes in the human brain. The published literature in humans consists of case reports, cross-sectional and prospective clinical studies which have utilised a variety of neuropathological, neurophysiological, neuropsychological and neuroradiological techniques and have provided supportive evidence for the existence of a "Diabetic Encephalopathy". The introductory section of the present thesis summarises the published data, with a particular focus on evidence accumulated from human studies, touching on pre-clinical studies where data from research in humans is unavailable.

1.2 DIABETES AND THE BRAIN: A HISTORICAL PERSPECTIVE

Type 1 diabetes mellitus is a chronic disorder characterised by absolute insulin-deficiency, which can be acutely associated with intermittent metabolic decompensation, in the form of hypoglycaemic or hyperglycaemic crises, and the long-term development of microvascular complications traditionally reported to target the microvasculature of the retina, peripheral nervous system and the kidneys. However, it has long been recognised by the medical literature that Type 1 diabetes mellitus may also be associated with central nervous system abnormalities. Indeed, the association between "diabetes mellitus of the juvenile type" and abnormalities of the central nervous system was first reported by DeCalvi in 1864 (Marchal (deCalvi), 1864). DeCalvi observed neuropathological changes at post-mortem in a young patient with "diabetes of the juvenile type" who had exhibited a variety of neurological symptoms during life. DeCalvi hypothesised that the brain abnormalities observed in this case may be the cause of diabetes, or more likely represent a consequence of the disorder itself (Marchal (deCalvi), 1864). Seegen et al reported corroborative neuropathological findings in a similar case report published in 1893 (Seegen J, 1893) but reports dwindled thereafter. Following the discovery of insulin by Banting and Best in 1921 and its early clinical utilisation it became quickly apparent that insulin therapy came with the potential for serious side-effects, in the form of a propensity towards severe

hypoglycaemia. By 1922 Woodyatt and colleagues had reported a series of fatalities as a consequence of severe hypoglycaemia secondary to the use of insulin therapy in juvenile-type diabetes (Woodyat RT, 1922). Wohlwill (1928) first provided a detailed account of the neuropathological changes following a fatal case of hypoglycaemia where widespread brain and meningeal softening was observed (Baker AB, 1939). Baker (1939) and Lawrence (1942) subsequently reviewed the neuropathological literature published in the 1920's and 1930's (Baker AB, 1939;Lawrence RD et al., 1942) and identified a key pattern of selective neuropathological abnormalities following severe hypoglycaemia: selective loss of neurones predominantly in layers 2/3 of the neocortex, the basal ganglia and to a lesser extent the dentate nucleus of the cerebellum. In 1943 Murphy and Purtell summarised the literature pertinent to descriptions of permanent impairment of cerebral function in diabetic survivors of severe hypoglycaemia: personality changes and intellectual decline sometimes to the extent of "idiocy" were reported (Murphy FD and Purtell J, 1943), with similar reports observed by Fineberg who reported permanent neurological damage in a survivor of severe hypoglycaemia (Fineberg S.K. and Altschul A., 1952). Fischer and Dolger thereafter suggested that cumulative exposure to severe hypoglycaemia may be deleterious to intellectual capacity (Fischer AE and Dolger H, 1946), a concept which Jones et al furthered by positing the existence of a 'post-hypoglycaemia encephalopathy' in 1947. However, it was not until 1950 that DeJong (DeJong, 1977) proposed the existence of a "diabetic encephalopathy" as a clinical complication of Type 1 diabetes, having observed multiple cerebral abnormalities at post-mortem in a young patient with long-duration Type 1 diabetes, which were not solely in keeping with the pattern of hypoglycaemia-related neuropathological damage previously summarised by Baker (1939) and Lawrence (1942), and further explored by Lawrence in 1952 (Lawrence RD et al., 1942). Alex et al (Alex et al., 1962) reported a higher prevalence of cerebro-vascular disease at post-mortem in those with diabetes mellitus and Grunnet and co-workers reported an earlier age of onset of cerebro-vascular disease in subjects who had Type 1 diabetes mellitus (Grunnet, 1963), each inferring that macrovascular

disease of the cerebral circulation may also contribute central nervous system abnormalities in Type 1 diabetes, even in chronologically young individuals. In 1961 Ack and colleagues identified an early childhood onset of diabetes as a further potential aetiological factor to any detrimental effects that Type 1 diabetes may confer upon the brain. Ack identified significantly poorer scores on a test of general intelligence in children who had developed Type 1 diabetes before their 5th birthday relative to their siblings, observing that those diagnosed in early life bore a greater cumulative exposure to acute metabolic disturbances in the form of hypoglycaemia or keto-acidosis. Reske-Nielsen et al (1963) described multiple neuropathological and histological findings in three cases of "juvenile-type diabetes mellitus" and then extended the scope of the initial study to perform a more detailed examination of the neuropathological consequences of juvenile-type diabetes and its associated clinical complications in a post-mortem examination of a series of 16 patients (Reske-Nielsen and Lundbaek, 1963). Reske-Nielsen observed gross pathological differences affecting gray matter, white-matter and the lepto-meninges co-existent with multiple histopathological abnormalities, some of which were in keeping with diabetes complications in other tissues. However, the cohort evaluated by Reske-Nielsen had extensive co-morbidity and included patients with a long duration of diabetes generally diagnosed in early childhood, advanced microangiopathic complications, previous exposure to severe hypoglycaemia, uraemia and overt psychiatric symptomatology. The post-mortem observations could not be directly correlated to the presence of any particular clinical complications of Type 1 diabetes and Reske-Nielsen and colleagues concluded that multiple different pathogenic factors may be responsible for the gross changes described at autopsy. Bale used neuropsychological tests to evaluate the cognitive ability of people with Type 1 diabetes relative to healthy controls (Bale, 1973) using a test of short-term memory performance: lower scores were identified in participants with diabetes, with a significant association observed between lower scoring and previous hospital treatment for hypoglycaemic coma. Further studies provided data supportive of

Bale's observations but did not provide further insight as to causality (Franceschi et al., 1984;Meuter et al., 1980).

The early studies summarised above provided data to support the concept of a "Dabetic Encephalopathy" as a complication of Type 1 diabetes and prompted further research, aimed at dissecting which diabetes-specific factors may mediate the cerebral disadvantage reported in case reports and early cross-sectional studies. Factors identified as potential mediators of disadvantage by these early studies included recurrent exposure to Severe Hypoglycaemia, the development of Macrovascular and Microvascular complications, the onset of diabetes during early childhood neurodevelopment and a long disease duration of diabetes. Subsequent research has hypothesised that other factors, including those known to play a role in the development of peripheral neuropathy such as the accumulation of Advanced Glycation End products (AGE) or increased Aldose Reductase Pathway activity (Tesfaye et al., 2005) may also be of importance. Recent advances in genetic techniques have identified specific gene polymorphisms which may confer relative susceptibility to cerebral disadvantage as a consequence of exposure to many pathological processes known to affect the central nervous system. The following chapters summarise the pertinent literature which supports the validity of each of the potential mediators identified above.

CHAPTER 2

SEVERE HYPOGLYCAEMIA AND THE BRAIN

2.1 HISTORICAL PERSPECTIVE

The negative consequences of severe hypoglycaemia induced by exogenous insulin first became apparent shortly after the discovery and clinical utilisation of insulin as a treatment for Type 1 diabetes, as described above. As well as usage in Type 1 diabetes mellitus, Insulin was also used in non-diabetic individuals, as "Insulin shock" therapy, a treatment administered for otherwise incurable psychoses and drug addiction to patients in the 1930's (Sakel, 1994). This procedure involved the induction of profound degrees of insulin-induced severe hypoglycaemia to produce protracted cerebral glucodeprivation and resultant hypoglycaemic coma. It soon became recognised that "Reversible coma" could be expected if the duration of profound hypoglycaemia was less than 30 to 60 minutes, whereas more protracted glucodeprivation was noted to result in neurological sequelae, permanent and "Irreversible coma", or in some cases death (Baker AB, 1939;Fazekas et al., 1951), leading to the withdrawal of "insulin shock" therapy from the psychiatric treatment armamentarium in the 1950's.

2.2 DEFINITION OF SEVERE HYPOGLYCAEMIA

Severe hypoglycaemia is clinically defined as any episode of hypoglycaemia of sufficient severity such that external third party assistance is necessary to facilitate recovery from the episode (1991;1997). This definition in not dependent on a specific blood glucose concentration but rather depends on the individual with diabetes having hypoglycaemia of sufficient severity to lose control and normal volition. The term "Severe hypoglycaemia" therefore encompasses a variety of episodes of hypoglycaemia, ranging in severity from "milder" episodes where a loss of volition to self-treat has occurred yet consciousness is maintained, through to more profound episodes of glucodeprivation which can result in coma and eventually seizure as the blood glucose concentration falls further.

2.3 FREQUENCY OF SEVERE HYPOGLYCAEMIA

The frequency of severe hypoglycaemia in Type 1 diabetes varies according to the population sampled but prospective reports vary from 35 episodes per 100 patient-years (Donnelly et al., 2005) in a UK patient group, to 62 episodes per 100 patient-years in the Diabetes Control and Complications Trial (DCCT) (1991), through to 170 events per 100 patient-years (MacLeod et al., 1993). Approximately one-third of insulin-treated patients will experience severe hypoglycaemia at least annually, one-third of whom will experience coma (10%), and one-third of whom will repeatedly experience severe hypoglycaemia on a recurrent basis (2-3%) (Tattersall RB, 1999).

2.4 SEVERE HYPOGLYCAEMIA AND BRAIN METABOLISM

2.4.1 Introduction

The human brain is a highly metabolically active tissue, estimated to have a metabolic and therefore has a high obligate demand for energy. Despite only representing approximately 2% of body weight, the human brain utilises approximately 20% of the oxygen delivered via the lungs, receives 15% of cardiac output and metabolises 50-80% of circulating glucose in the fasting state (Sokoloff, 1991). The processes responsible for this high obligate glucose consumption include the biosynthesis and re-uptake of neurotransmitters, the generation of neuronal action potentials, the maintenance of intra- and extra-cellular ion concentration gradients, axoplasmic flow and protein synthesis, all of which are reliant on the oxidative metabolism of glucose for their energy demands (McCall, 2004).

2.4.2 Uptake of Glucose into the Brain

The adult human brain is almost exclusively dependent on glucose as its principal fuel and has a limited capacity to utilise alternative sources of fuel. The brain is therefore almost entirely dependent upon the delivery of a continual supply of glucose via the cerebral circulation and

rapidly dysfunctions when this is interrupted. Glucose is transported into the brain interstitium across the blood-brain barrier by the process of passive diffusion, a process which does not in itself consume energy, but which is facilitated by a specific glucose transport protein (GLUT 1) (McEwen and Reagan, 2004;Pardridge, 1991). Within the central nervous system other members of the GLUT superfamily of glucose transporters, most notably GLUT 3, appear to be responsible for facilitating the uptake of glucose by neurones, astrocytes and glial cells (McEwen and Reagan, 2004). Under normal metabolic conditions, where the plasma glucose concentration is strictly controlled within a tight reference range, the facilitated transport of glucose into the brain does not limit the rate of neuronal glucose metabolism. However, in the setting of Type 1 diabetes mellitus, where plasma glucose concentrations can fluctuate between subnormal (hypoglycaemia) or supra-normal concentrations (hyperglycaemia) the rate of facilitated glucose diffusion from the plasma into the brain can influence the rate of neuronal metabolism. During hypoglycaemia glucose transport across the blood-brain barrier becomes rate limiting resulting in neuroglycopenia with prompt deterioration in neuronal metabolism (McCall, 2004) and the development of cognitive dysfunction (Warren and Frier, 2005).

2.4.3 Glucose Metabolism in the Brain

As in other tissues Glucose undergoes oxidative metabolism via the Kerbs cycle to yield Adenosine Tri-Phosphate (ATP), the cellular energy substrate. Each molecule of Glucose yields 42 ATP molecules when oxidatively metabolised. The anaerobic metabolism of Glucose to Lactate can also occur which yields only 2 molecules of ATP per Glucose molecule.

2.4.4 Metabolic Consequences of Neuroglycopenia

As the transport of glucose into the brain occurs via carrier-mediated facilitated diffusion, rather than via an active pump system, the plasma glucose concentration is the main determinant of glucose availability for brain metabolism. As the plasma glucose falls during hypoglycaemia, the

concentration of glucose in the brain interstitium falls accordingly in a progressive manner. During severe neuroglycopenia, a state of partial cellular energy depletion develops where the concentration of Adenosine Tri-Phosphate (ATP), the principal energy molecule, falls to approximately one third of euglycaemic concentrations (Bischof et al., 2004). The partial neuronal energy depletion that ensues following severe hypoglycaemia is in contrast to the absolute energy failure that occurs during cerebral ischaemia, and may reflect the use of alternative fuels by the brain during times of limited glucose availability (see Section 2.4.5). Partial neuronal energy depletion has consequences for all of the active metabolic processes upon which brain functionality depends. During hypoglycaemia a compensatory switch to the catabolism of lipid and protein substrates occurs and cellular membrane pumps dysfunction, resulting in diffusion of cellular ions to equilibriate with their extracellular concentrations (Auer and Siesjo, 1993). The activity of the glycolytic pathway, which is responsible for the normal oxidative metabolism of glucose, is markedly suppressed due to lack of substrate availability, resulting in a fall in the concentration of Lactate and Pyruvate. Consequent upon this the activity of the Krebs cycle is also minimal, such that the normal accumulation of hydrogen ions that accompanies cellular metabolism does not occur, a degree of intracellular alkalosis develops and Aspartate accumulates within neurones (Auer and Siesjo, 1993).

The term 'excitotoxic brain injury' was first proposed by Olney and colleagues in 1969 following their observation that the application of the stimulatory neurotransmitter Glutamate to brain tissue resulted in selective neuronal necrosis in mice (Olney, 1969). Glutamate and Aspartate are the two major excitatory neurotransmitters in the human brain and each bind to *N*-Methyl-D-Aspartate (NMDA) receptors on post-synaptic membrane on the neuronal dendrite to produce downstream signalling and cellular effects. During neuroglycopenia failure of neuronal and glial cellular membrane pumps results in leakage of excitatory neurotransmitters into the synaptic cleft which combined with a lack of active neurotransmitter re-uptake by the pre-synaptic neurone

results in prolonged accumulation of excitatory neurotransmitters in the synapse. Subsequent uncontrolled activation of stimulatory NMDA receptors results in the setting of partial neuronal energy depletion results in a large inward calcium current which triggers downstream signalling pathways triggering mitochondrial swelling, degradation and damage to the cellular cytoskeleton by free radicals, breakdown of the cell membrane and apoptosis if the duration of insult is protracted (Olney, 1994). During hypoglycaemia the excitatory neurotransmitter that accumulates in the synaptic cleft is predominantly Aspartate, rather than Glutamate which is typical of most other type of 'excitotoxic' brain injury *e.g.* ischaemia, status epilepticus. The pattern of cellular death associated with Asparate induced 'excitotoxic' cell death produces the characteristic neuropathological appearances associated with hypoglycaemic brain injury (Auer and Siesjo, 1993).

2.4.5 Alternative Fuel Use by the Human Brain

Despite near total dependence upon glucose for its entire metabolic demands the human brain has little capacity to synthesise glucose from precursors and has extremely limited carbohydrate stores, in the form of glial glycogen (Gruetter, 2003;Suh et al., 2007). The adult human brain also appears to have a limited capacity to utilise energy sources other than glucose to support its obligate metabolic requirements. Limited evidence suggests that the brain can utilise Ketone Bodies (Amiel et al., 1991;Veneman et al., 1994), Lactate (Maran et al., 1994;Maran et al., 2000;Smith et al., 2003), Amino Acids (Evans et al., 2004) and Lipids (Evans et al., 1998) to support brain metabolism, at least to a limited extent.

Lactate may be used as an alternative metabolic fuel by the brain, through its ability to be converted to Pyruvate by the enzyme Lactate Dehydrogenase. The entry of Lactate into the brain is facilitated by the Monocarboxylate Transporter protein (MCT1), a protein which is developmentally regulated (Vannucci and Vannucci, 2001). Lactate can partly compensate for

neuroglycopenia and has been estimated to provide approximately one-third of the energy required to support brain metabolism in the neonate (Vannucci and Vannucci, 2001) and a continuous intravenous infusion of Lactate has been demonstrated blunt the degree of hypoglycaemia-induced cognitive dysfunction and counter-regulatory hormone release in adult humans (Smith et al., 2003). In both of these settings the availability of Lactate can only partly compensate for neuroglycopenia and cerebral dysfunction is not reversed.

Following a period of adaptation, the oxidative metabolism of Ketone Bodies can chronically provide two-thirds of the cellular energy necessary to support brain metabolism during periods of prolonged fasting (Owen et al., 1967), and infusion of Ketone Bodies during experimentally induced hypoglycaemia has been shown to blunt, but not prevent, the decrement in cognitive performance and counter-regulatory hormone release that is characteristically observed during hypoglycaemia (Amiel et al., 1991). More recently, it has become apparent that astrocytes, a type of glial cell which supports neurones, have keto-genic abilities and could potentially shuttle Ketone Bodies to neurones during times of glucodeprivation (Guzman and Blazquez, 2001).

The ability of the human brain to utilise alternative energy sources appears to be developmentally regulated. In the neonatal period the brain appears to be more flexible in fuel utilisation. During starvation, such as periods between suckling, ketone bodies can be readily utilised to maintain neuronal metabolism. During periods of hypoglycaemia the neonatal brain appears to be able to utilise amino acids, pyruvate, lactate and free fatty acids, all of which are transported into the central nervous system by the Monocarboxylate Transporter protein (MCT1), a transporter appears to be highly expressed in early life but decreases around the time of weaning (Vannucci and Vannucci, 2001). However, recent evidence suggests that a degree of cerebral metabolic adaptation may occur in adults with Type 1 diabetes who appear to have an enhanced ability to

transport and cerebrally metabolise Acetate which is transported into the brain by MCT1, which is double that of the non-diabetic (Mason et al., 2006).

For several reasons, the above discussion of alternative substrate use by the human brain may be of academic interest rather than having practical meaning and applicability. Firstly, hypoglycaemia in the individual with diabetes is induced by the presence of excessive concentrations of insulin: hyperinsulinaemia potently inhibits the synthesis of Ketone Bodies, Free Fatty Acids and Amino Acids, the very precursors which are necessary to support brain metabolism during times of neuroglycopenia. Secondly, the entry of alternative fuels into the adult human brain may be limited. Amino Acids, Pyruvate, Lactate and Free Fatty Acids are transported into the central nervous system by the Monocarboxylate Transporter protein (MCT1), a transporter which appears to be highly expressed in early life but which decreases around the time of weaning (Vannucci and Vannucci, 2001). The developmental expression of this transported may partly account for the flexibility of substrate usage apparent in the neonatal period.

2.5 NEUROPATHOLOGICAL STUDIES OF SEVERE HYPOGLYCAEMIA

Hypoglycaemic brain damage is characterised by 'selective neuronal necrosis' with the distribution of neuronal necrosis determined by the expression of the NMDA receptor on the dendrite of the neurone. This selective apoptosis generally spares some cellular populations within the brain but targets neurones and glia. However, all cellular types are affected if the duration of profound hypoglycaemia is protracted. This has been demonstrated in the rodent model of hypoglycaemic brain injury where neuronal necrosis occurs only after the Electro-Encephalogram (EEG) has been iso-electric for at least 10 minutes and continued durations of profound glucodeprivation beyond this threshold result in greater necrosis, beginning with

NMDA expressing neurones and glia, but developing into non-specific generalised necrosis thereafter which is difficult to distinguish from ischaemic brain injury (Auer and Siesjo, 1993).

Characteristic histopathological appearances have been described following hypoglycaemic brain injury in laboratory animals and humans: neurones in layer 2 of the cerebral cortex appear to be most vulnerable (Auer et al., 1989;Lawrence RD et al., 1942;Patrick and Campbell, 1990) along with the Hipocampus, particularly neurones in the dentate gyrus (Auer et al., 1985a), with relative sparing of cerebellar Purkinje cells(Kalimo and Olsson, 1980;Lawrence RD et al., 1942;Patrick and Campbell, 1990), the brain stem and white matter (Auer et al., 1985c;Auer et al., 1985b;Patrick and Campbell, 1990). The pattern of histopathological necrosis is thought to represent NMDA receptor expression (Olney, 1994) and exposure of those neurones in close contact with CSF to secondary 'excitotoxic' injury following diffusion of neurotransmitters (Auer and Siesjo, 1993).

2.6 SEVERE HYPOGLYCAEMIA, COGNITION AND INTELLIGENCE

2.6.1 Case Reports

There are many case reports in the medical literature which report permanent cognitive impairment and disability as a consequence of exposure to protracted and profound degrees of severe hypoglycaemia. Such protracted and profound episodes of severe hypoglycaemia are rare and their damaging effect on the central nervous system is without doubt. The importance of these initial case reports is that they stimulated many research groups to investigate the consequences of the more commonly encountered recurrent exposure to episodic severe hypoglycaemia in a more structured and analytical manner. A detailed summary of these initial case reports will not be provided as a sufficient body of structured evidence has now superseded these early published reports.

2.6.2 Cross-Sectional Studies

A number of cross-sectional studies have explored the relationship between previous exposure to severe hypoglycaemia and subsequent cognitive ability as determined by neuropsychological tests.

Bale first reported an association between a history of hospital treatment for hypoglycaemic coma and poorer mental abilities (Bale, 1973). Bale evaluated performance on a test of word learning, the Walton Black New Word Learning Test, in people with Type 1 diabetes relative to healthy controls (Bale, 1973): lower scores were identified in participants with diabetes, with a significant association observed between lower scoring and previous requirement for hospital treatment of hypoglycaemic coma. However, Bale did not assess the pre-morbid ability of participants and the differences observed between could reflect differing pre-morbid functional level between the Type 1 diabetes group and healthy controls. Bale's conclusions that "Brain Damage in Diabetes Mellitus" was related to exposure to severe hypoglycaemia were at best overstated and perhaps misleading. Skenazy et al applied a battery of cognitive ability tests in a case-control study which evaluated the abilities of a small number of people with Type 1 diabetes (n=19) relative to healthy controls and a chronic illness control group: poorer performance on the Weschler Performance IQ subtests, which measure current intellectual performance, and a complex test of cognitive flexibility and visuomotor scanning was observed in the diabetes group. Furthermore, the authors identified an association between the frequency of preceding severe hypoglycaemia, retrospectively determined from case records, and poorer Performance IQ scores (Skenazy and Bigler, 1984). Similar caveats apply to these observations as pre-morbid ability was not evaluated. Wredling examined a small group of patients with Type 1 diabetes (n=17) pre-selected from a group of 600 patients due to their high cumulative severe hypoglycaemic burden, and compared neuropsychological performance with an identical number of control subjects with

Type 1 diabetes who were naïve to severe hypoglycaemia. Groups were matched with respect to diabetes-specific factors, educational level and socio-economic estimates, to provide roughly matched groups with respect to pre-morbid attainment (Wredling et al., 1990). Wredling observed lower scores in those who had experienced severe hypoglycaemia on tests of simple motor speed (finger tapping), short-term and associative memory and visuospatial tasks assessing ability in general problem-solving, but did not report differences in Trail Making performance or Performance IQ, with the exception of Digit Symbol Substitution scores. However, methodological limitations could still not separate whether the ability differences observed related to pre-morbid ability differences or reflected the consequences of severe hypoglycemia and recruitment bias also seemed possible. In a study of 100 patients with Type 1 diabetes diagnosed in adulthood Langan et al (Langan et al., 1991) explored the relationship between preceding exposure to severe hypoglycaemia and performance on a range of cognitive ability tests relative to pre-morbid ability, assessing the crystallised intelligence of participants with the National Adult Reading Test (NART) (Nelson and Willison, 1991): performance on the NART is highly resistant to the detrimental confounding effects of age and organic disease noted with many other tests of cognitive ability and reliably estimates the 'best ever' intelligence of an individual. Langan compared the performance of two sub-groups of patients, a group naïve to severe hypoglycaemia (n=23) and a group who had experienced in excess of five cumulative lifetime episodes (n=24). The frequency of preceding severe hypoglycaemia, as determined by a retrospective questionnaire, was the sole measure of estimated preceding exposure to severe hypoglycaemia that was associated with aspects of intellectual performance: significantly poorer performance of the order of 0.4 SD (6 IQ points) on the Performance IQ subtests of the WAIS-R and slower information processing speed (Choice reaction time, decision component) was observed in the hypoglycaemia-exposed group. Other aspects of cognitive ability, including Verbal IQ scores, tests of memory, attention and concentration and frontal and executive function appeared unaffected by preceding severe hypoglycaemia exposure (Langan et al., 1991). The

observations of Langan and colleagues were the first to robustly exclude disparities in pre-morbid ability, age, or diabetes duration as being responsible for the relatively modest performance differences observed between those exposed to severe hypoglycaemia and control groups. Sachon performed a similar examination of a variety of cognitive abilities in a cross-sectional study of three groups of patients matched for social class and age: a small healthy control group and 55 patients with Type 1 diabetes, 30 of whom had impaired awareness of hypoglycaemia and had experienced repeated episodes of severe hypoglycaemia, with the remainder naïve to severe hypoglycaemia (Sachon et al., 1992). Sachon reported poorer performance in four of the seven tests administered in those with Type 1 diabetes, relative to healthy controls, but also observed a significant performance difference on two ability tests, measuring word learning and verbal fluency respectively, where those previously exposed to severe hypoglycaemia performed significantly less well than those with Type 1 diabetes naïve to severe hypoglycaemia. Again, difficulties with pre-morbid ability assessment confound interpretation but the findings did infer cognitive disadvantage consequent upon severe hypoglycaemia. In a further evaluation of the cohort examined by Langan, Deary and colleagues extended the cognitive domains examined by their previous assessment and included 100 healthy volunteers as a control group (Deary et al., 1993). After controlling for pre-morbid IQ (NART) the diabetes participants exhibited modest but statistically significant lower WAIS-R scores for both Performance and Verbal IQ, equivalent to a 5 IQ point deficit. Further statistical adjustment for the preceding frequency of severe hypoglycaemia eliminated the differences observed in Performance IQ scores, suggesting that the differences observed between the diabetes and healthy control groups were accounted for by detrimental effects of severe hypoglycaemia (Deary et al., 1993). In 1996 Lincoln and colleagues replicated the earlier findings of Langan et al in an identically designed study of 70 patients with Type 1 diabetes which had developed in adulthood. Lincoln identified a modest correlation between a calculated IQ decrement, as the difference in Z-scores between pre-morbid ability and current performance, and preceding exposure to severe hypoglycaemia (Lincoln et al., 1996).

Cross-sectional studies exploring the hypothesis that repeated exposure to severe hypoglycaemia may be disadvantageous to aspects of human cognitive abilities have not been universally supportive. In a carefully designed and thorough study, Ryan *et al* evaluated a large group of patients with Type 1 diabetes (n=142) who had developed diabetes before the attainment of full intellectual maturity and compared performance across a number of cognitive domains with ability-matched controls (n=100) (Ryan et al., 1993). Sustained attention, visual scanning and decision making, mental flexibility, psychomotor speed and short-term memory were examined and multiple regression techniques were utilised to identify independent predictors of cognitive performance. Ryan did not identify any independent detrimental effects relating to preceding severe hypoglycaemia exposure across a broad selection of cognitive domains, although a statistically significant interaction between severe hypoglycaemia exposure and the presence of peripheral neuropathy was observed. However, this interaction predicted only a small additional amount of variance in Trail Making (mental flexibility) and Embedded Figures (visual scanning and rapid decision making) performance, the magnitude of which may be of statistical significance rather than of clinical importance (Ryan et al., 1993). Snoeke and Heine assessed aspects of attentional processes in a small study of 18 individuals free of microvascular complications who had developed Type 1 diabetes in adulthood, nine of whom had a history of recurrent severe hypoglycaemia (defined as greater than five lifetime episodes) with the remainder being naïve to severe hypoglycaemia, relative to nine healthy controls. No significant differences in a two-choice reaction time vigilance test or in WAIS-R Digit Span scores were observed between groups, leading the authors to conclude that the components of attention assessed were unlikely to be negatively affected by previous exposure to severe hypoglycaemia (Snoek et al., 1998). However, definitive conclusions are difficult to conclude from this underpowered study which only evaluated select aspects of attention abilities. Kramer and Grimm, utilising a strict definition of severe hypoglycaemia, studied the effects of hypoglycaemic

coma on aspects of cognitive ability and neuro-physiological measures in 108 adult subjects with Type 1 diabetes, of whom 55 had experienced severe hypoglycaemia (Kramer et al., 1998). Kramer utilised the Trail Making (Part A) Test, performance on which had been previously reported as being compromised by preceding severe hypoglycaemia, as well as the Mini Mental State Examination (MMSE), a structured screening test designed to detect the degree of cognitive impairment that could be reasonably expected in elderly individuals consistent with an underlying diagnosis of dementia. The MMSE is an insensitive test for the detection of subtle differences in cognitive ability of the type that could be reasonably expected, from published observations (Deary et al., 1993;Langan et al., 1991;Lincoln et al., 1996;Sachon et al., 1992;Wredling et al., 1990), to be associated with preceding exposure to severe hypoglycaemia in a young adult population. Unsurprisingly, Kramer did not identify significant differences in MMSE scores in relation to preceding hypoglycaemic coma, but did identify a trend towards poorer Trail Making performance in those exposed to coma (Kramer et al., 1998).

Each of the studies outlined above utilised retrospective techniques, usually as a combination of formatted questionnaires and case record validation, to estimate the preceding exposure to severe hypoglycaemia of those studied. Such methodologies are only capable of providing an estimate of the actual hypoglycaemic burden of an individual although these estimates do appear to correlate well with prospective measures, at least over a relatively short 18-month period (Deary et al., 1993). With this caveat, the majority of cross-sectional studies discussed above offer some support for the hypothesis that severe hypoglycaemia may disadvantage select aspects of cognitive ability in people who have Type 1 diabetes. In particular, those abilities evaluated by the WAIS-R Performance IQ sub-tests and measures of psychomotor speed may be the most sensitive to disruption. However, where performance differences were reported in the studies summarised above the magnitude of difference between those exposed and naïve to severe hypoglycaemia appeared modest at best. The largest cross-sectional study within this research

area, which was of robust design and sound interpretation, found no such association (Ryan et al., 1993), confirming that the hypothesis that episodic exposure to severe hypoglycaemia is harmful to intellectual abilities in adulthood remains contentious and certainly not proven.

2.6.3 Prospective Studies

Three prospective studies have examined the effects of repeated exposure to severe hypoglycaemia on cognitive ability in people who had developed Type 1 diabetes in adulthood. The Stockholm Diabetes Intervention Study was the first to prospectively evaluate the cumulative effects of severe hypoglycaemia exposure on a limited range of cognitive abilities (Reichard et al., 1991). In this study Reichard and co-investigators sequentially examined 97 patients over a 7.5 year period, randomised into two well-matched subgroups: one received intensified insulin therapy (IT, n=44), the remainder conventional insulin treatment (CT, n=53). The cumulative burden of severe hypoglycaemia experienced by participants over the duration of study was modest such that the two insulin treatment groups differed little in their experience of severe hypoglycaemia: 1.1 episodes per patient per annum was observed in the IT group relative to 0.4 episodes in the CT group. Furthermore, the battery of cognitive ability tests utilised by the researchers was limited to the assessment of simple reaction time, an assessment of short-term memory, visuo-spatial ability and frontal lobe function. Performance IQ and more detailed measures of psychomotor speed, identified as perhaps being vulnerable to deterioration following severe hypoglycaemia by cross-sectional reports, were not assessed in this study which was designed and executed before this knowledge was published. Reichard and co-investigators reported no significant changes in the cognitive domains examined with respect to severe hypoglycaemia exposure (Reichard et al., 1991). In addition to the limitations inherent in the cognitive domains examined, the study had limited power to detect differences in cognitive ability between groups. Because of these limitations the Stockholm Diabetes Intervention Study may not have been optimal to address its main neuropsychological aims. In contrast to these

limitations, the Diabetes Control and Complications Trial (DCCT) investigators prospectively evaluated the impact of repeated exposure to severe hypoglycaemia across a wide range of cognitive abilities using a thoughtfully constructed neuropsychological test battery. This landmark diabetes study prospectively evaluated 1441 participants who were randomised to receive either intensified insulin therapy (n=711) or conventional insulin treatment (n=730) over an assessment period spanning 6.5 years, equating to 9300 patient years of observation. This provided more than adequate statistical power to identify even modest changes in ability in association with severe hypoglycaemia. The DCCT studied young adults of greater than average intelligence, of higher than average social class, who had diabetes of relatively short duration, each of whom underwent neuropsychological evaluation on three separate occasions: after two, five and seven years from randomisation. The neuropsychological test battery provided 25 individual test scores which were summated into 8 subjectively determined cognitive domains labelled as problem solving, learning, immediate memory, delayed recall, spatial ability, attention, psychomotor efficiency and motor speed. Multiple regression analyses identified no measure of cumulative severe hypoglycaemia exposure as being a statistically significant independent determinant of performance within any cognitive domain. However, despite impressive statistical power to detect change, the DCCT excluded from study those who may could perhaps be considered to be at the greatest risk of developing cognitive disadvantage as a consequence their diabetes-related problems: those who had experienced in excess of two episodes of severe hypoglycaemic coma in the year leading up to study, or who were prone to repeated episodes of Diabetic Keto-Acidosis, or who had advanced microangiopathy. Through the application of these exclusion criteria the sensitivity of the DCCT to detect cognitive change as a consequence of any of the clinical complications associated with Type 1 diabetes is likely to have been truncated but this carefully executed prospective study does provide reassurance that intensified insulin treatment and its clinical bedfellow, recurrent severe hypoglycaemia, is not detrimental to the cognitive abilities of young patients with Type 1 diabetes who have a limited

microangiopathic burden (1996). The analytical methodologies utilised by the DCCT investigators in their approach to domain allocation were criticised by Deary et al who argued that the allocation of subtests to each of the 8 pre-defined cognitive domains was performed on an ad hoc basis rather than through robust statistical data reduction methodologies. Austin and Deary thereafter re-analysed the entire DCCT dataset utilising validated psychometric techniques. Principal components analysis identified four underlying key factors corresponding to spatial ability, memory, processing speed and verbal ability. However, despite the differing methodological approach to analysis this psychometric re-analysis did not identify any evidence to support the hypothesis that recurrent exposure to severe hypoglycaemia is detrimental to human cognitive abilities, at least in the patient group studied in the DCCT (Austin and Deary, 1999).

The EDIC Study, the on-going research product of the Diabetes Control and Complications Trial (DCCT) recently re-evaluated the effects of intensive insulin therapy on neuropsychological function in 1144 participants from the original DCCT cohort after a median duration of diabetes of 18 years. A detailed battery of neuropsychological tests were utilised which encompassed all recognised cognitive domains. The EDIC follow up validated the observations of the earlier DCCT paper which addressed neuropsychological function: no detrimental effect of repeated exposure to severe hypoglycaemia was detected despite 40% of participants having experienced coma due to severe hypoglycaemia (Jacobson et al., 2007).

In summary, there is no evidence from prospective studies in relatively young adults with Type 1 diabetes that severe hypoglycaemia is detrimental to cognitive abilities, although the prospective studies reported to date may not perhaps have evaluated those at the highest risk. The hypothesis that repeated exposure to moderate degrees of severe hypoglycaemia is damaging to cognitive abilities in adults with Type 1 diabetes remains contentious and unproven.

2.6.4 Meta-Analyses

Brands et al performed a meta-analysis of 33 studies in which the cognitive abilities of those with Type 1 diabetes were contrasted to those of healthy controls. Whilst the primary objective of this meta-analysis was to define and determine the presence and magnitude of any cognitive disadvantage associated with Type 1 diabetes *per se*, the authors also performed a meta-analysis of the eight studies, from which sufficient information was available for the purpose of meta-analysis, which evaluated the long-term sequelae of repeated exposure to severe hypoglycaemia on cognitive abilities. Cognitive abilities were categorised into an overall summary measure of cognitive performance and seven separate categorical sub-domains: intelligence, learning and memory, speed of information processing, attention, mental flexibility and visual perception. The authors concluded that evidence to support the existence of a negative effect of preceding severe hypoglycaemia exposure on overall cognitive ability, or on any of the cognitive domains examined, was lacking with the caveat that those with multiple diabetes complications were excluded from participation in published reports (Brands et al., 2005).

2.7 NEUROIMAGING ASSOCIATIONS OF SEVERE HYPOGLYCAEMIA

2.7.1 Case Studies

Severe hypoglycaemia has been recognised as being potentially injurious to the human brain since the discovery, purification and initial clinical usage of insulin. However, permanent neurological deficits or obvious cognitive impairment are extremely uncommon following a single episode of severe hypoglycaemia, most commonly being observed following alcohol ingestion (Arky et al., 1968), which disables effective hypoglycaemic counter-regulation, or consequent upon deliberate attempts at self harm. Those adults who go on to develop permanent neurological sequelae following a single episode of profound hypoglycaemia are almost always

comatose on presentation. Richardson and colleagues first described the neuroimaging sequelae of profound severe hypoglycaemia (Richardson et al., 1981). A comatose male patient with Type 1 diabetes complicated by profound severe hypoglycaemia and circulatory collapse was observed to have generalised infarction of cortical gray matter on CT neuroimaging in the days following initial presentation. However, it was not until the 1988 that further case reports detailing the neuroimaging consequences of exposure to a single episode of profound severe hypoglycaemia began to emerge, at a time when Magnetic Resonance Imaging (MRI) techniques were in their relative infancy. Meer et al first described the MRI neuroimaging associations of severe hypoglycaemic coma in a 33 year old woman with Type 1 diabetes. An initial non-contrast enhanced CT scan of the brain identified no abnormalities but subsequent contrast-enhanced CT neuroimaging identified transient enhancement in the region of the right Putamen. This corresponded to identically placed high-intensity signal abnormalities subsequently observed on MRI of the same case (Meer et al., 1988). In another patient with Type 1 diabetes, Chalmers et al thereafter reported high-intensity signal abnormalities on T1-weighted MRI images in the left hippocampus and medial temporal lobe following an episode of protracted severe hypoglycaemia. The patient exhibited a permanent short-term memory deficit and a persistent Hippocampal abnormality which remained evident 6-months later when the MRI examination was repeated (Chalmers et al., 1991). Koppel reported the development of a transient left internal capsule hypodensity visible on CT scanning of the brain in a middle-aged patient with Type 1 diabetes who developed severe hypoglycaemia whilst fasting pending a procedure whilst a hospital in-patient (Koppel and Daras, 1993). Boeve et al reported a further case following coma induced by severe hypoglycaemia in which a male with Type 1 diabetes developed bilateral hippocampal high-intensity signal lesions evident on MRI, with scattered abnormalities also evident in the temporal and occipital gray matter. These abnormalities were not visible on CT scanning and the patient developed permanent cognitive impairment with prominent learning and memory deficits (Boeve et al., 1995). Holemans et al reported a similar case where bilateral Hippocampal

hyperintensities were observed on T2-weighted MR images in a young male with Type 1 diabetes who also experienced learning and memory deficits subsequent to hypoglycaemic coma. Yet again, no abnormalities were observed on initial CT brain imaging in this case (Holemans et al., 2001). Gold and co-workers thereafter reported a case of permanent cortical blindness and marked cognitive impairment following severe hypoglycaemic coma in a young woman with Type 1 diabetes. Initial CT imaging in this case identified no abnormalities but repeat CT scanning 4 months later revealed severe global cerebral atrophy with low attenuation areas suggestive of infarction of the posterior parietal lobe (Gold and Marshall, 1996). Perros et al described permanent pontine dysfunction in association with an abnormal high-intensity lesion in the pons following hypoglycaemic coma (Perros et al., 1994) and Rajbhandari and colleagues observed similar radiological changes consistent with central pontine myelinolysis in a further case report of pontine dysfunction following hypoglycaemic coma (Rajbhandari et al., 1998). Bakshi reported the presence of high-intensity signal lesions in the caudate and Putamen in a 41 year old woman with Type 1 diabetes following severe hypoglycaemic coma which subsequently proved fatal. No abnormalities were apparent on CT imaging, MRI abnormalities were subtle on T2-weighted images but were readily apparent when FLAIR sequences were utilised, inferring a greater sensitivity for this sequence in detecting hypoglycaemia-related lesions relative to the other MRI sequences utilised (Bakshi et al., 2000).

Finelli and colleagues first utilised Diffusion-Weighted Imaging (DWI) techniques in their evaluation of hypoglycaemia-related brain damage (Finelli, 2001). DWI is an advanced MRI echo planar technique, which allows contrast to be applied across relative proton diffusion gradients, thereby highlighting areas where proton diffusion is increased or restricted relative to the surrounding normal brain tissue. DWI is capable of highlighting focal areas of cerebral energy depletion and cytotoxic oedema and has been demonstrated to be superior to conventional MRI neuroimaging methodologies in the early diagnosis of stroke, particularly in identifying

early ischaemia or small foci of ischaemia. Finelli reported a fatal case of hypoglycaemic coma in a middle-aged male who in addition to Type 1 diabetes, had other co-morbidity known to impact upon the central nervous system in the form of HIV and a history of previous intravenous substance misuse. An initial CT brain scan in this case was unremarkable, but 4 hours later a further CT brain scan revealed subtle reduced attenuation in the basal ganglia. MRI incorporating DWI at 48 hours revealed widespread bilateral high-signal intensity in the basal ganglia, hippocampi and neocortex, whereas standard T2-weighted images revealed only the basal ganglia changes, and T1-weighted images were unremarkable (Finelli, 2001). Chan described a further case of fatal hypoglycaemic coma complicating Type 1 diabetes in a middle-aged male in which MRI neuroimaging was performed. Consistent with Finelli's findings T1-weighted sequences were unremarkable, T2-weighted images revealed subtle areas of increased signal, whereas DWI revealed diffusion restriction in the cerebral cortex, most marked in the temporo-occipital areas and basal ganglia (Chan et al., 2003). Yoneda and colleagues described a case of fatal hypoglycaemic coma complicating Type 1 diabetes in which diffusion-weighted MRI appearances suggested widespread laminar necrosis of the neocortex, most prominently in the frontal lobes. DWI identified the gross widespread cortical abnormalities, similar to those observed by Chan et al (2003) whereas T2-images revealed only subtle areas of high signal intensity and T1-weighted images were normal (Yoneda and Yamamoto, 2005). Maekawa and colleagues described a case of severe hypoglycaemic coma in a middle-aged male with Type 1 diabetes in which transient MRI abnormalities and clinical recovery were observed. Initial CT scanning of the brain revealed slight cerebral atrophy, as did repeat CT at 48 hours, whereas MRI evaluation at the time of the second CT revealed multiple abnormalities apparent on T2, FLAIR and DWI in the occipital cortex and basal ganglia. The temporal evolution of the MRI changes revealed a diminution of DWI abnormalities by day 7 and near resolution by day 14, whereas the T2-weighted and FLAIR high-intensity lesions remained apparent (Maekawa et al., 2006). A

similar temporal evolution of MRI abnormalities was previously described in a case of hypoglycaemic coma complicating Type 2 diabetes (Aoki et al., 2004).

The generalised pattern of brain injury reported in the neuroimaging case studies summarised above, which demonstrate cortical gray matter injury, followed by injury to deep gray matter structures such as the hippocampus and basal ganglia, thereafter by damage to the midbrain before finally involving the cerebellum and spinal cord appears to be consistent with the neuropathological observations from animal and human post-mortem work (Auer and Siesjo, 1993).

These cases also identify beyond doubt that profound severe hypoglycaemia can be permanently damaging to the adult brain in Type 1 diabetes. In addition to this somewhat obvious conclusion, the neuroimaging studies give some insight to the relative sensitivity of the radiological techniques utilised in the evaluation of hypoglycaemia-related brain damage. Uncontrasted Computerised Tomography (CT) appears a relatively insensitive test for the evaluation of early hypoglycaemia-related brain injury, at least in the first 24-48 hours following the brain insult. Contrast enhanced CT techniques appear to offer greater sensitivity but consistently appear inferior to MRI methodologies, although both techniques were not employed simultaneously in the majority of cases summarised above. Many of the reports which MRI techniques demonstrated abnormalities also utilised CT scans. In many of the above cases CT techniques failed to identify any focal abnormalities in association with severe hypoglycaemia (Chalmers et al., 1991;Finelli, 2001;Holemans et al., 2001;Maekawa et al., 2006;Perros et al., 1994;Yoneda and Yamamoto, 2005) when abnormalities were readily apparent on MRI. These observations infer that MRI may offer greater sensitivity in detecting subtle structural brain changes related to severe hypoglycaemia. Some forms of MRI neuroimaging may be more sensitive in detecting more subtle degrees of hypoglycaemia-related brain injury. Case reports infer that DWI may be

the present optimal technique in the acute assessment of hypoglycaemic brain injury, followed by FLAIR, T2-weighted, T1-weighted images, contrast CT and finally CT in descending order of sensitivity. This hierarchical interpretation may be over simplistic for two reasons. Firstly, the timing of neuroimaging studies in many of the cases may partly confound the interpretation offered above: many of the cases had CT brain scans performed on admission with MRI techniques performed days to weeks later. However, the more recent case described by Maekwa and colleagues (Maekawa et al., 2006) may mitigate against this as a confounder and does infer true superiority for MRI based techniques. Secondly, selection bias may have occurred as only those cases in which initial CT imaging was negative or inconclusive in the face of neurological compromise would have been likely to have MRI neuroimaging, thereby potentially adding bias in the favour of these techniques.

Irrespective of the relative sensitivity of the MRI techniques outlined above, the presence of structural brain abnormalities on MRI appears to be indicative of a poor prognosis. However, reporting and publication bias could confound this interpretation.

2.7.2 Cross-Sectional Studies

Only two cross-sectional studies have attempted to delineate the structural brain correlates of repeated exposure to severe hypoglycaemia in people with Type 1 diabetes. In a small underpowered cross-sectional study Lunetta and co-workers examined brain structure using MRI in ten patients with Type 1 diabetes, who had a relatively short median duration of diabetes, variable hypoglycaemia exposure and differing degrees of microangiopathy and contrasted the appearances observed with a control group (Lunetta et al., 1994). The MRI sequences utilised included T1 and T2-weighted images and post-scan analysis included calculation of the cerebro-ventricular index (CVI), a surrogate measure of dilatation of the lateral ventricles. Lunetta reported a significantly increased CVI in 70% of the diabetes group, as well as a degree of

subjectively-rated cortical atrophy. The manuscript provides scant demographic details of the control group, may rely on a definition of an elevated CVI that is not robust, and reported no significant statistical correlation between preceding history of severe hypoglycaemia and the presence of central brain atrophy, defined by the relatively greater CVI observed in the diabetes group. However, the study was underpowered to address any of its primary aims and its negative findings should be viewed accordingly. Perros et al examined 22 participants of a previously published cohort (Langan et al., 1991), in whom the preceding frequency of severe hypoglycaemia was associated with poorer current intellectual performance (WAIS-R Performance IQ). This modestly sized study examined MRI brain structure in two equally sized (n=11) well-matched groups of adult participants with Type 1 diabetes of long duration; a group naïve to severe hypoglycaemia and a group with more than five cumulative episodes of severe hypoglycaemia. Perros identified two types of structural brain abnormalities in those with Type 1 diabetes: high-intensity lesions in the peri-ventricular white matter and generalised cortical atrophy. Cortical atrophy was observed more frequently (45.5% vs. no cases) in those with a previous history of exposure to severe hypoglycaemia and the presence of any structural brain abnormality (defined as either white matter lesions or atrophy) was also significantly more frequent in this group (63.6% vs. 18.2%) (Perros et al., 1997). The preliminary observations of this modestly sized pilot study were the primary driver for the subsequent neuroimaging studies presented in this thesis.

2.7.3 Prospective Studies

No studies have been published to date that have attempted to prospectively evaluate the structural brain correlates of repeated exposure to severe hypoglycaemia in people with Type 1 diabetes.

CHAPTER 3

CHILDHOOD ONSET TYPE 1 DIABETES AND THE BRAIN

3.1 HISTORICAL PERSPECTIVE

Since 1961 it has been recognised that the onset of Type 1 diabetes in early childhood may confer disadvantage upon the development and attainment of certain intellectual abilities (Ack et al., 1961). Ack's seminal cross-sectional study identified significantly lower general intelligence scores relative to non-diabetic sibling controls in those who had developed T1DM before their 5th birthday, observing that those diagnosed in early life bore a greater cumulative exposure to acute metabolic disturbances in the form of hypoglycaemia or keto-acidosis. These metabolic decompensations of T1DM were suggested as potential organic mediators of the differences observed between cases and their sibling pairs. However, others have posited socio-educational factors, rather than the consequences of acute metabolic deteriorations, as being responsible for such differences. The following sections introduce the basics of structural brain development across childhood and adolescence before summarising the evidence base from neuropsychological and neuroradiological studies.

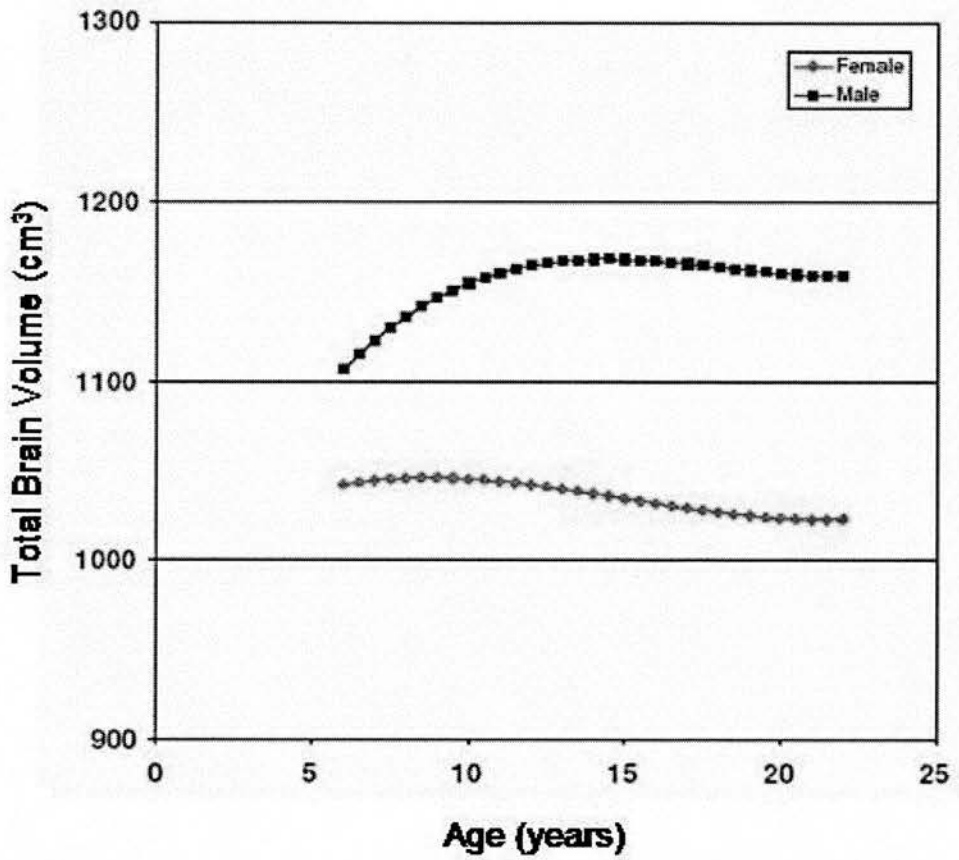
3.2 NEUROIMAGING OF NORMAL CHILDHOOD NEURODEVELOPMENT

Since the emergence of Magnetic Resonance Imaging (MRI) techniques in the late 1980's it has become possible to determine the neuroimaging associations of childhood and adolescent neurodevelopment in health and disease on a sequential basis, without the negative consequences incumbent upon previous X-ray based methodologies. MRI has allowed the chronological development of cortical gray matter, sub-cortical gray matter, white matter and the cerebral ventricles to be determined in detail and the use of computer-based volumetric analysis has provided further insights into the subtleties of regional neurodevelopment, details which were previously unknown. Using such techniques investigators have begun to explore the structural brain changes that accompany the development of cognitive abilities, identifying regional differences in the timing and rate of brain growth associated with cognitive maturation (Durston et al., 2001).

3.2.1 Whole Brain Volume and Neurodevelopment

At the time of birth the size of the human brain is disproportionate to that of the remainder of the body and the total brain volume of the newborn is approximately 80% of final adult volumes. In early childhood total brain volume steadily increases to attain 95% of peak volume at the age of 5 years with volume increases thereafter being limited to a further 5%. Despite this tailing off of cerebral enlargement neurodevelopment is far from complete (Lenroot and Giedd, 2006). After the age of five, subtle differential changes in the growth and regression of gray and white matter occurs. The processes underlying these relatively modest changes appear to reflect selective glial and neuronal apoptosis and ongoing myelination which continues into the 3rd decade of life (Durston et al., 2001). The temporal changes in total brain volume are illustrated in Figure 3.1.

Figure 3.1 Temporal Development of Whole Brain Volume in 532 Subjects.



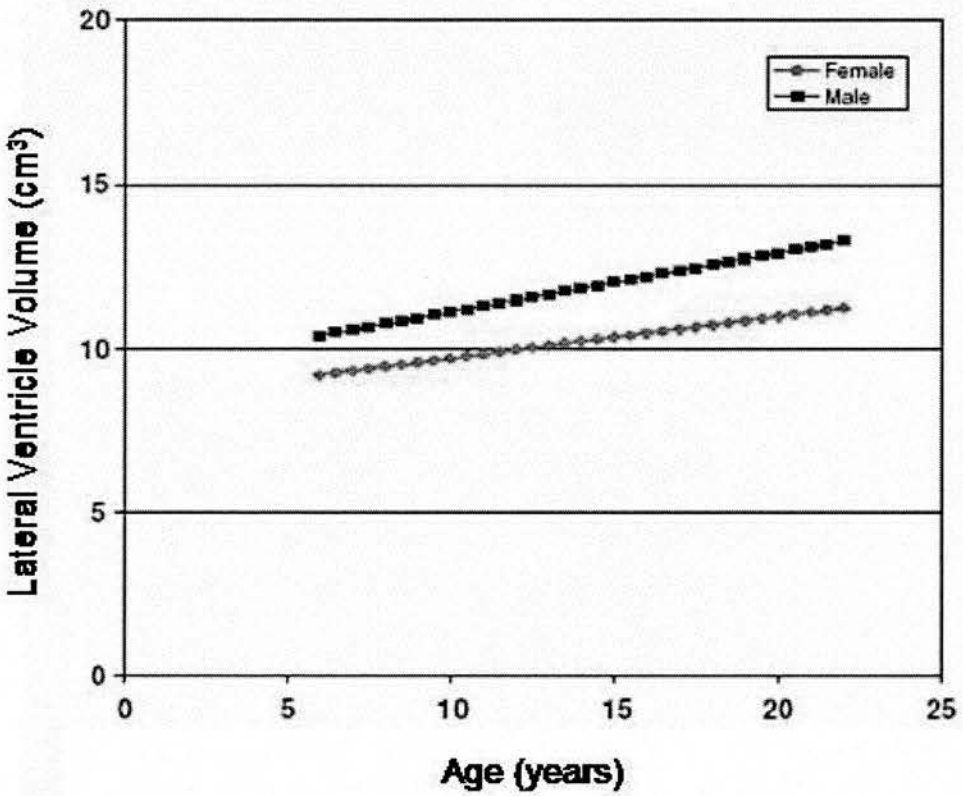
Adapted from Lenroot et al 2006.

3.2.2 Cortical Gray Matter Volumes and Neurodevelopment

Cortical gray matter, consisting primarily of the cell bodies of neurones and their supporting glial cells, increases in volume by approximately 10-15% from birth across the first five years of life, thereafter reaching peak volumes in a regionally-specific manner. Peak frontal and parietal cortical gray matter volumes are attained at age 10-12 years, whereas peak temporal gray matter volumes are not attained to age 16-17 years. The last area to attain peak volume is the pre-frontal

cortex, an area associated with higher intellectual functioning and reasoning (Lenroot and Giedd, 2006). The overall changes observed from the age of five to adulthood reflect a reduction in volume of the order of 20-30% (Durston et al., 2001). At least two distinct processes appear to account for the overall reductions in gray matter from early childhood to adulthood. Neuropathological studies confirm that selective programmed neuronal necrosis, a process termed "pruning", combined with necrosis of supporting glial cells does occur between early childhood and adulthood and contributes to some of the reduction in gray matter volume observed in neuroimaging studies. This loss of glia and neurones is thought to underlie the 60-150% increase observed in the volume of the lateral ventricles that has been observed across the same timeframe and which appears more marked in males relative to females (Durston et al., 2001). In addition to neuronal loss, a further contributor to the apparent reduction in cortical gray matter volume observed using MRI appears to be sub-cortical myelination which occurs late in neurodevelopment and which gives the neuroradiological appearance consistent with that of white matter, whereas the tissue appears to be an admixture of gray and white matter components on histological analysis. A summary diagram of the developmental changes which occur in gray matter volumes is provided in Figure 3.2.

Figure 3.2 Temporal Development of Lateral Ventricle Volumes in 532 Subjects.



Adapted from Lenroot et al 2006.

3.2.3 Sub-Cortical Gray Matter Volumes and Neurodevelopment

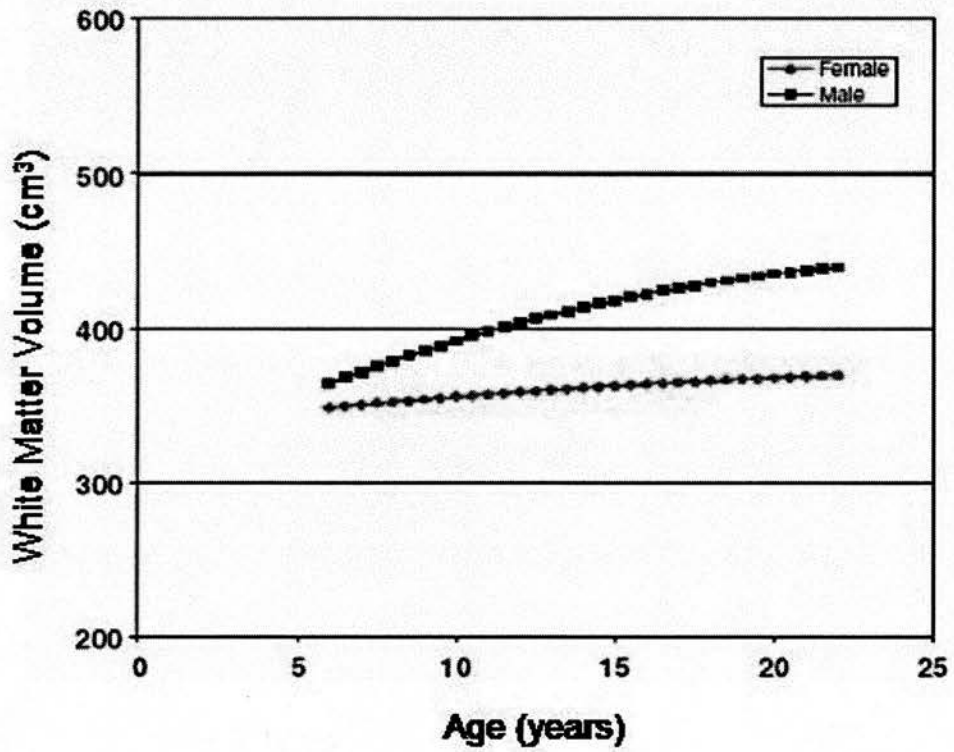
The sub-cortical gray matter consists of the basal ganglia and the amygdalo-hippocampal complex. The temporal changes observed in the development of sub-cortical gray matter structures across neurodevelopment appear to differ from those affecting the cortical gray matter. The amygdala and hippocampus, located in the medial temporal lobe, appear to modestly increase in volume (10-20% for Hippocampus; 40-50% for Amygdala) from childhood to adulthood. The

basal ganglia, which consist of five separate nuclei; the caudate nucleus, putamen, globus pallidus, substantia nigra and the sub-thalamic nucleus decrease in volume from their peak volumes at age 7-10 years (Lenroot and Giedd, 2006) by 10-15% (Durston et al., 2001) to reach adult volumes in a manner similar to that observed in accordance with cortical gray matter.

3.2.4 Cerebral White Matter Volumes and Neurodevelopment

Cerebral white matter is composed of the axons of neurones, linking different regional cortical and sub-cortical brain areas, oligodendrocytes and the myelin sheaths that they synthesised to enhance the speed of conduction of action potentials following neuronal depolarisation. Much of the process of myelination appears to have taken place by the time of birth. In the post-natal period white matter volumes rapidly increase, notably so during early childhood, with an increment of 75% being observed from birth to 5 years. The majority of this increase is observed during the first and second year of life (Hermoye et al., 2006). Thereafter, a further increase of the order of 10-20% occurs from the age of five until adult life. This increase in volume is though to represent the progressive myelination of axons by oligodendrocytes. Thereafter, the process of myelination continues at a lesser rate into the 3rd decade of life, generally proceeding from inferior to superior and posterior to anterior brain regions. Notable exceptions to this over simplification exist, particularly with respect to the corpus callosum, the largest white matter tract within the human brain which connects the cerebral hemispheres. The volume of the corpus callosum increases well into adulthood with the posterior aspects maturing last. The overall change in white matter volume which occurs with age is illustrated in Figure 3.3.

Figure 3.3. Temporal Development of White Matter Volumes in 532 Subjects.



Adapted from Lenroot et al 2006.

3.2.5 Gender-Specific Neurodevelopment and Brain Volumes

Gender is a powerful determinant of total and regional brain volumes. In all published series and across all age groups, from birth to adulthood, the male brain is consistently larger in volume than that of the female brain by approximately 10% and the majority of intra-cranial structures are proportionally smaller in women. However, certain regional brain areas differ in their proportional volume (*i.e.* the absolute volume relative to the intra-cranial volume) between the genders. Women have proportionally larger caudate nuclei, hippocampi and smaller amygdala relative to males but absolute volumes are similar (Lenroot and Giedd, 2006).

3.3 AGE AT BRAIN INJURY AND NEURODEVELOPMENTAL OUTCOME

There are two distinct outcomes that have been recognised with respect to the timing and type of cerebral insult during childhood and the subsequent risk of cognitive impairment: the developing brain appears to have the ability to adapt and correct for loss of specific functions, at least in part, through the process of synaptic plasticity, whereas generalised cerebral insults, such as those which could be expected to occur in association with severe hypoglycaemia or during Diabetic Keto-Acidosis, may potentiate sufficient brain injury to result in permanent neurological sequelae, developmental delay or cognitive impairment which may only become apparent in later life. Insults occurring within the first two years of life, during the period of relatively rapid cerebral growth are likely to result in a greater magnitude of permanent disability and dysfunction than those which occur during adolescence when the neurodevelopment is by comparison a relatively subtle process. This phenomenon has been clinically apparent for decades but was first documented in children with Epilepsy but thereafter has been described in association with severe hypoglycaemia in the neonatal period (Persistent Hyperinsulinaemic Hypoglycaemia of Infancy) as well as in a spectrum of other pathological processes known to affect the central nervous system (Tranel and Eslinger, 2000). In theory at least, the clinical consequences of decompensated T1DM, manifest as Severe Hypoglycaemia or Diabetic Keto-Acidosis, are

therefore most likely to incur negative consequences for the central nervous system if they occur early in life, particularly within the first two years where brain growth is at its most rapid. This concept is largely supported by data from clinical and pathological studies in humans. Severe Hypoglycaemia appears to be a reasonable example with a sufficient evidence base to support this theory. The occurrence of severe hypoglycaemia during the neonatal period, particularly when complicated by seizures, is associated with a poor clinical outcome with developmental delay, disability or death (Vannucci and Vannucci, 2001). Repeated exposure to severe hypoglycaemia during early childhood appears to incur subtle disadvantage upon the acquisition of certain cognitive abilities (Northam et al., 2001;Rovet and Ehrlich, 1999), whereas prospective studies of adults with T1DM have shown no detrimental association between repeated exposure to severe hypoglycaemia and subsequent cognitive abilities (Reichard et al., 1991; Austin and Deary, 1999; Jacobson et al., 2007).

3.4 NEUROPATHOLOGICAL STUDIES IN CHILDREN WITH T1DM

No studies have been published which have evaluated the neuropathological consequences of the development of Type 1 diabetes in early life. However, data from post-mortem studies of neonates who had experienced profound and protracted severe hypoglycaemic episodes appear to confirm a similar pattern of hypoglycaemic brain damage to that reported in adults, with severe neuronal injury to the Neocortex, Basal Ganglia and Hippocampus and a lesser degree of injury to the Thalamus, Brain Stem and Spinal Cord (Vannucci and Vannucci, 2001). In childhood, a single published case report describes the neuropathological appearances consequent upon insulin-induced severe hypoglycaemia in a previously healthy 8 year old non-diabetic girl who was rendered hypoglycaemic in a Munchausen's by Proxy case. The child developed a permanent right hemiparesis followed by the development of refractory epilepsy necessitating a excision of the left cerebral hemisphere. Histological examination of the excised hemisphere revealed a complete absence of cortical neurones, reactive gliosis, vacuolation of the white

matter, yet with sparing of the Basal Ganglia, Cerebellum and Brain Stem (Christiaens et al., 2003). The neuropathological sequelae of severe hypoglycaemia cannot be determined from this case study as repeated status epilepticus may potentially confound the histopathological appearances.

3.5 NEUROPSYCHOLOGICAL STUDIES IN CHILDREN WITH T1DM

3.5.1 Cross-Sectional Studies

Ack et al (Ack et al., 1961) first described the association between the development of Type 1 diabetes in early childhood, defined as an onset before the 5th birthday, and relatively poor performance on a test of general intelligence. Ack studied 38 sibling pairs discordant for Type 1 diabetes and compared performance on the Stanford-Binet Intelligence Scale and identified a correlation between general intelligence performance and the age of diagnosis of diabetes: in the 13 sibling pairs which included a child who had developed Type 1 diabetes before their 5th birthday, significantly poorer scores were observed, a difference equivalent to 10 IQ points (0.66 SD). In exploring the reasons underlying these observations, Ack noted that those diagnosed with diabetes in early life exhibited a trend towards a greater exposure to acute metabolic decompensations, in the form of severe hypoglycaemia or diabetic keto-acidosis, hypothesising that these two potential aetiological organic factors could explain some of the performance differences observed between cases and controls. Ryan and colleagues extended the breadth of knowledge within the field with their examination of cognitive abilities and intelligence in a study of 116 adolescents with T1DM, 46 of whom were diagnosed before their 5th birthday. Ryan found corroborative evidence that severe hypoglycaemia may be detrimental to the developing brain: deficits in attention, learning and memory, visuo-spatial ability, psychomotor speed, motor speed and verbal intelligence were observed in those who developed diabetes aged less than 5 years relative to those with a later onset of diabetes. Deficits were sufficient in magnitude to

classify a proportion of "early onset" children as cognitively impaired (Ryan et al., 1985a). This led Ryan and many subsequent investigators to assume that exposure to severe hypoglycaemia was primarily responsible for the broad-based deficits observed in association with "early onset" diabetes. This hypothesis was not without foundation as a greater cumulative burden of metabolic decompensations, particularly as severe hypoglycaemia, has been consistently reported for those diagnosed with T1DM in early life, relative to those diagnosed later in childhood (Daneman et al., 1989;Davis and Jones, 1998;Golden et al., 1985;Ternand et al., 1982). Following on from the observations of Ryan, others have expanded the breadth of cognitive domains that appear to be affected by an "early onset " of T1DM and attempted to separate the specific effects of severe hypoglycaemia and early disease onset *per se.* Rovet examined the intellectual consequences of the development of T1DM in early childhood in 27 children who had developed the disorder before their 4th birthday, 24 children with T1DM who had developed diabetes at a later age and 30 sibling controls. An early onset of T1DM was associated with broad-based intellectual deficits in girls who exhibited poorer performance IQ scores, poorer reading and spelling abilities. Although the early onset group in this study had a slightly greater cumulative exposure to severe hypoglycaemia, the poorer performance of the "early onset" group could not be directly attributed to severe hypoglycaemia (Rovet et al., 1987a). Bjorgaas and colleagues performed a detailed neuropsychological assessment in a small study of 28 children with T1DM and 28 age and 28 ability-matched controls, relating their observations to the occurrence of severe hypoglycaemia. Those children who had experienced severe hypoglycaemia (n=15) consisted of a group who were diagnosed before their 5th birthday (EOD-SH, n=5) with the remainder diagnosed after this age (LOD-SH, n=10). The exposure of the T1DM group to severe hypoglycaemia was very modest and no child experienced severe hypoglycaemia before the age of 4 years. Bjorgaas identified significantly poorer performance on tests of attention and psychomotor efficiency in the EOD participants, all of whom had experienced severe hypoglycaemia. Rather oddly, the performance of the EOD-SH group was thereafter compared

relative to the LOD-SH and remaining T1DM participants, rather than an EOD group naïve to severe hypoglycaemia which was not possible. These groups were not matched for educational or pre-morbid ability. The authors concluded, somewhat inappropriately, that severe hypoglycaemia exposure was causative of the deficits in attention and psychomotor efficiency (Bjorgaas et al., 1997). Rovet examined general intelligence and attentional abilities in detail in a large group of children and adolescents with T1DM (n=103) relative to matched controls (n=100). The attentional abilities of the diabetes group differed little from those of control subjects. However, sub-analysis identified a broader spectrum of deficits in several aspects of attention in association with an early onset of T1DM, defined as an onset before the 6th birthday and a history of preceding exposure to severe hypoglycaemia. An early onset of diabetes specifically affected the 'select' aspect, whereas severe hypoglycaemia exposure affected the 'inhibit' and 'focus' subcomponents. Those with an early onset of diabetes and exposure to severe hypoglycaemia exhibited lower verbal IQ scores and multiple attentional deficiencies. However, the magnitude of the differences observed in attentional processes was small and of the order of 0.2SD when present. An early onset of diabetes was not associated with any difference in general intelligence, Performance IQ or Verbal IQ, whereas a history of preceding exposure to severe hypoglycaemia was associated with a small difference in verbal IQ scores of the order of 0.3SD (4.5 IQ points) (Alvarez and Rovet, 1997). In a small and perhaps underpowered study Hershey et al examined medial temporal function, as declarative memory ability, in 25 children with T1DM randomised to receive either "intensive" insulin treatment, geared towards the attainment of strict glucose control, or "conventional" insulin treatment where glycaemic targets were more lax. The "intensive" group experienced a three-fold increased incidence of severe hypoglycaemia, defined as an episode of sufficient severity to necessitate 3rd party intervention to facilitate recovery (The DCCT Research Group,1991; The DCCT Research Group, 1997). Hershey observed poorer delayed spatial memory function in those children receiving "intensive" insulin treatment although the statistically significant disadvantage in performance was small in

magnitude. However, when comparing those children who had experienced severe hypoglycaemia relative to those who were naïve to severe hypoglycaemia no significant difference in abilities was observed. Whilst this modestly powered study identified inferred that a specific aspect of memory function may be associated with "intensive" insulin therapy, no detrimental effects of severe hypoglycaemia *per se* were identified (Hershey et al., 1999).

Other cross-sectional studies have consistently identified relatively poorer performance across a broad base of cognitive domains in children who had developed Type 1 diabetes in early life (Hagen et al., 1990;Holmes and Richman, 1985;Wolters et al., 1996). However, studies which have examined the effects of severe hypoglycaemia exposure in children have provided inconsistent results. Deficits in declarative memory (Hershey et al., 1997;Kaufman et al., 1999;Rovet and Ehrlich, 1999), spatial memory (Hershey et al., 2004), word recall (Wolters et al., 1996), attention (Alvarez and Rovet, 1997;Rovet and Ehrlich, 1999) and Verbal IQ (Alvarez and Rovet, 1997;Rovet and Ehrlich, 1999) have been reported in association with severe hypoglycaemia. Other studies have found no association between severe hypoglycaemia exposure and poorer neuropsychological functions (Bjorgaas et al., 1997;Strudwick et al., 2005;Wysocki et al., 2003). Drawing definitive conclusions from these studies are difficult due to the heterogeneity of neuropsychological tests used and the differing definitions of "early onset" diabetes.

Others have postulated alternative hypotheses to account for the differences observed, placing an emphasis on socio-economic factors rather than organic factors. Children with T1DM diagnosed early in life have been shown to have poorer school attendance and are more likely to exhibit behavioural difficulties. Children with diabetes have been demonstrated to have poorer school attendance than their peers (Hagen et al., 1990;McCarthy et al., 2002;McCarthy et al., 2003;Parker et al., 1994;Rovet et al., 1987b;Ryan et al., 1985b). However, reports on the school

performance of children with T1DM are conflicting. Rovet observed poorer school performance, perhaps related to deficits in the ability to learn, in children with T1DM (Rovet et al., 1993) and also reported that children with T1DM were more likely to require to repeat a school year (Rovet et al., 1987a). However, McCarthy and colleagues examined the learning and memory abilities and academic prowess of children in a large study of significant statistical power of three groups of children: a Type 1 diabetes group (n=244), sibling controls (n=110) and matched-classmate controls (n=209). No differences between those with T1DM and matched controls were observed. No difference in academic performance was associated with the presence of diabetes relative to siblings and classmates (McCarthy et al., 2002;McCarthy et al., 2003). Children with T1DM have also been shown to exhibit greater difficulties with behaviour in school. Fallstrom first demonstrated behavioural difficulties and anxiety problems, relative to non-diabetic children, which correlated with exposure to severe hypoglycaemia (Fallstrom, 1974). Others have since corroborated the higher prevalence of behavioural problems in children with an early onset of diabetes, although not consistently associated with severe hypoglycaemia (McCarthy et al., 2002;Rovet et al., 1987b).

Separating the disease-specific organic factors, such as contributions from severe hypoglycaemia and chronic hyperglycaemia, from the socio-economic and behavioural contributors to the cognitive differences observed in association with an early onset of diabetes has proved difficult and beyond the design limitations of cross-sectional studies, necessitating the collection of prospective data.

3.5.2 Prospective Studies

Rovet et al first prospectively examined the associations of exposure to severe hypoglycaemia in early life with later cognitive abilities and general intelligence scores in a small study of 16 subjects with T1DM evaluated over a 7 year period. Rovet identified that subjects exposed to

hypoglycaemic seizures at or before the age of five (n=4 subjects) exhibited a selective performance deficit restricted to lower verbal IQ scores and poorer scores on tests of attention and memory abilities (Rovet and Ehrlich, 1999). However, limitations inherent upon the statistical power of this study and the retrospective collection of severe hypoglycaemia data prevent definitive conclusions.

Hershey and colleagues examined the effects of two different insulin treatment strategies on a range of cognitive abilities in 25 older diabetic children (average age 12 years) were followed from diagnosis for a mean 36 months (Hershey et al., 1999). Participants were randomised to receive "intensive insulin" therapy, with the aim of attaining near normoglycaemia, or "conventional insulin" treatment. Those children receiving intensive insulin therapy had a threefold increased risk of hypoglycaemia and performed slightly less well on a single test of declarative memory, from an extensive battery which included several other assessments of memory. Across many of the other cognitive abilities examined those receiving "intensive insulin" therapy performed better than those receiving "conventional insulin". The investigators concluded that the "intensive insulin" strategy may be harmful to aspects of cognitive ability in developing children, not addressing the potential for Type 1 statistical in their interpretation of their results (Hershey et al., 1999). Had multivariate statistical techniques been utilised the observations from this small study may have been reported in an entirely different manner.

Northam and colleagues thereafter evaluated the impact of a childhood diagnosis of T1DM on the attainment of general intelligence and specific neuropsychological abilities in a well-characterised cohort of children with diabetes relative to age and ability-matched healthy controls. At the outset of this prospective study no differences in general intelligence, as determined by the Weschsler Intelligence Scale for Children, were observed between cases and controls. Two years following the diagnosis of T1DM differences in the processing speed and learning abilities of

those with T1DM were apparent (Northam et al., 1998). By the third evaluation after 6 years of prospective assessment significant differences between cases (n=92) and controls (n=84) were clearly evident and specific effects relating to diabetes-related variables discernable. Multivariate analysis identified diabetes *per se* as an independent predictor of lower general intelligence scores, predominantly determined by poorer performance on the verbal IQ subtests of the WISC, with a small magnitude of deficit. Those with an early onset of diabetes, defined as onset before the 4th birthday, also exhibited a broad range of neuropsychological deficits with poorer performance on tests of attention, processing speed and executive function being apparent. By the 6 year assessment 25% of the cohort had experienced severe hypoglycaemia, but only one child had experienced an episode of hypoglycaemic seizure before their 4th birthday. Exposure to hypoglycaemic seizure was associated with significantly poorer verbal IQ scores and lower FSIQ scores. However, the amount of variance explained by exposure to severe hypoglycaemia was modest, equating to 0.4SD for verbal IQ and 0.2SD for FSIQ scores. Unlike the findings with respect to an early onset of diabetes, the influence of severe hypoglycaemia exposure was limited to detrimental effects on verbal IQ and was not associated with poorer performance on any of the neuropsychological test battery (Northam et al., 2001). Due to the temporal occurrence of severe hypoglycaemia in this cohort, the hypothesis that severe hypoglycaemia exposure in early life is especially injurious to the human brain remained untested.

Schoenle and colleagues prospectively evaluated the general intelligence of a Austrian cohort of children age 7-16 years with T1DM relative to healthy controls, using the German version of the Weschsler Intelligence Scale for Children (WISC) to determine Verbal IQ, Performance IQ and Full-Scale IQ (FSIQ) scores. IQ scores were evaluated with respect to the disease-specific variables of interest: severe hypoglycaemia and degree of metabolic control. Schoenle reported a gender-specific pIQ deficit in boys who developed T1DM before their 6th birthday, most notably so in those with poor glycaemic control. In contrast to the observations of Northam, no

differences in verbal IQ or FSIQ were observed in either gender and no detrimental effects of severe hypoglycaemia on general intelligence were identified. Schoenle concluded that an early onset of diabetes and poor metabolic control may be disadvantageous to current intellectual performance in males. Despite these apparently reassuring negative findings with respect to severe hypoglycaemia, it should be borne in mind that no participant in this Austrian cohort was exposed to severe hypoglycaemia before the age of five years, again failing to test the hypothesis that early life severe hypoglycaemia is particularly detrimental to the brain (Schoenle et al., 2002).

The prospective studies summarised above strongly suggest that the development of diabetes in early childhood may confer modest disadvantage on the acquisition of a broad range of neuropsychological abilities, most notably attention, information processing speed, aspects of memory abilities and executive functions. The cognitive disadvantage associated with childhood exposure to severe hypoglycaemia appears to be more specific and may selectively affect the attainment of verbal intelligence. The hypothesis that early life exposure to severe hypoglycaemia, before the 6th birthday, may be more deleterious than exposure in later childhood remains unproven largely through the lack of severe hypoglycaemia exposure in early life of the participants of the above studies.

3.6 NEUROIMAGING ASSOCIATIONS OF CHILDHOOD ONSET T1DM

Ack and colleagues in 1961 proposed two major organic factors that could, at least in part, account for the significantly poorer intellectual performance observed in children who had developed diabetes before their 5th birthday: exposure to episodes of severe hypoglycaemia and hyperglycaemic decompensations in the form of Diabetic Keto-Acidosis (Ack et al., 1961). The neuroimaging associations of these acute metabolic decompensations have been described in case reports and small cross-sectional studies and will be discussed separately as they appear to differentially impact upon the central nervous system.

3.6.1 Neuroimaging Sequelae of Severe Hypoglycaemia in Childhood

No cases describing permanent hypoglycaemic brain damage with associated neuroimaging abnormalities have been published in children and adolescents with Type 1 diabetes in the mainstream medical literature, in contrast to the body of literature which exists in adults. Whether this reflects reporting bias, difficulties in the practicalities of the neuroimaging of children, the smaller absolute number of children with Type 1 diabetes relative to adults, or a genuine reflection of a lesser propensity towards brain injury following severe hypoglycaemia in this age group remains unclear. Christiaens and colleagues described a single case of hypoglycaemic coma associated with the development of hemiparesis, expressive aphasia, cognitive impairment and neuroimaging abnormalities in a non-diabetic 8 year old girl, rendered comatose through hypoglycaemia in a Munchausen's by proxy case. Early CT imaging revealed loss of the normal differentiation between gray and white matter and increased density of the left cerebral hemisphere. Subsequent MRI revealed gross abnormalities of the entire left neocortex on Proton Density/T2-weighted sequences with sparing of the Basal Ganglia, Brain Stem and Cerebellum and entire right cerebral hemisphere (Christiaens et al., 2003).

3.6.2 Neuroimaging Sequelae of Diabetic Keto-Acidosis in Childhood

Diabetic Keto-Acidosis (DKA) remains the leading cause of death during childhood and adolescence in people with Type 1 diabetes (Edge et al., 1999). In contrast to the paucity of published evidence to support the hypothesis that episodes of severe hypoglycaemia during childhood may adversely affect brain structure in children with Type 1 diabetes, a large number of published case reports and several case series document the neuroimaging abnormalities associated with hyperglycaemic decompensation, as DKA. Children and adolescents with Type 1 diabetes are known to develop cerebral oedema more frequently and with modest degrees of metabolic derangement during DKA relative to adults with T1DM. Cerebral oedema is said to classically present 4-16 hours following the commencement of active treatment for DKA, with intra-venous insulin and fluid resuscitation, and is heralded by the onset of confusion, localising neurological signs or a reduction in the conscious level of the child (Edge et al., 2006a;Figueroa et al., 2005). The development of clinically apparent cerebral oedema, which complicates approximately 1% of childhood DKA cases (Edge et al., 2006b;Roe et al., 1996) is a poor prognostic feature with a reported mortality of 24% and disability or developmental delay in 35% of those who survive (Edge et al., 2001). Due to the obvious propensity for neurological sequelae in the acute phase of treatment of DKA an extensive body of literature has accumulated. Krane et al first reported subtle structural brain abnormalities in association with DKA in children. In a small case series serial CT imaging of the brain was performed in neurologically asymptomatic children with DKA: an initial image was acquired during the early phase of treatment with intravenous fluids and insulin, with a follow up scan obtained around the time of discharge from hospital. Krane identified temporal changes in the cross-sectional diameter of the cerebral ventricles which were interpreted as reflecting a degree of sub-clinical brain swelling present during the early stages of DKA treatment (Krane et al., 1985). An alternative interpretation would be that reductions in the diameter of the cerebral ventricles may represent partial collapse of these structures consequent upon dehydration and reduced CSF volumes in untreated DKA,

with re-expansion on the attainment of euvolaemia. Irrespective, these changes were observed in children in whom no apparent neurological or cognitive deficit was apparent. Hoffman and colleagues also utilised serial CT scans to evaluate the temporal evolution of brain structure changes during the active treatment of DKA in nine children. Hoffman also observed smaller volumes of the lateral and 3rd ventricles before treatment was commenced, relative to subsequent scans obtained 6-8 hours later and at seven days following treatment (Hoffman et al., 1988). Again, the authors interpreted the smaller ventricular volumes observed at presentation as being representative of sub-clinical brain swelling. Rogers et al described the neuroimaging sequelae observed in three non-fatal cases of DKA presenting between 1984-1987, each complicated by neurological deterioration of the child. CT and MRI scans obtained in these clinically more severe cases revealed haemorrhagic infarction of the thalamus, basal ganglia and lentiform nuclei associated with permanent physical and developmental disabilities (Rogers et al., 1990). McAloon (McAloon et al., 1990) and Greene (Greene et al., 1990) reported similar CT abnormalities and adverse developmental outcomes in a small number of cases of childhood DKA complicated by neurological deterioration after the commencement of treatment. Lebrun and colleagues subsequently reported the MRI associations of a case of hemiparesis as a consequence of cerebral oedema in the setting of DKA (Lebrun et al., 1994). Roe et al reported the neuroimaging and clinical associations observed in a retrospective series of 5 cases of DKA complicated by neurological sequalae. Consistent with previous reports neuroimaging revealed infarction in the region of the thalamus, basal ganglia and lentiform nuclei but also infarction of the cingulate gyrus and occipital lobes were observed in some patients. Roe suggested that these specific territories were vulnerable to infarction due to their dependency on perforator branches of the major cerebral arteries for blood supply. The thalamus receives its vascular supply from branches of the posterior cerebral artery, whereas the basal ganglia and lentiform nuclei receive supply from the middle cerebral artery, yet each of these subcortical gray matter structures is reliant on perforator branches for arterial supply (Roe et al., 1996). Shrier reported a fatal case of

cerebral oedema complicating DKA resulting in central brain herniation, infarction of the medial temporal lobe structures and central brain atrophy in a nine year old girl (Shrier et al., 1999). Hoffman utilised conventional MRI sequences to evaluate the neuroradiological appearances at two time points in six children with DKA: initial scans revealed increased T2-weighted signal in the cortex, which had resolved by the second scan on day 14. Hoffman also reported an increase in the size of the lateral ventricles across the same time frame and interpreted this as being secondary to the resolution of subclinical brain oedema which was compressing the ventricular cavity on the initial scan (Hoffman et al., 1999). However, no evidence of cerebral oedema was observed on the initial imaging and two other explanations could be offered: firstly, the dehydration incumbent upon the development of DKA may reduce CSF production and hence cerebro-ventricular volumes, or secondly, that the temporal increase in volume could reflect the development of central brain atrophy. Neither of these alternative hypotheses were entertained by the authors. Keane reported a case of venous sinus thrombosis of the straight sinus complicating a case of DKA, where the child deteriorated following the commencement of replacement fluid and insulin, resulting in characteristic appearances on CT imaging and a temporary hemiparesis which resolved to leave mild learning difficulties (Keane et al., 2002). Cameron described the serial MRI appearances observed in 5 cases of DKA in children in whom no signs of cerebral oedema were clinically apparent and reported transient increases in signal on FLAIR and T2-weighted sequences in the frontal lobes, which resolved over a period of days (Cameron et al., 2005). Glaser utilised Diffusion (DWI) and Perfusion (PWI) Weighted neuroimaging techniques in a small study of 14 children with DKA, obtaining images shortly after the initiation of treatment and 48 hours later. Changes on DWI in the frontal, temporal and parietal cortices were suggestive of vasogenic oedema in these areas. None of those children studied had concerning features, inferring that sub-clinical vasogenic oedema may be more common than previously considered (Glaser et al., 2004). Figueroa utilised multiple MRI sequences, which included Diffusion Weighted (DWI) and T2-weighted imaging, to examine the neuroradiological

associations of DKA in a small series of children. MRI scans were obtained at 6-12 hours and repeated at 96 hours and revealed a transient increase in T2-weighted signals in cortical areas which had resolved by the second scan. In contrast to the observations of Glaser, no DWI abnormalities were observed inferring that oedema may not have been present (Figueroa et al., 2005). Glaser used MRI methodologies to sequentially assess pre and post-treatment lateral ventricular diameters in 41 children undergoing treatment for DKA. In keeping with the earlier reports from Hoffman (Hoffman et al., 1988;Hoffman et al., 1999) an increase in the diameter of the lateral ventricular cavity was observed following treatment of DKA, with 50% of children exhibiting narrowing of the lateral ventricles during the early phase of DKA treatment. Glaser interpreted this observation as being indicative of sub-clinical cerebral oedema in a considerably greater proportion of children than was previously recognised (Glaser et al., 2006).

In summary, Diabetic Keto-Acidosis is frequently accompanied by measurable neuroradiological abnormalities even in asymptomatic children. DKA can be complicated by neurological deterioration which is associated with a poor prognosis, subsequent developmental delay and permanent neuroradiological abnormalities, most commonly as cerebral infarction in severe cases. The pattern of brain injury differs significantly from that associated with hypoglycaemic brain damage.

3.6.3 Prospective Studies

No studies have prospectively examined the neuroimaging correlates of Type 1 diabetes diagnosed during childhood, with respect to evaluating the brain structural associations of repeated exposure to Severe Hypoglycaemia, Diabetic Keto-Acidosis, or any other disease specific factors.

CHAPTER 4

VASCULAR DISEASE, GENETICS AND NEUROIMAGING

4.1 BASICS OF NEUROIMAGING

4.1.1 Introduction to Magnetic Resonance Imaging

Magnetic resonance (MR) imaging generates images by applying a varying magnetic field to the body, which aligns the hydrogen atoms of the tissue under evaluation in accordance with the polarity of the applied magnetic field. When the field is released, radio waves are generated as the polarised hydrogen atoms return to their resting state. The frequency of the emitted radio waves is related to the chemical environment of the atoms as well as to their location. With computer analysis of these data, MR images can be generated.

Magnetic Resonance imaging systems are formed from several key components. These include a large magnet supplemented by shim coils to generate a sufficiently uniform powerful magnetic field. A radiofrequency (RF) coil is used to transmit controlled radio signals into the body tissue under evaluation. The characteristics of the transmitted radio signals are altered as they penetrate tissues, according to the properties of each specific tissue, and such alterations are detected by the receiver coil. Additional gradient coils assist in delineating the spatial localisation of the returning radio signals, which are then computer integrated to generate reconstructed images for interpretation by a radiologist. A major advantage of MR techniques is the ability to alter radio signal settings to selectively enhance and delineate intra-cranial tissues. With the notable exception of bony structures most tissues within the skull vault can be outlined. This allows the accurate definition of white matter, gray matter, cerebrospinal fluid, adipose tissue and their interfaces, which facilitates volumetric analysis of specific tissue types. Alteration of the plane of imaging can also be manipulated to the specific anatomical brain area being studied, allowing regional brain areas to be outlined with a greater degree of accuracy than that permitted by previous neuroimaging techniques.

4.1.2 Alteration of Radio Signal Intensity and MR Sequences

Different image contrasts can be achieved by using specific RF pulse sequences and imaging parameters. The basic signal intensity parameters that can influence imaging are:

Proton Density

The proton density (PD) of a tissue reflects the concentration of protons in the tissue, in the form of water and contained within macromolecules.

T1 Relaxation Time

The T1 relaxation time reflects the longitudinal relaxation time taken by protons to revert back to their resting states after exposure to an RF pulse.

T2 Relaxation Time

The T2 relaxation time reflects the transverse relaxation time taken by protons to revert back to their resting states after exposure to an RF pulse.

Blood Flow

Blood flow can affect signal acquisition as rapidly flowing arterial blood produces deficits in signal. Exploitation of this characteristic underlies the development of specific sequences that facilitate MR angiography techniques.

The contrast on MR images can be manipulated by changing the parameters which characterise a pulse sequence. A pulse sequence defines the specific strength, number, timing and gradient of the RF pulses applied to the body tissue under evaluation. The two most important parameters are the repetition time (TR) and the echo time (TE). The TR is the time between consecutive 90 degree RF pulses. The TE is the time between the initial 90 degree RF pulse and the echo. T1-weighted sequences use a short TR and short TE (TR < 1000msec, TE < 30msec). T2-weighted sequences use a long TR and long TE (TR > 2000msec, TE > 80msec). T2-weighted sequences can be employed as a dual echo sequence to further enhance the contrast between tissues of interest. The first or shorter echo (TE < 30msec) is proton density-weighted, or a mixture of T1 and T2-weighted images. This image is very helpful for evaluating changes in the peri-ventricular white matter, such as those observed in demyelination or cerebral microangiopathy, because hyperintense white matter lesions are contrasted against the lower signal observed in the CSF. FLAIR (Fluid Attenuated Inversion Recovery) sequences are a further enhancement of the basic PD imaging sequence. FLAIR images are a special inversion recovery sequence in which the TI time is adjusted to match the relaxation time of the tissue whose image should be suppressed. This sequence is an important technique used to differentiate lesion type within the central nervous system.

4.1.3 Magnetic Resonance Imaging of the Brain

Magnetic resonance (MR) imaging is an ideal modality for the imaging of brain structure, and has several advantages over X-ray dependent technologies such as CT scanning. The advantages of MR imaging include:

1. MR imaging does not involve the delivery of a dose of ionising radiation;
2. The imaging study can be tailored to the brain region of interest and to the disease process being studied. Radio signal intensities observed on T-1, T-2, and proton density-weighted images relate to specific characteristics of the tissues being evaluated.

Due to its high sensitivity for brain water, MR is generally more sensitive for detecting subtle brain abnormalities, during the early stages of disease, than CT based techniques. In addition, MR imaging is exquisitely sensitive for the detection of white matter changes, such as those caused by demyelination and cerebral microvascular disease. Patients with substantial white matter abnormalities on MR imaging may have an apparently normal CT scan.

4.2 STRUCTURAL BRAIN ABNORMALITIES

Two major types of structural brain abnormalities are commonly reported in neuroimaging epidemiology studies: high-intensity white matter lesions and cerebral atrophy.

4.2.1 Neuropathology of High Intensity White Matter Lesions

White matter lesions differ in their type, size, location within the brain, neuropathological associations and hence prognosis.

Types of White Matter Lesions:

1. *Peri-ventricular White Matter Lesions*

Peri-ventricular White Matter Hyperintensities (PVWH) are abnormalities of the white matter, located in the white matter adjacent to the lateral ventricles. PVWH vary in severity: early punctate lesions and peri-ventricular rims or caps (mild); patchy white matter with early confluence (moderate); large confluent white matter lesions (severe). Pathologically, early punctate lesions are characterised by peri-vascular hyalinosis and do not appear to have the same associations with stroke, myocardial infarction and vascular death associated with more advanced lesions. Progression of PVWH appears proportional to lesion burden at baseline and data for the effects of hypertension are inconsistent (De Leeuw et al., 2001;Schmidt et al., 1992;Schmidt et al., 2003). The neuropathological appearances of moderate PVWH are those of demyelination, reactive gliosis without substantial changes in blood vessels, whereas severe PVWH, which are associated with advancing age and hypertension, exhibit similar pathology but with the additional features described with DWMH, as described below. The Rotterdam Scan Study suggested that moderate to severe PVWH were more closely associated with cardiovascular risk factors rather than DWMH (De Leeuw et al., 2001).

2. *Deep White Matter Lesions*

Deep White Matter Hyperintensities (DWMH), also termed 'Leukoaraiosis', are typically located adjacent to areas of cortical infarction or within the deep white matter. Epidemiological studies have identified an association between DWMH and the subsequent risk of CVA and death from

IHD (Launer, 2004). The presence of DWMH can be associated with cognitive impairment, although this is generally noted for gross changes leading some experts to propose a "threshold" effect, such that a certain cumulative white matter burden is required before cognition suffers. Similar to PVWH, progression of DWMH appears proportional to lesion burden at baseline and data for the effects of hypertension are inconsistent (De Leeuw et al., 2001;Schmidt et al., 1992;Schmidt et al., 2003). The neuropathological appearances of DWMH reveal reduced myelin and oligodendrocytes, reduced axonal density, axonal fragmentation and small blood vessels show hyalinosis and variability of luminal cross-sectional diameter. The pathological findings are consistent with focal microvascular ischaemia with reactive gliosis and demyelination (Englund, 2002).

3. *Small Punctate White Matter Lesions*

Small Punctate White Matter Lesions (SPWML) are small hyperintense white matter abnormalities, 1-2mm in diameter, that can be observed throughout the entire brain, although are most typically observed in the region of the Basal Ganglia and Centrum Semi-Ovale. The clinical associations of SPWML appear less well characterised, relative to PVH and DWMH, but this type of lesion is considered to be relatively benign abnormalities due to the lack of a strong association with the subsequent risk of macrovascular disease in the few studies that have assessed SPWML (Fazekas et al., 1991;Schmidt et al., 2003). The risk of progression to more severe manifestations of white matter abnormalities appears small, at least in elderly populations at high risk of cerebrovascular disease (Schmidt et al., 2003). Histologically, SPWML consist of areas of focal demyelination and rarefaction of myelinated fibres with hyalinosis of small arterioles (Fazekas et al., 1991). The peri-vascular appearance of SPWML share significant commonality with the limited evidence from histological studies of the brain in Type 1 diabetes (Reske-Nielsen and Lundbaek, 1963a).

4.2.2 Epidemiology of White Matter Lesions

White Matter Lesions are present within the brain from an early age, with their frequency being partly dependent on the mode of neuroimaging utilised. CT imaging is relatively insensitive for detecting white matter pathology, whereas MRI based techniques are more sensitive. MRI pulse sequences also differ in their sensitivities in detecting of WML: T-1 weighted images are relatively insensitive, whereas sensitivity increases stepwise for Proton Density, T2-weighted, FLAIR and DWI techniques. The type of sequence utilised therefore impacts upon the epidemiological interpretation of WML distribution and burden (Fazekas et al., 2002) as inter-observer variability in the rating of MRI scans (Wardlaw et al., 2004).

WML are apparent on MRI scans from a relatively young age. The CAMERA study identified a point prevalence of 42% in normal healthy adults aged 30-40 years, rising to 70% in those aged 50-65 years (Kruit et al., 2004), whereas the ARIC study identified that 88% of 55 year olds had some degree of WML, increasing to 92% in 65 year olds (Liao et al., 1997). The progressive rise in WML incidence with age was confirmed by the large Rotterdam Scan Study of adults aged 60-90 years in which 95% of participants had some degree of WML: WML incidence and burden increased progressively with chronological age (De Leeuw et al., 2001).

Risk Factors for WML:

1. Hypertension

The presence of hypertension appears to be the most important determinant of white matter abnormalities in middle age and later life. The presence of hypertension, blood pressure variability, isolated systolic hypertension (Liao et al., 1996), or the development of Left Ventricular Hypertrophy (De Leeuw et al., 2001) as a consequence of hypertension are major risk factors for white matter pathology. These factors are modified by the degree of anti-hypertensive control and the presence of the APOE ε4 allele; those with good anti-hypertensive control have

fewer WML (Dufouil et al., 2001;Liao et al., 1996), whereas those with the ε4 allele (De Leeuw et al., 2004) appear to develop a greater WML burden when hypertensive. The degree of hypertension also appears to influence the rate of progression of WML (Schmidt et al., 2003).

2. *Diabetes and WML*

The development of microvascular complications of diabetes, particularly as diabetic retinopathy, has been hypothesised as being a likely surrogate for the presence of intra-cerebral microangiopathy elsewhere. However, little epidemiological evidence exists to support this hypothesis. Of the major large scale population-based MRI neuroimaging studies in middle-aged and elderly subjects only the Cardiovascular Health Study (Manolio et al., 1999) identified an association between the presence of Type 2 diabetes and WML. In Type 1 diabetes limited neuroimaging data exist. In a smaller case-control study Dejgaard and colleagues described the Magnetic Resonance neuroimaging correlates of the clinical complications of Type 1 diabetes a long-duration T1DM group with extensive microangiopathy (n=16) and an age-matched healthy control group (n=19) (Dejgaard et al., 1991a). The MRI techniques utilised by Dejgaard were commensurate with the year of study and could be considered relatively insensitive compared to current methodologies. White-matter lesions (69%) were significantly more common in the microangiopathic group relative to healthy age-matched controls (12%), being distributed evenly throughout the white matter of the cerebral hemispheres (as PVWH and DWMH), cerebellar hemispheres and brain stem. WML were small in size, infrequent in number and modest at worse. Dejgaard et al interpreted the higher prevalence of white-matter lesions observed in the T1DM group as being indicative of the presence of intra-cranial microangiopathy, complicating Type 1 diabetes, or alternatively as being indicative of small foci of ischaemia, known as 'Leukoaraiosis', as part of a subcortical arteriosclerotic encephalopathy (Dejgaard et al., 1991a). Youssem compared the frequency of white-matter lesions in 25 adult patients with Type 1 diabetes complicated by proliferative diabetic retinopathy (severe retinopathy) relative to 10 age-

matched healthy control subjects using MRI techniques consisting of T1-weighted and Proton Density images. In contrast to the observations of Dejgaard, no white-matter lesions were identified in any study participant and Youssem concluded that proliferative diabetic retinopathy was not associated with white-matter pathology and that microangiopathy of the brain was unlikely in T1DM (Yousem et al., 1991). Advances in MRI technology in the intervening years since publication have since revealed that the imaging sequences utilised by Youssem are not ideal for the determination and quantification of white-matter changes, being insensitive to detect subtle differences of the type that could be expected in young adults. Lunetta and co-workers thereafter examined the MRI neuroimaging appearances (T1 and T2-weighted sequences) in a small heterogeneous group of ten patients with Type 1 diabetes, who had relatively short diabetes duration and varying degrees of microangiopathy. No mention of white-matter abnormalities were discussed in the manuscript (Lunetta et al., 1994). The relationship between Type 1 diabetes and white matter lesions remains under evaluated at present with limited data inferring that the prevalence of white matter change may be greater in those with T1DM complicated by microangiopathy.

4.2.3 Neuropathology and Subtypes of Cerebral Atrophy

Cerebral atrophy is a hallmark of many of the diseases that affect the brain and reflects a loss of neuronal bodies, their axonal interconnections, and their supporting glial cells. Atrophy can be generalised, such that a symmetrical reduction in brain tissue volume occurs, or it can be focal, affecting only a limited area of the brain and resulting in a decrease of the functions that area of the brain controls. Additionally, cerebral atrophy is frequently subdivided into two types: Sulcal atrophy with widening of the cerebral sulci; Ventricular atrophy with an increased cerebral ventricle cavity size. The pattern and rate of progression of cerebral atrophy depends on the underlying aetiological process. Potential aetiologies are multiple, the most common of which

are vascular disease and Alzheimer's disease. Almost any pathological process that affects the brain can result in a degree of brain atrophy.

4.2.4 Epidemiology of Cerebral Atrophy

Cerebral atrophy is most commonly associated with vascular disease (Mungas et al., 2001) and Alzheimer's disease (Silbert et al., 2003) and its incidence rises progressively with age (Longstreth, Jr. et al., 2000). The Atherosclerosis Risk In Communities (ARIC) study examined the epidemiological associations of brain atrophy in a large sample of middle-aged individuals and identified the independent predictors of cerebral atrophy. The sole independent predictors included age and the presence of "diabetes": age increased the risk of subjectively-rated Ventricular and Sulcal atrophy, whereas the presence of "diabetes" was independently associated with Ventricular atrophy, such that a 63% increased risk of Ventricular atrophy was reported for those with "diabetes", although the sub-type of diabetes (Type 1 or 2) was not further defined (Knopman et al., 2005). The Cardiovascular Health Study, another large population-based neuroimaging study which examined an older cohort (aged > 65 years), noted that age and gender were significant predictors of subjectively-rated atrophy and that diabetes was associated with Sulcal atrophy in women [1288]. The Framingham Offspring Study used volumetric analyses and reported independent associations for age, smoking, diabetes, hypertension, the presence of cardiovascular diseases and global cerebral atrophy (Seshadri et al., 2004). Schmidt and colleagues reported a strong association between the presence of Type 2 diabetes, an interaction with hypertension and cortical brain atrophy in a large cross-sectional study (Schmidt et al., 2004).

In summary, the major risk factors for the development of brain atrophy appear to be advancing age and factors associated with the development of vascular disease and their interaction: Type 2 diabetes, hypertension and cigarette smoking.

4.3 CEREBRO-VASCULAR DISEASE IN T1DM

4.3.1 Epidemiology of Cerebrovascular Disease in T1DM

Relatively few studies have examined the relationship between Type 1 diabetes and the subsequent risk of developing cerebral macrovascular disease. Alex et al (Alex et al., 1962) identified a higher prevalence of cerebro-vascular disease at post-mortem in those with juvenile-type diabetes mellitus relative to controls and Grunnet and co-workers reported an earlier age of onset of cerebro-vascular disease in subjects who had Type 1 diabetes mellitus (Grunnet, 1963), both inferring that macrovascular disease of the cerebral circulation may be a significant contributor to any central nervous system abnormalities observed in Type 1 diabetes, even in chronologically young individuals. Krowelski et al suggested that the relative risk of developing cerebro-vascular disease in people who have T1DM was approximately double that observed in healthy age-matched controls (Krolewski et al., 1977) Laing et al reported the mortality from cerebrovascular disease in a very large cohort of 23,751 patients with T1DM recruited from the UK and analysed over a median 17 year review period. Laing reported the Standardised Mortality Rate (SMR) from cerebrovascular disease for the diabetes group relative to SMR from the non-diabetic population: a significantly increased SMR for cerebrovascular death was observed in all age groups of those with insulin-treated diabetes. Eighty deaths in the diabetes group were attributed to cerebrovascular disease, reflecting 6% of deaths and 4% of deaths in the under 40 years age group. Despite identifying SMR's as high as 7.6 for cerebrovascular deaths in the 20-39 year age group, the absolute risk of cerebrovascular death was small at 12 deaths per 100,000 patient-years of follow up for the entire cohort, with considerably lower absolute risks in those under the age of forty. The overall incidence of fatal cerebrovascular disease in this cohort was extremely small, of the order of 0.37%, despite the analyses including participants up to the age of 84 years (Laing et al., 2003).

The Diabetes Control and Complications Trial (DCCT) compared the incidence of diabetes-related complications in a large cohort of 1441 participants with T1DM who were of relatively young age and largely free of co-morbid diseases. The DCCT evaluated the risks and benefits two alternative insulin treatment strategies, "conventional" insulin treatment versus "intensified" insulin treatment, the latter of which aimed to attain near normal glucose concentrations. Those who were randomised to receive "intensified" insulin treatment had persistently better long-term glucose control as determined by Glycosylated Haemoglobin (HbA_{1c}) concentrations during the 6.5 years of study. The DCCT reported the incidence of new microvascular and macrovascular complications in participants who were subdivided into a primary prevention cohort, who had no evidence of complications of diabetes at entry into the study, and a secondary prevention cohort in whom modest microvascular complications were already present. No cerebro-vascular events occurred during the median 6.5 years of follow up in the DCCT in either the primary prevention cohort, or the secondary prevention cohort, presumably as a consequence of the relatively young age and modest complication burden of those studied (1995). The Epidemiology of Diabetes Interventions and Complications (EDIC) study extended the evaluation of the participants of the DCCT to a mean of duration of follow up of 17 years and reported the incidence of macrovascular complications of T1DM: a total of 144 cardiovascular events occurred in 83 patients, with a significantly higher incidence of events reported in those initially allocated to the "conventional" arm of the study, with 0.80 events per 100 patient-years observed as opposed to 0.38 events per 100 patient-years in the "intensive" group. Risk factors associated with the development of macrovascular disease in the EDIC study included the traditional risk factors of age, dyslipidaemia, obesity as well as diabetes-specific predictors such as the presence of retinopathy, albuminuria and degree of glycaemic control (Nathan et al., 2005). The incidence of cerebro-vascular events was low being recorded at 0.44%, of the EDIC cohort who had a mean age of 45 years, consistent with previous observations that overt cerebro-vascular disease is uncommon in young and middle-aged people with Type 1 diabetes.

Fuller et al examined the risk factors for the development of macrovascular complications in 1260 individuals with Type 1 diabetes who participated in the World Health Organisation Multinational Study of Vascular Disease in Diabetes (WHO MSVDD). Those studied were middle-aged, had a long duration of diabetes and were evaluated over a 12 year period. Using a Cox Proportional Hazards Model, the major independent clinical risk factors for fatal and non-fatal cerebrovascular events were the presence of isolated systolic hypertension and proteinuria, present as either microalbuminuria or dipstick positive proteinuria. Other microvascular complication of Type 1 diabetes, such as retinopathy or peripheral neuropathy, did not independently predict the occurrence of overt cerebrovascular disease (Fuller et al., 2001).

Klein and colleagues reported the incidence of cerebrovascular disease in 996 participants of the Wisconsin Epidemiologic Study of Diabetic Retinopathy over a 20 year follow up period . Those studied had T1DM of long duration and at baseline 36% were aged 20-45 years, with half of the cohort aged greater than 50 years. At the 20 year follow up 5.9% of the entire study cohort had overt cerebrovascular disease, although the incidence was only 1.3% in those aged less than 40 years at the time of follow up. Cerebrovascular disease was significantly more prevalent in older participants, especially those with poorly-controlled diabetes, diabetes of long duration, essential hypertension or a wide pulse pressure. Those with microvascular complications of diabetes had a greater incidence of cerebrovascular disease, with the severity of diabetic retinopathy being correlated to the incidence of cerebrovascular disease: those with proliferative diabetic retinopathy were at particularly elevated risk.

Davis and colleagues reported the incidence of new clinically apparent cerebro-vascular events, for fatal and non-fatal cerebrovascular disease, in 126 patients with Type 1 diabetes evaluated over a median duration of follow up of 7.2 years in the Freemantle Diabetes Study. Six new

cerebro-vascular events occurred within the period of follow up of which 5 were thrombotic strokes. Cerebro-vascular events were uncommon with an estimated incidence of 7.0 per 100,000 person years of follow up and tended to occur in middle-aged participants known to have essential hypertension, or who were receiving treatment with anti-hypertensive medications. The only independent predictor of incident cerebro-vascular disease was a low HDL concentration at baseline, although treated hypertension may mask any effect of essential hypertension in this study (Davis et al., 2005).

In a meta-analysis exploring the relationship between degree of glycaemic control and risk of macrovascular disease Stettler and colleagues evaluated eight studies in people with Type 1 diabetes (Stettler et al., 2006). Despite including 1800 patients and reporting observations over 11,293 person-years of follow up only six cerebrovascular events occurred in the eight studies included for meta-analysis. The finding from this meta-analysis, although heavily influenced by the DCCT cohort, confirm that the incidence of clinically apparent cerebrovascular disease is very low, at least in the studies of younger adults up to an approximate age of 50 years which were included in the meta-analysis (Stettler et al., 2006).

In summary, the incidence, prevalence and absolute risk of clinically apparent fatal and non-fatal cerebrovascular disease appears to be very low in Type 1 diabetes, particularly those aged less than 50 years. Despite this low absolute risk, the relative risk of cerebrovascular disease in T1DM approximates double that observed in the non-diabetic population.

4.4 Neuroimaging Studies of Cerebral Macrovascular Disease in T1DM

4.4.1 Case Studies

Bellassoued et al described a case where the acute onset of hemiballism occurred in a 26 year old male with T1DM of 13 years duration, complicated by advanced microvascular manifest as proliferative retinopathy, peripheral neuropathy and diabetic nephropathy. Infarction of the Caudate Nucleus and anterior part of the Putamen was confirmed with neuroimaging, utilising CT and MRI techniques. Bellassoued concluded that advanced microvascular complications may be associated with similar disease affecting the lenticulo-striatal arteries which provide the arterial supply to the Caudate Nucleus (Bellassoued et al., 2001). No other case reports appear to have been published to assist in identifying which territories of the intra-cranial vasculature appear most vulnerable to macrovascular disease in those with T1DM.

4.4.2 Cross-Sectional Studies

No neuroimaging studies have aimed to determine the prevalence of the structural correlates of cerebrovascular disease in people with Type 1 diabetes relative to controls. In particular, the prevalence of silent cerebrovascular disease in Type 1 diabetes mellitus is not known.

4.4.3 Prospective Studies

No studies have prospectively examined the radiological neurovascular associations of Type 1 diabetes.

4.5 MICROVASCULAR DISEASE IN TYPE 1 DIABETES

The microangiopathic complications of Type 1 diabetes have long been recognised to have a predilection for the retinal circulation, the *vasa nervosum* which provide the arterial supply to

peripheral nerves, and the renal microvasculature contributing to the respective development of Diabetic Retinopathy, Peripheral Neuropathy and Diabetic Nephropathy.

4.5.1 Epidemiology of Microvascular Disease in T1DM

The Diabetes Control and Complications Trial (DCCT) showed that the cumulative lifetime exposure to hyperglycaemia, as measured by the Glycosylated Haemoglobin (HbA_{1c}) concentration, was the principal determinant of the risk for the development of microangiopathy in Type 1 diabetes (The DCCT Investigators, 2002), and that any degree of hyperglycaemia above the normal biological range was associated with an increased relative risk of microangiopathy. *e.g.* The relative risk of retinopathy increases by 39% for every 1% rise in the HbA_{1c} above the normal reference range (The DCCT Investigators, 1996). The lack of a glycaemic threshold above normality beneath which microvascular complications could be prevented was first identified by the EURODIAB research group (Chaturvedi et al., 2001), and thereafter confirmed by the DCCT investigators (The DCCT Investigators, 1996). The DCCT compared two insulin treatments strategies, "intensive" and "conventional" insulin therapy, which achieved a 1.8% mean difference in the HbA_{1c} concentrations across the study. This improvement in glycaemic control was associated with substantial benefits for those in the "intensive" treatment group. In the Primary Prevention subgroup the incidence of diabetic retinopathy was reduced by 76%, microalbuminuria by 39% and neuropathy by 69%. The progression of diabetic microangiopathy was retarded by "intensive" insulin treatment in the Secondary Prevention subgroup: progression of retinopathy was reduced by 54%, progression to albuminuria was reduced by 56% and neuropathy by 57%. However, despite the strict glycaemic control attained in the DCCT microangiopathy still developed in a significant proportion of participants such that 24% of the "intensive" group had developed Retinopathy and 23% early Diabetic Nephropathy (as microalbuminuria) by 9 years of follow-up (The DCCT Investigators, 2000a). However, despite the DCCT/EDIC study clearly demonstrating clinically meaningful

reductions in the incidence and severity of microangiopathic complications and lasting benefits associated with the period of "intensive" treatment during the DCCT study, the most recent paper from the EDIC cohort published highlights the relative inevitability of diabetic microangiopathy in Type 1 diabetes: 89% of the "intensive" treatment group had evidence of Diabetic Retinopathy relative to 97% of the "conventional" group after 18 years of follow-up (Jacobson et al., 2007). This observation is entirely consistent with the earlier observations of the Pittsburgh Epidemiology of Diabetes Complications Study which prospectively evaluated a cohort of 657 subjects with T1DM and identified that almost all participants has at least some degree of Diabetic Retinopathy after 14 years (Orchard et al., 1990). Orchard attempted to quantify the degree of cumulative exposure necessary for the development of clinically detectable microvascular complications in 353 young adults with Type 1 diabetes of long duration, sequentially evaluated over a six year period in the Pittsburgh Epidemiology of Diabetes Complications Study. Orchard identified that an increasing burden of glycaemic exposure, defined in terms of HbA_{1c} months, was associated with the sequential development of clinical microangiopathic complications, such that early Background Diabetic Retinopathy was the first manifestation, followed by Proliferative Diabetic Retinopathy, thereafter by early Diabetic Nephropathy (microalbuminuria), overt proteinuria and eventually Peripheral Neuropathy as the cumulative hyperglycaemic burden increased. The observations of Orchard et al infer that the development of Diabetic Retinopathy is the first clinically detectable manifestation of microangiopathy, reflecting a lesser cumulative glycaemic burden than that necessary for the development of other microvascular complications of T1DM (Orchard et al., 1997).

4.5.2 Aetiology and Pathogenesis of Microangiopathy

As was clearly demonstrated by the DCCT, the principal driver for the development of microvascular complications in Type 1 diabetes is cumulative hyperglycaemia exposure, such that the degree of glycaemic control and duration of disease are important clinical factors.

Hyperglycaemia appears to be the fundamental risk factor for microangiopathy and the attainment of near normoglycaemia dramatically retards, but does not completely abolish the development of clinically detectable microangiopathy (1996). The importance of hyperglycaemia in the pathogenesis of microangiopathy is demonstrated by the Primary Prevention Cohort of the DDCT: maintenance of strict glycaemic control, equivalent to a Glycosylated Haemoglobin (HbA_{1c}) concentration of 7.5% or less, reduced the incidence of new cases of diabetic retinopathy by 76% over a mean duration of review of 6.5 years. However, despite the attainment of strict glycaemic control in the DCCT "intensive" group, microangiopathy still developed in some participants, inferring either that degree of glycaemic control attained during the DCCT was insufficiently strict to normalise microvascular function, or that factors other than hyperglycaemia *per se* may also influence the susceptibility to and progression of microangiopathy. The former assertion is supported by epidemiological evidence from the EURODIAB (Chaturvedi et al., 2001) and DCCT cohorts (1996). A brief overview of the sub-cellular mechanisms thought to underpin the effects of hyperglycaemia and an introduction to the effects of other potential factors is provided subsequently.

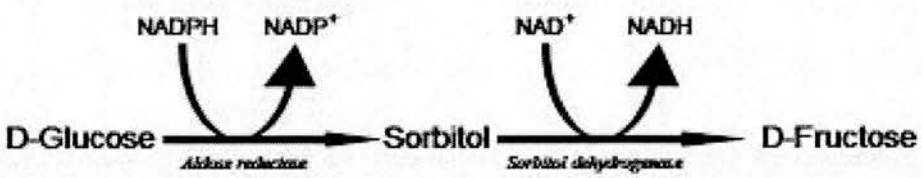
4.5.3 Hyperglycaemia and the Development of Microangiopathy

Exposure of the cell to intermittent hyperglycaemia results in a sequence of alterations of cellular metabolism, some of which may predispose to diabetic microangiopathy. Metabolic alterations induced by hyperglycaemia which have been implicated in the pathogenesis of diabetic microangiopathy include alterations in the cellular $NADH/NAD^+$ ratio, the accumulation of Sorbitol accumulation via overactivity of the Aldose-Reductase Pathway, intra-cellular Myoinositol depletion and the cumulative accumulation of Advance Glycosylation End-Products (Williams, 2006).

Aldose-Reductase (Polyol) Pathway

D-Glucose can be metabolised to Sorbitol catalysed by the enzyme Aldose Reductase, the rate of which is determined primarily by the prevailing cellular concentration of substrate, D-Glucose, such that during hyperglycaemia Sorbitol synthesis is accelerated.

Figure 4.1. The Polyol Pathway.



In some tissues Sorbitol is further metabolised to D-Fructose, catalysed by the enzyme Sorbitol dehydrogenase, an energy requiring reaction which consumes NAD^+. Sorbitol does not easily cross cell membranes and accumulation within the cell predisposes to cellular damage through osmotic swelling of the cell, or through altering the $NADH/NAD^+$ ratio which results in a degree of cellular energy depletion similar in pattern to that observed during hypoxia-ischaemia. The intra-cellular accumulation of Sorbitol also has detrimental effects on Myoinositol metabolism. Despite there being considerable evidence in laboratory animals of the importance of the Aldose Reductase pathway in the pathogenesis of diabetic microangiopathy, the evidence in humans is lacking. Trials of Aldose Reductase inhibitors, which potently inhibit hyperglycaemia-induced cellular Sorbitol accumulation, have failed to reveal any clinically meaningful benefits in human studies of neuropathy and retinopathy despite earlier work in animal models which inferred the potential for clinical utility (Chung and Chung, 2005).

Myoinositol Metabolism

The majority of Myoinositol is derived from dietary intake and is present in high intra-cellular concentrations, with considerably lower extra-cellular concentrations. Myoinositol is the precursor for Phoshotidylinositol, an important regulator of the cellular membrane Na^+-K^+-ATPase via a signalling pathway which also involves Diacylglycerol and Protein Kinase C. The cellular membrane Na^+-K^+-ATPase regulates intra-cellular Sodium and Potassium concentrations and maintains the gradient of these ions between the intra-cellular and extra-cellular compartments. The accumulation of intra-cellular Sorbitol during hyperglycaemia inhibits the entry of Myinositol into cells and can produce relative intra-cellular Myoinositol depletion. This deficiency reduces membrane Na^+-K^+-ATPase activity, promoting the intra-cellular retention of Sodium and interfering with the generation of action potentials, the most notable effect of which is to slow peripheral nerve conduction velocities. The effects of Myoinositol depletion on the neurophysiology of central nervous system neurones remains poorly defined (Chung and Chung, 2005).

Protein Kinase C

Protein Kinase C (PKC) is a ubiquitous cellular enzyme and one of a large super-family of serine-threonine kinases. PKC is known to have at least 12 protein isoforms, the expression of which is tissue-specific and tightly regulated. Physiological activators of PKC include specific hormonal, neuronal and growth factor stimuli. However, intra-cellular hyperglycaemia through an indirect effect which increases Diacylglycerol concentrations can result in increased activation of some PKC isoforms in specific cell types. In particular, increased activity of the PKC isoforms β and δ have been implicated in the development of a pro-microangiopathic state through at least three separate mechanistic pathways. Firstly, activation of the β and δ isoforms of PKC induces the expression of Transforming Growth Factor β (TGFβ), the net effect of which is increased synthesis of extracellular matrix and thickening of the cellular basement membrane. Secondly,

PKC activates NADPH oxidase which leads to the production of oxygen free radicals and resultant tissue oxidative stress. Thirdly, PKC activation appears to inhibit Nitric Oxide synthesis, a potent vasodilator and determinant of vascular smooth muscle relaxation, resulting in a degree of microvascular hypertension and endothelial dysfunction (Tooke et al., 1996). The development of basement membrane dysfunction and permeability, oxidative stress and endothelial dysfunction appear to be integral factors in the development of diabetic microangiopathy (Williams, 2006). In rodent models Ruboxistaurin, a potent of inhibitor of the PKC isoforms implicated in the pathogenesis of diabetic microangiopathy, has been shown to substantially reduce many of the histological abnormalities characteristic of diabetic microangiopathy. Studies in the setting of human diabetic nephropathy are underway but clinical trials of PKC inhibition in diabetic retinopathy have shown modest beneficial effects at best (Williams, 2006).

Advanced Glycosylation End-Products

Glucose is able to become non-enzymatically attached to the amino acid side chains of proteins, the rate of which is determined by the ambient glucose concentration. During hyperglycaemia this non-enzymatic glycosylation is accelerated and initially results in the formation of potentially reversible intermediate products, termed Schiff bases, which then undergo rearrangement in the setting of continuing hyperglycaemia to form more stable glycation products, known as Amadori products. This process is identical to that which is responsible for the non-enzymatic glycosylation of Haemoglobin, to form Glycated Haemoglobin (HbA_{1c}), which is presently accepted as the gold-standard surrogate measure of glycaemic exposure over preceding weeks. Non-enzymatic glycosylation of proteins affects many protein sub-classes across many tissues and such glycation may interfere with functionality. In the face of chronic hyperglycaemia, Amadori products can form irreversible glycosylated protein structures called Advanced Glycation End-Products (AGE), through the formation of glucose-derived cross-links with other

glycosylated proteins. Such AGE's cannot be enzymatically degraded and accumulate in affected tissues. AGE accumulation is particularly notable in proteins with a long lifespan such as those present in the skin and connective tissues, including the basement membrane and adventitia of blood vessels. Accumulation of AGE within these sites can interfere with the normal synthesis, metabolism and enzymatic degradation of cellular and extracellular proteins, thereby affecting their functional characteristics. In blood vessels and capillaries AGE accumulation promotes thickening of the basement membrane and altered membrane permeability, which is one characteristic of diabetic microangiopathy. Circulating AGE also have the ability to bind to receptors for AGE (RAGE) which are expressed on endothelial cells, macrophages, renal mesangial cells and other cell types. Activation of RAGE receptors modulates the behaviour of target cell types, promote the release of vasoactive factors, cytokines and growth factors, all of which can modify microvascular function, membrane permeability and induce cellular proliferation in a disadvantageous manner (Williams, 2006). The clinical relevance of the tissue accumulation of AGE was first demonstrated by Schalkwijk and colleagues who reported an independent association between the concentration of circulating AGE-Albumin and the development of diabetic retinopathy and microalbuminuria in the 447 T1DM participants of the EUCLID study (Schalkwijk et al., 2002). The DCCT investigators confirmed the potential clinical utility of AGE in T1DM: baseline concentrations of skin glycosylation products, notably as Furosine (AGE-Collagen) predicted the 10-year risk of developing diabetic retinopathy or nephropathy with considerably greater accuracy than that predicted by the presently accepted gold-standard methodology for assessing cumulative glycaemic exposure, the glycated haemoglobin, HbA_{1c} (Genuth et al., 2005). The effects on microangiopathy of inhibitors of glycation, AGE cross-link breakers and other modifiers of AGE biology are presently under evaluation in laboratory animals and the clinical efficacy of such compound remains untested.

4.5.4 Factors other than Hyperglycaemia

Apart from cumulative exposure to hyperglycaemia, which explains the majority of variance observed in the development of microangiopathy, other factors appear to play a role of lesser importance. These factors may increase the vulnerability to developing microangiopathy, or alternatively, may accelerate the progression of established microvascular disease. This section will briefly review those factors of relevance, with a particular focus on their impact upon diabetic retinopathy, which is a potential surrogate marker for the presence of intra-cranial microangiopathy.

Essential Hypertension and Microangiopathy

Hypertension is itself an independent risk factor for the *de novo* development of microangiopathy, independent of any disturbances of glucose tolerance. Hypertensive retinopathy, the development of microvascular abnormalities of the retinal circulation, is a common complication of untreated essential hypertension. Hypertension causes progressive abnormalities of the retinal microvasculature which are graded by severity using the Keith-Wagner Scale (Dodson et al., 1996). Clinically these microvascular changes are manifest as Silver Wiring of arterioles (Grade 1 retinopathy), Arterio-Venous Nipping (Grade 2 retinopathy), breakdown of the blood-retinal barrier to produce flame-shaped intra-retinal haemorrhages, lipid leakage termed as 'hard exudates' and ischaemia of the nerve fibre layer known as 'soft exudates' (Grade 3 retinopathy). The most severe degree of hypertensive retinopathy is associated with the development of cerebral oedema and ischaemia of the optic nerve head to produce papilloedema (Grade 4 hypertensive retinopathy). These abnormalities are commonly observed in the non-diabetic population over the age of 40 years, with a reported prevalence range from 2-14% in epidemiological studies (Wong and Mitchell, 2007). In addition, the presence of hypertensive retinopathy increases Stroke risk two to four-fold, after adjustment for confounding effects of diabetes, age, blood pressure and hyperlipidaemia, strongly supporting the concept that

hypertensive retinopathy is a marker for microangiopathy elsewhere in the brain (Wong and Mitchell, 2007). Furthermore, the presence of hypertensive retinopathy is a marker of generalised target organ hypertensive damage being associated with the presence of microalbuminuria, renal impairment and left ventricular hypertrophy in Type 1 diabetes (Chaturvedi et al., 2001).

In addition to its *de novo* effects on the microvascular circulation, essential hypertension appears to accelerate the rate of progression of diabetic microvascular disease. The EUCLID study examined the effects of the Angiotensin-Converting Enzyme Inhibitor (ACEI) in a double-blind placebo-controlled study of the incidence and progression of diabetic retinopathy and nephropathy in a population with T1DM, 15% of whom had early nephropathy in the form of microalbuminuria. The EUCLID study identified that treatment with the blood pressure lowering ACEI was independently associated with a statistically significant 50% reduction in the progression of diabetic retinopathy or the development of proliferative diabetic retinopathy, as defined by the use of a standardised grading system (Chaturvedi et al., 1998). Klein et al in the 14 year report from the Wisconsin Epidemiology of Diabetic Retinopathy Study identified hypertension as a clinically significant factor in the progression to proliferative retinopathy [(Klein et al., 2003).

Dyslipidaemia and Microangiopathy

Limited data exist which address the relationship between dyslipidaemia and the development of microvascular complications. In a large cross-sectional cohort of 1362 subjects with T1DM from the EURODIAB study, Karamanos observed significant differences in the fasting lipid profiles of those with microangiopathy relative to those free of microangiopathy: the presence of microangiopathy was associated with lower HDL-cholesterol and higher Triglyceride concentrations (Karamanos et al., 2000). The EURODIAB group also identified a modest independent association between fasting hypertriglyceridaemia, defined as triglycerides >

1.7mmol/L, and the prospective development of retinopathy in a cohort of 764 T1DM participants evaluated over a 7 year period (Chaturvedi et al., 2001): the relative risk of retinopathy was 24 % greater in those with baseline hypertriglyceridaemia. Thomas and colleagues reported similar associations between an adverse lipid profile and the progression of diabetic nephropathy in a prospective study of 152 individuals with T1DM (Thomas et al., 2006). In a more detailed analysis of lipid sub-fractions and their relationship to the development of diabetic retinopathy, the DCCT/EDIC investigators reported a similar association between hypertriglyceridaemia and a low HDL-cholesterol in a study of 988 subjects with T1DM but also identified the presence small dense LDL particles, small dense HDL particles and Apolipoprotein-B concentrations as independent predictors of the presence and severity of diabetic retinopathy. Epidemiological data appear to suggest that a pro-atherogenic lipid profile may also be disadvantageous to the microvascular circulation, in addition to the well described effects of such a lipid profile on the risk of macrovascular disease. This has led to the hypothesis that lipid-lowering drugs may have the potential to reduce the incidence diabetic microangiopathy (Leiter, 2005) a hypothesis which has not as of yet been formally tested.

Pregnancy and Microangiopathy

The association between pregnancy and the potential for rapid progression of diabetic retinopathy has been common clinical knowledge for decades. The DCCT investigators confirmed this supposition in a sub-study of 270 women who became pregnant during their participation relative to 500 female controls who did not: a tripling of the risk of clinically meaningful progression of retinopathy was observed in pregnant women, but longer term follow up indicated that the risks conferred by pregnancy were transient, rapidly returning to baseline risk within the first post-partum year. Despite accelerated progression of retinopathy during pregnancy no long-term negative impact on visual function was observed, perhaps relating to the careful ophthalmological care provided. Pregnancy therefore appears to be a state which may significantly accelerate pre-

existing retinopathy or promote the development of early retinopathy in those without any existing retinopathy (2000b).

Smoking and Microangiopathy

Smoking is known to adversely affect vascular biology [(Lehr, 2000) and the accumulation of AGE is greater in smokers (Nicholl et al., 1998), factors which could potentially influence the development and rate of progression of microvascular complications. Reichard reported an association between smoking and the progression of microvascular complication of T1DM in the 96 participants of the Stockholm Diabetes Intervention Study (Reichard, 1992). The EURODIAB group identified that microvascular complications, as retinopathy and microalbuminuria, were more prevalent in smokers relative to non-smokers with Type 1 diabetes (Chaturvedi et al., 2001) and demonstrated that smoking independently predicted the development of early retinopathy in T1DM (Karamanos et al., 2000). Chase et al evaluated the impact of smoking on the development and progression of microangiopathy in T1DM and identified an association between cigarette smoking and a higher prevalence of diabetic retinopathy, microalbuminuria (an early manifestation of diabetic nephropathy) and the rate of progression of these complications. Following multivariate adjustment for the potential confounding effects of age, glycaemic control, blood pressure and gender the detrimental influence of smoking persisted with respect to the deterioration of diabetic nephropathy but no significant effect on retinopathy progression was apparent (Chase et al., 1991). However, these findings conflict with the observations of the EURODIAB group who found no independent association between cigarette smoking and the progression of diabetic nephropathy in a 7 year prospective evaluation of 532 subjects with T1DM complicated by microalbuminuria (Giorgino et al., 2004). Data which have evaluated the relationship between diabetic retinopathy and cigarette smoking are contradictory and the case for a definitive detrimental appears unproven.

Genetic Polymorphisms and Diabetic Retinopathy

Genetic susceptibility to the development of diabetic retinopathy has been described but the associations identified between potential candidate genes studied and the development or progression of retinopathy have been statistically weak and often not replicated by other studies (Warpeha and Chakravarthy, 2003). The association between diabetic retinopathy and polymorphisms of the following genes have been examined. Studies have reported weakly positive associations between polymorphisms of the HLA genes and the Immunogloblin-G gene and retinopathy in T1DM, whereas mixed findings have been reported in association with polymorphisms of the promoter region of the Aldose Reductase gene. No associations between diabetic retinopathy were reported in association with polymorphisms of the GLUT-1, Plasminogen-Activator-1 Apolipoprotein-E, Type IV Collagen, Angiotensin Converting Enzyme, Nitric Oxide Synthase or Endothelin-1 genes (Warpeha and Chakravarthy, 2003).

4.6 DIABETIC RETINOPATHY

The retinal circulation, although technically extra-cranial in site, is derived from the Ophthalmic Artery, a terminal branch artery of the Middle Cerebral Artery. The retinal circulation therefore shares embryological commonality with the remainder of the intra-cranial vasculature which fostered the hypothesis that the presence of diabetic retinopathy may be a marker for microangiopathy elsewhere within the cerebral brain. Diabetic retinopathy varies in its clinical severity and is formally categorised from the most minor changes termed "Background Diabetic Retinopathy" (BDR) through to "End-Stage Diabetic Eye Disease". The progression from the early changes of BDR through to more advanced degrees of sight-threatening retinopathy tend to occur in a stepwise manner, driven predominantly by hyperglycaemia (2000a), although essential hypertension (Klein et al., 2003), pregnancy (2000b), and perhaps smoking (Reichard, 1992) appear to accelerate the rate of progression. Counter intuitively, sudden improvements in glycaemic control have also been long recognised to have the potential to accelerate diabetic

retinopathy. This supposition was confirmed by the DCCT investigators (1998) and may account for at least part of the negative consequences of pregnancy on retinopathy progression. Genetic polymorphisms have been posited as potential mediators and could be of some influence, although studies to date have not identified any association which appears to have more than a minor influence on the development or progression of diabetic retinopathy (Warpeha and Chakravarthy, 2003).

4.6.1 Grading the Severity of Diabetic Retinopathy

The most widely accepted grading system for the epidemiological study of diabetic retinopathy is the modified Airlie House Grading system (1991). The development and progression of Diabetic Retinopathy is primarily determined by cumulative glycaemic exposure and the prevailing degree of glycaemic control, as determined by the Glycated Haemoglobin concentration (HbA_{1c}) (The DCCT Investigators 2000a; The DCCT Investigators, 2002). The accepted gradings of severity of Diabetic Retinopathy are:

1. *Background Diabetic Retinopathy*

Characterised by the presence of two or more microaneurysms in at least one eye as a minimum diagnostic criteria, often with scattered areas of exudation, superficial retinal haemorrhages, minor venous changes and occasionally areas of focal retinal ischaemia, present as 'Cotton Wool Spots', of which no more than 5 lesions are present.

2. *Pre-Proliferative Retinopathy*

Pre-proliferative Diabetic Retinopathy has all of the features of Background Retinopathy but with additional features which are indicative of severe retinal ischaemia. These include the presence of Intra-Retinal Microvascular Abnormalities (IRMA), the presence of deep intra-retinal haemorrhages termed Cluster Haemorrhages, > 5 Cotton Wool Spots and the development of

venous changes. Venous changes are particularly pathognomic of severe retinal ischaemia and are characterised by venous beading (irregular sausage-like irregularities of luminal diameter), venous dilatation or venous reduplication.

3. *Proliferative Diabetic Retinopathy*

Proliferative Diabetic Retinopathy is characterised by the development of fragile new blood vessels, formed in the environment of a severely ischaemic retina. These new vessels have a propensity to bleed spontaneously to produce pre-retinal or vitreous haemorrhage, with the subsequent risk of visual loss and retinal detachment.

4. *End-Stage Diabetic Eye Disease*

The most advanced stage of diabetic eye disease is characterised by Retinal Detachment, Vitreous Heamorrhages, Neovascular Glaucoma and blindness.

4.6.2 Neuropathological Studies of Cerebral Microangiopathy

For many years the cerebral circulation was thought to be resistant to the development of diabetic microangiopathy and to this day very little is known with respect to the histological effects of Type 1 diabetes on the human brain. DeJong and colleagues had reported the presence of multiple cerebral abnormalities, observed at post-mortem in a young patient with long-duration Type 1 diabetes, which were not consistent with the pattern of hypoglycaemia-related neuropathological damage previously described by Baker (1939) and Lawrence (1942), and further explored by Lawrence in 1952 (DeJong, 1977;Reske-Nielsen and Lundbaek, 1963b). In 1963 Reske-Neilsen and colleagues reported the cerebral histological abnormalities observed in an autopsy study of three young patients with Type 1 diabetes. The same research group thereafter performed a more detailed examination of the neuropathological consequences of "juvenile-type diabetes" and its associated clinical complications in a larger post-mortem series of

16 patients and observed gross pathological abnormalities affecting gray-matter, white-matter and the lepto-meninges which were co-existent with multiple histopathological abnormalities. These histological changes included changes in the structure of the cerebral microvessels where hyalinosis of vessel walls, thickening of the capillary basement membrane and increased peri-vascular connective tissue was observed, in keeping with the microscopic findings associated with diabetic microangiopathy in other tissues, inferring that the cerebral circulation may also be vulnerable to microangiopathy in T1DM. However, in addition to advanced microangiopathic complications the cohort evaluated by Reske-Nielsen had other extensive co-morbidity including previous exposure to severe hypoglycaemia, hypertension, chronic renal failure and clinically manifest psychiatric symptomatology. The post-mortem observations could not be directly correlated to the presence of any particular clinical complications of Type 1 diabetes and Reske-Nielsen and colleagues concluded that multiple different pathogenic factors may be responsible for the gross changes described at autopsy (Reske-Nielsen and Lundbaek, 1963b). Since this report, published in excess of 40 years ago, no other histo-pathological analysis of the effects of Type 1 diabetes on the cerebral circulation has been published in the main stream medical literature.

4.6.3 Neuroimaging Associations of Retinal Microangiopathy

In disease states other than diabetes, the presence of retinopathy, defined as pathological changes in the vessels of the retinal microcirculation, is associated with the presence of microangiopathy of the cerebral circulation. Mitchell and colleagues evaluated the relationship between retinal markers of microvascular disease, present as microaneurysms or retinal haemorrhages, in a non-diabetic elderly cohort and reported an association with the incidence and mortality from cerebrovascular disease (Mitchell et al., 2005). This has been best described in essential hypertension where the presence of hypertensive retinopathy is associated with the presence of high-intensity white matter lesions and their rate of progression (Ikram et al., 2006). In a study of

elderly subjects, Longstreth and colleagues reported a higher incidence of white matter lesions observed on MRI in those with retinal small vessel disease, interpreting the presence of WML as being indicative of cerebral microvascular disease (Longstreth, Jr. et al., 2007). Wong and colleagues reported the neuroimaging associations of the presence of retinal microvascular abnormalities in a middle-aged and elderly cohort of 1684 healthy individuals participating in the Atherosclerosis Risk In Communities (ARIC) study: those with retinopathy exhibited greater degrees of sulcal and ventricular atrophy (Wong et al., 2003) and also had a significantly higher prevalence of high-intensity lesions of the cerebral white matter [(Wong et al., 2002). The ARIC study included some participants with diabetes, predominantly as Type 2 diabetes, but the relationship between retinopathy and structural brain abnormalities persisted following statistical adjustment for diabetes. Wong concluded that the presence of retinal microangiopathy was indicative of microangiopathy of the brain, a conclusion supported by the higher incidence of stroke subsequently observed in the prospective arm of the ARIC study in those who had retinopathy at baseline (Wong et al., 2002). In keeping with this observation, Inoue and colleagues reported an independent association between the presence of retinopathy and lacunar infarcts, in a middle-aged to elderly cohort some of whom had Type 2 diabetes (Inoue et al., 1996). The Wisconsin Epidemiologic Study of Diabetic Complications reported an increased incidence of cerebrovascular episodes in association with the presence of diabetic retinopathy in the 996 participants with Type 1 diabetes: severity of retinopathy significantly predicted the incidence of Stroke, with a significantly higher incidence reported in those with proliferative or advanced background retinopathy. This association was strongly influenced by age, such that the correlation reported between the severity of diabetic retinopathy and Stroke was only significant in those aged more than 50 years, as a paucity of end-points was observed in younger participants (Klein et al., 2004)

4.7 NEUROPSYCHOLOGICAL STUDIES AND MICROANGIOPATHY

4.7.1 Case Reports

In contrast to the literature that exists summarising the cognitive deficits observed in case reports of the acute metabolic decompensations of Type 1 diabetes, as either severe hypoglycaemia or diabetic keto-acidosis, there are no published case reports describing cognitive deficits in association with microangiopathy. This may reflect the insidious and chronic progressive nature of microangiopathy per se which does not become clinically evident as acute neuropsychological or neurological deterioration.

4.7.2 Cross-Sectional Studies

Relatively few studies have explored the relationship between the microvascular complications of Type 1 diabetes, defined as retinopathy, peripheral neuropathy or nephropathy and their association with cognitive performance. Rennick and colleagues (Rennick PM et al., 1968) first reported relative cognitive disadvantage in association with diabetic microangiopathy. Rennick evaluated the cognitive performance of 30 adults with Type 1 diabetes utilising two validated neuropsychological test batteries: the Wechsler Adult Intelligence Scale (WAIS) and the Halsted-Reitan Neuropsychological Battery. The 15 participants with microangiopathy, present as diabetic retinopathy, performed significantly more poorly on the sub-components of the test batteries which appraised abstract reasoning skills and complex problem solving, relative to the remaining participants who were free of microangiopathy. Performance on the other aspects of cognitive ability evaluated appeared unaffected by microangiopathy (Rennick PM et al., 1968). Skenazy and Biggler used the same neuropsychological test battery to study two groups, defined as "less severe" and "more severe", according to the accumulation and severity of the microangiopathic complications of Type 1 diabetes (Skenazy and Bigler, 1984). The "more severe" group, which had the greater microvascular burden, demonstrated relative deficits in

mental flexibility and psychomotor speed. The pattern of dysfunction observed by Skenazy and Biggler in association with microangiopathy differed to that reported by Rennick et al, which may reflect the diversity of the microangiopathic disease of those studied, or the psychometric tests utilised. Dejgaard and colleagues described the neuropsychological, neurophysiological and Magnetic Resonance neuroimaging correlated of the clinical complications of Type 1 diabetes in three groups: a short-duration T1DM group free of microvascular complications (n=20); a long-duration T1DM group with established microangiopathy (n=20) and age-matched healthy control group (n=19) (Dejgaard et al., 1991b). Cognitive performance, relative to that of a reference population of 120 healthy controls, was significantly poorer in the long-duration T1DM group who exhibited relative deficits in verbal learning, visual memory, fluid intelligence (WAIS performance sub-tests) and frontal lobe and executive function, whereas visuo-motor speed and visuo-spatial abilities appeared preserved (Dejgaard et al., 1991b). Ryan administered a battery of neuropsychological tests to 75 adults with T1DM and a demographically matched non-diabetic control group. Relative to control subjects, those with T1DM performed significantly more poorly on measures of psychomotor efficiency and spatial information processing, whereas performance with respect to verbal intelligence, learning, memory, problem solving and simple motor speed were equivalent. Multiple regression analyses identified microvascular complications, particularly the presence of distal symmetrical polyneuropathy, as an independent determinant of psychomotor slowing, leading to the hypothesis that a "central neuropathy" may also potentially complicate Type 1 diabetes (Ryan et al., 1992). In the largest study to date to examine the cognitive associations of microangiopathy Ryan and colleagues thereafter examined the performance of 142 subjects with T1DM and 100 age and ability-matched healthy controls on measures of general intelligence, sustained attention, visual scanning and rapid decision making, mental flexibility, memory and motor speed (Ryan et al., 1993). The 142 T1DM subjects had been diagnosed with diabetes before their 17th birthday and had diabetes of long duration. Independent determinants of cognitive performance were identified using regression techniques.

Ryan identified the presence of distal symmetrical polyneuropathy as the most powerful disease-related determinant of cognitive performance. The presence of neuropathy was disadvantageous to all of the cognitive abilities examined with the exception of memory. The presence of diabetic retinopathy also independently influenced motor speed, despite the presence of neuropathy and retinopathy being moderately correlated (r^2=0.39) and sharing pathogenic commonality. The presence of microangiopathic complications of Type 1 diabetes was associated with poorer performance on tests of sustained attention, visuospatial ability and psychomotor speed (Ryan et al., 1993).

In contrast to the widely divergent findings previously described in studies evaluating the effects of recurrent exposure to severe hypoglycaemia on cognitive abilities in T1DM, only a single study has failed to report significant ability differences in association with microangiopathy. Lawson and co-workers compared the performance of 40 subjects with T1DM complicated by peripheral and autonomic neuropathy, relative to a control group and observed no between-group differences in performance across a number of cognitive domains (Lawson et al., 1984). Lawson concluded that the presence of advanced microvascular complications of diabetes did not appear harmful to cognitive performance. However, the T1DM and healthy control groups studied by Lawson and colleagues were not matched for pre-morbid ability, which undermines the interpretations and conclusions of this study, as the lack of differences observed may reflect inherent differences in the crystallised intelligence of the study groups.

In summary, the presence of microvascular complications of Type 1 diabetes, particularly as peripheral neuropathy, may be associated with a degree of cognitive disadvantage which appears to affect performance across a broad variety of cognitive domains.

4.7.3 Prospective Studies

No prospective studies have examined the cognitive consequences of the development of microvascular complications of Type 1 diabetes of adult onset. In particular, analyses of the DCCT have not explored this research question to date, as all published analyses addressing cognitive ability have been performed on an "intention to treat" basis, or in relation to summary variable of exposure to severe hypoglycaemia. The most recent publication from the DCCT investigators pertaining to the cognitive abilities of participants assessed after a mean total duration of 18 years of follow up in the EDIC study has indicated that the vast majority of the original DCCT participants now have established microangiopathy (Jacobson et al., 2007). The EDIC study is therefore not best placed to address the cognitive associations of microangiopathy in Type 1 diabetes.

4.7.4 Meta-Analyses

Formal meta-analysis techniques have not been applied to explore the relationship between the microangiopathic complications of T1DM and cognitive ability, due to the relatively small number of studies examining this hypothesis and their heterogeneity in terms of those studied and the diversity of the neuropsychological instruments utilised.

4.8 NEUROIMAGING STUDIES AND MICROANGIOPATHY IN T1DM

4.8.1 Case Reports

In stark contrast to the multiplicity of case reports which provided an outline to guide researchers exploring the likely structural brain correlates of severe hypoglycaemia or diabetic keto-acidosis, there are scant case reports published which describe the neuroimaging associations of microangiopathic complications, apart from the single case report of Bellassoued and colleagues (Bellassoued et al., 2001) which was discussed previously. This may reflect the chronic and

insidious nature of microangiopathy relative to the clinically dramatic acute neurological decompensations that can accompany the presentation of Severe Hypoglycaemia or Diabetic Keto-Acidosis.

4.8.2 Cross-Sectional Studies

Dejgaard and colleagues (Dejgaard et al., 1991b) first explored the neuroimaging correlates of microangiopathy in evaluating the MRI appearances of sixteen patients with long-duration Type 1 diabetes, complicated by diabetic retinopathy and peripheral neuropathy, relative the appearances observed in non-diabetic healthy controls (n=40). The diabetes group had a substantial microvascular burden: each had peripheral neuropathy and a degree of background or proliferative retinopathy, with a proportion also having clinically apparent autonomic neuropathy and overt diabetic nephropathy, yet none had previously experienced severe hypoglycaemia. The MRI techniques utilised were commensurate with the year of study and relative to current techniques could be considered relatively insensitive for detecting abnormalities of white matter. Despite this caveat, high-intensity white matter lesions were observed in 69% of the diabetes group, of whom 31% were also considered to have a degree of subjectively-rated cerebral atrophy. The white matter lesions observed by Dejgaard were non-specific in their distribution, being located throughout the cerebral hemispheres, cerebellar hemispheres and brain stem, were small in size and infrequent in number in each subject. White-matter lesions (69%) were significantly more common in the microangiopathic group relative to healthy age-matched controls (12%) and no neuroimaging evaluation was performed in the short-duration Type 1 diabetes controls. Dejgaard et al interpreted the higher prevalence of white-matter lesions observed in the microangiopathic group as being indicative of the presence of intra-cranial microangiopathy, complicating Type 1 diabetes, or alternatively as being indicative of small foci of ischaemia, known as leukoaraiosis (Pantoni and Garcia, 1995) as part of a subcortical arteriosclerotic encephalopathy (Dejgaard et al., 1991b). Youssem and colleagues (Yousem et

al., 1991) in a study published in the same year also hypothesised that the presence of proliferative diabetic retinopathy may be associated with co-existent white-matter abnormalities of the brain visible on MRI as a neuroimaging indicator of intra-cranial microangiopathy. Youssem compared the frequency of white-matter lesions in 25 adult patients with Type 1 diabetes complicated by proliferative diabetic retinopathy (severe retinopathy) relative to 10 age-matched healthy control subjects using MRI techniques consisting of T1-weighted and Proton Density images. In contrast to the observations of Dejgaard, no white-matter lesions were identified in any study participant and Youssem et al concluded that proliferative diabetic retinopathy was not associated with white-matter pathology and that microangiopathy of the brain was unlikely in T1DM. Advances in MRI technology in the intervening years since publication have since revealed that the imaging sequences utilised by Youssem are unsuited for the quantification of white-matter changes, being unlikely to detect subtle differences.

In a small underpowered cross-sectional study Lunetta and co-workers examined a heterogeneous group of ten patients with Type 1 diabetes, who had a relatively short median duration of diabetes, variable hypoglycaemia exposure and differing degrees of microangiopathy (Lunetta et al., 1994). MRI brain appearances were compared to control subjects, of which little detail is provided in the paper. The MRI sequences utilised included T1 and T2-weighted images and the cerebro-ventricular index (CVI) was calculated as a surrogate measure of dilatation of the lateral ventricles. Lunetta reported an increased CVI in 70% of the diabetes group, as well as a degree of subjectively-rated cortical atrophy, interpreting these observations as being indicative brain atrophy in the diabetes group relative to control subjects. No mention of white-matter abnormalities were reported in the manuscript. The study was underpowered to address any of its primary aims, the definition of an abnormal CVI was suspect, the control group undefined and the conclusions should be interpreted accordingly.

In summary, the limited neuroimaging data available suggest that the microangiopathic complications of Type 1 diabetes may be associated with structural abnormalities of the central nervous system, perhaps as high intensity white matter lesions and possibly cerebral atrophy. However, the heterogeneous nature, small sample sizes and methodological flaws of some published studies preclude definitive interpretation.

4.8.3 Prospective Studies

No prospective studies have examined the structural brain correlates of Type 1 diabetes and associated microvascular complications.

4.9 APOLIPOPROTEIN-E POLYMORPHISMS AND THE BRAIN

Genetic factors have been identified as significant modulators of outcome in a variety of neurological diseases (Wright, 2005) and could partly mediate the susceptibility towards the development of cognitive impairment in Type 1 diabetes. The role of genetic factors as mediators of cognitive dysfunction following severe hypoglycaemia, or chronic hyperglycaemia has not been previously examined.

4.9.1 Genetic Polymorphisms and Cognitive Ageing

Genetic factors have been identified which appear to influence cognitive ageing and which may additionally modify the rate of age-related decline in specific cognitive abilities. Genes identified to date include the Apolipoprotein-E gene (APOE), the Brain Derived Neurotrophic Factor gene (BDNF), the Catechol-O-Methyl Transferase gene (COMT) and the multiple genes coding for subtypes of the Dopamine receptor (Savitz et al., 2006). Only the role of the APOE gene will be discussed further.

4.9.2 Neurobiology of Apolipoprotein-E (APOE)

Polymorphism of the gene for Apolipoprotein-E (APOE) is the most important single genetic determinant of late-onset Alzheimer's disease (Corder et al., 1993). The APOE gene has three common alleles (ε2, ε3, ε4) coding for three corresponding protein isoforms, designated as E2, E3 and E4 respectively, each of which vary in their biological activity. The APOE E4 isoform has the least biological activity and the bioactivity of the protein isoform appears to be of clinical importance. Apolipoprotein-E (APOE) mediates central nervous system cholesterol transport in an isoform-specific manner and is an important determinant of neuronal repair and cytoskeletal maintenance: the likelihood of neuronal recovery and survival following brain injury appears to be mediated in an isoform-specific manner with the least active E4 isoform conferring a disadvantage (Smith, 2000).

4.9.3 APOE Polymorphisms and Cerebral Glucose Metabolism

Small and colleagues first identified regional cerebral metabolic abnormalities in a small study of healthy middle-aged relatives of patients with Alzheimer's disease which utilised PET and MRI techniques: reduced parietal lobe metabolism and increased right-left hemisphere asymmetry was observed only in those at risk relatives who had one or more ε4 allele (Small et al., 2000). Reiman et al further refined this observation by studying regional cerebral glucose metabolism in healthy younger adults, aged 20-49 years, utilizing a combination of PET and MRI techniques. Symmetrical reductions in regional cerebral glucose utilisation (CMR_{glu}) were observed in ε4 heterozygotes in the posterior cingulate, parietal, temporal, and prefrontal cortex. The reduction observed in CMR_{glu} was apparent decades before any clinical presentation of Alzheimer's disease could reasonably be expected and was detectable in the absence of any measurable difference in cognitive ability, as determined by a neuropsychological test battery (Reiman et al., 2001;Reiman et al., 2004;Reiman et al., 2005).

4.9.4 APOE Polymorphisms and Healthy Ageing

Polymorphism of the APOE ε4 allele appears to influence aspects of cognitive ageing, even in apparently healthy individuals, although no negative consequences have been reported as yet in childhood or adulthood. Children and young adults with an ε4 allele appear not to exhibit any cognitive disadvantage on tests of general intelligence (Turic et al., 2001) and the relative frequencies of APOE genotypes appears not to differ between high and low-IQ children (Plomin et al., 1994). In another study of young Chinese women superior fluid intelligence was observed in association with the ε4 allele (Yu et al., 2000). However, by middle-age possession of the ε4 allele appears to confer disadvantage. Apparently healthy middle-aged adults possessing an ε4 allele exhibit subtle deficits in learning and memory abilities relative to those with other genotypes (Flory et al., 2000). APOE genotypes also appear to influence the rate at which certain cognitive abilities decline with normal ageing: healthy elderly subjects with an ε4 allele have poorer information processing speed and memory abilities (Staehelin et al., 1999) but do not appear to undergo a more rapid rate of loss of cognitive ability than that observed in those with no ε4 allele (Pendleton et al., 2002;Staehelin et al., 1999).

4.9.5 APOE Polymorphisms in Neurological Disease

Clinical research studies evaluating cognitive ability, morbidity and in some cases mortality following a variety of central nervous system insults have consistently identified a significant disadvantage in outcome in association with possession of an ε4 allele in middle-aged and elderly subjects (Smith, 2000). However, carriage of the ε4 allele, and consequently the least biologically-active E4 isoform, appears only to be disadvantageous when pathological processes affect the central nervous system: measurable negative associations of the ε4 allele are not evident during childhood or young adult life (Plomin et al., 1994;Turic et al., 2001;Yu et al.,

2000). This observation infers that the negative consequences associated with possession of the ε4 allele are not mediated through a neuro-developmental mechanism, only becoming manifest when age-related decline or detrimental pathological processes affect the central nervous system.

An association between polymorphism of the APOE gene and outcome differences has been reported following a broad spectrum of brain injuries. The APOE ε4 allele has been associated with poorer cognitive and neurological outcome in head injury (Crawford et al., 2002;Teasdale et al., 1997), rapidity of disease progression, white-matter lesion burden, mortality and disability in multiple sclerosis (Pinholt et al., 2006); reduced verbal fluency following sub-arachnoid hemorrhage and cardio-pulmonary bypass surgery (Louko et al., 2006); poorer general cognitive function in ex-professional Boxers (Jordan et al., 1997); impaired attention, information processing speed and general cognitive ability in ex-US Football players (Kutner et al., 2000); and a higher incidence of dementia following Stroke (Sudlow et al., 2006).

In summary, carriage of the APOE ε4 allele may influence glucose utilisation in certain brain regions, appears to be disadvantageous to the normal cognitive ageing process and may significantly increase the risk of subsequent cognitive impairment, death or morbidity following a variety of brain injuries. Whether this has relevance for brain vulnerability in Type 1 diabetes has not been explored.

CHAPTER 5

HYPOTHESES FOR STUDIES

5. STUDY HYPOTHESES

5.1 Influence of Diabetes Specific Factors on Cerebral Structure and Function in Type 1 Diabetes

The present study was designed to ascertain the neuroimaging and cognitive associations of the clinical complications and disease-specific factors unique to Type 1 diabetes. In particular, the relationship between the neuropsychological performance and neuroimaging correlates of preceding exposure to recurrent severe hypoglycaemia, the presence of microvascular disease (as retinopathy), the duration of diabetes and the age of onset of diabetes were of particular interest. A body of evidence exists which suggests that each of these diabetes-related factors may be disadvantageous to the brain. The present study was not designed to examine the effects of diabetes *per se* on cognitive function, rather was focused on determining the consequences of diabetes-specific disease factors on the neuropsychological and neuroimaging outcomes of interest. The hypotheses were:

1. Preceding exposure to recurrent severe hypoglycaemia may be associated with differences in cognitive ability and brain structure, particularly changes affecting gray matter structures in the cerebral cortex and deep gray matter.
2. Diabetic retinopathy may be a surrogate marker for microangiopathy elsewhere within the brain, apparent as high-intensity white matter lesions.
3. The onset of Type 1 diabetes during early childhood neurodevelopment may be disadvantageous and if associated with measurable differences in brain structure then an organic pathogenesis would be likely.

To test these hypotheses 71 young people with Type 1 diabetes of relatively long duration, who had developed the disorder during childhood or adolescence, and as a consequence may therefore

be more vulnerable to the development of cognitive impairment (Northam et al., 2001;Ryan and Becker, 1999) were recruited for study.

5.2 Influence of Diabetes-Specific Factors on Objectively Rated Structural Brain Abnormalities in Type 1 Diabetes

The neuroimaging associations of Type 1 diabetes remain under explored. Several diabetes-specific factors have been reported in the literature as being associated with the presence of structural brain abnormalities in case reports and small case-control studies. These diabetes-specific factors include exposure to protracted and profound degrees of severe hypoglycaemia, the development of Diabetic Keto-Acidosis in children and adolescents, and the development of microvascular complications as a consequence of chronic exposure to hyperglycaemia.

The present study examined a group of 133 adults with Type 1 diabetes of relatively long duration: a subgroup had developed the disorder during childhood or adolescence, and as a consequence were exposed to the potential negative consequences of diabetes during neurodevelopment and a second subgroup in which the onset of diabetes had occurred after neurodevelopment was largely complete. The present study was designed to achieve a number of aims:

1. To determine the prevalence of structural brain abnormalities, as high-intensity white matter lesions and cerebral atrophy, in a population of young adults with T1DM.
2. To determine whether structural brain abnormalities, such as white matter lesions or cerebral atrophy, were related to cognitive ability in T1DM.
3. To explore whether exposure to the clinical complications of Type 1 diabetes, as severe hypoglycaemia and microangiopathy, or an early childhood onset of Type 1 diabetes independently influenced the presence of any structural brain abnormalities.

As no healthy age-matched control group was included, the aims were not to examine the effects of Type 1 diabetes *per se* on brain structural outcome measures, rather concentrated on

determining the consequences of the diabetes-specific disease factors of interest on macroscopic neuroimaging outcomes of interest.

5.3 APOE Genotype and Cognitive Function in Type 1 Diabetes

As summarised in Chapter 4 (Section 4.7), polymorphism of the Apolipoprotein-E (APOE) gene appears to be a moderator of successful cognitive ageing from middle-age in apparently healthy adults, such that those with one or more copies of the APOE ε4 allele appear disadvantaged and may undergo more rapid cognitive decline. In addition to a role in mediating successful cognitive ageing, polymorphism of the APOE gene appears to influence the recovery of the human brain following a variety of cerebral insults, including head injury, vascular insults, demyelination and other pathologies. Type 1 diabetes can affect cerebral function, most notably through the development of intermittent hypoglycaemia. The relationship between APOE genotype and cognitive function in patients with Type 1 diabetes is unknown. The primary hypothesis of the present study was that possession of an APOE ε4 allele would associated with measurable cognitive disadvantage in a group of adults who had Type 1 diabetes of long duration, sufficient to accrue some clinical complications of the disorder. The secondary hypothesis was that severe hypoglycaemia exposure may modify any differences observed in neuropsychological performance, such that those with an APOE ε4 allele who had been previously exposed to severe hypoglycaemia would perform less well.

CHAPTER 6

METHODS

6. METHODS

6.1 SUBJECTS

The clinical characteristics and individual study inclusion and exclusion criteria are explained in detail in the individual chapters.

6.2 ETHICAL PERMISSION

Ethical permission was granted for each of the studies comprising this thesis by the Lothian Health Board Medical Research Ethics Subcommittee for Medicine and Clinical Oncology. Following a detailed explanation of the nature of the studies written informed consent was obtained from all subjects. All studies comprising this thesis were performed in accordance with the Declaration of Helsinki.

6.3 ASSESSMENT OF CLINICAL COMPLICATIONS OF DIABETES

Type 1 diabetes may be complicated by the development of acute and chronic metabolic complications.

Acute metabolic complications of Type 1 diabetes

The acute metabolic complications of type 1 diabetes include transient episodes of hypoglycaemia, and hyperglycaemic decompensation in the form of diabetic keto-acidosis (DKA).

Chronic complications of Type 1 diabetes

Chronic long-term complications of type 1 diabetes include the development of microvascular disease (microangiopathy), which may be manifest as retinopathy, neuropathy or nephropathy; premature macrovascular disease (as ischaemic heart disease, cerebro-vascular disease, peripheral vascular disease and reno-vascular disease), and recurrent exposure to severe hypoglycaemia. As the aim of the present series of studies was to determine whether any cumulative effects were present as a consequence of exposure to either recurrent exposure to severe hypoglycaemia or microangiopathy on the structure and function of the brain, only the presence of these clinical complications were assessed.

6.3.1 Assessment of preceding exposure to severe hypoglycaemia

Severe hypoglycaemia was defined in a manner consistent with the criteria of the Diabetes Control and Complications Trial (DCCT), as an episode of hypoglycaemia sufficient in severity such that external assistance was necessary to facilitate recovery (The DCCT Research Group, 1997). In addition to this clinical definition, the blood glucose concentration had to be documented at less than 2.8 mmol/l and/or the clinical manifestations had to have been reversed with oral carbohydrate, intramuscular glucagon, or intravenous glucose (The DCCT Research Group, 1997). These definitions encompass episodes of severe hypoglycaemia of varying severity; from episodes during which the patient retains consciousness but lacks the insight and volition to reverse the episode with oral carbohydrate, through to more profound degrees of hypoglycaemia sufficient to render the patient unconscious or result in a seizure secondary to neuroglycopenia.

In the present series of studies the severe hypoglycaemia exposure of participants was retrospectively assessed using a validated and formatted hypoglycaemia questionnaire (Deary et al., 1993). Using this questionnaire Deary et al have previously demonstrated a high degree of

correlation between the prospective assessment of the frequency of severe hypoglycaemia and patients self-reported estimates of their severe hypoglycaemia exposure obtained 18 months later. To further improve the accuracy of estimates of severe hypoglycaemia exposure participants in the present series of studies were requested to discuss their severe hypoglycaemia history with relatives or friends before completing questionnaires. Estimates were corroborated with case records where possible. Details were recorded of the total lifetime number of episodes, the frequency of occurrence of episodes, the total number of episodes requiring glucagon or medical assistance for recovery, and the total lifetime number of episodes of coma and hypoglycaemia-associated seizure. However, using this retrospective method of exposure it was not possible to precisely document the exact number of episodes to which participants had been exposed, neither was it possible to definitively state with exactitude the age at which the exposure to severe hypoglycaemia had occurred. The retrospective ascertainment of severe hypoglycaemia utilised in the series of studies comprising this thesis is a methodological weakness relative to the gold-standard methodology of prospective ascertainment, although is sufficient to determine with a degree of accuracy those naïve to and those previously exposed to severe hypoglycaemia. To overcome this relative methodological deficit would take decades of meticulous prospective evaluation, which is neither practical or easily funded.

6.3.2 Assessment of microangiopathy

The presence or absence of the microvascular complications of diabetes was determined in all subjects. Details of the assessment of the presence or absence of degrees of diabetic retinopathy, diabetic peripheral neuropathy and diabetic nephropathy are summarised below.

Assessment of Diabetic Retinopathy

Subjects were examined for clinical evidence of diabetic retinopathy using a composite of two separate diagnostic techniques. Two techniques were used, direct ophthalmoscopy and digital

retinal photography, as their combination has been demonstrated to increase the sensitivity and specificity of determinations of the degree of diabetic retinopathy (Aldington et al., 1995). Direct ophthalmopathy was performed following mydriasis with 1% Pilocarpine. The sensitivity of this technique when used in isolation for the detection of minimal changes of diabetic retinopathy is relatively poor, and varies according to the clinical experience of the operator. Sensitivities have been reported between 35% for general practitioners to greater than 85% for consultant ophthalmologists. For this reason the examination by indirect ophthalmoscope was complemented by digital retinal photography. Digital retinal photography was performed after mydriasis (1% Pilocarpine) using a Sony® video camera within a Topcon® digital fundus photography system. A single 45^0 field centred on the macula and with a resolution of 1200×1600 pixels was obtained. This single field assessment, which was the clinical standard at the time that the present series of studies were designed, has been subsequently demonstrated to have the potential to miss early diabetic retinopathy in the peripheral retina. Accordingly, it is now standard clinical practice to obtain two digital retinal images per fundus. As such, the techniques employed in the present studies could potentially misclassify those individuals as with minor degrees of diabetic retinopathy in the peripheral retina, but not apparent within the central retina. Technical failures can result in images which are inadequate for the grading of diabetic retinopathy. However, in the young adult population examined by the present series of studies no such technical failures were encountered. Digital retinal images were examined and scored according to criteria of the modified Airlie House grading system (Early Treatment Diabetic Retinopathy Screening Group, 1991). Digital retinal photography has a greater sensitivity than ophthalmoscopy alone for the detection of diabetic retinopathy, and the combination of both techniques when utilised together improves the sensitivity and specificity for the detection of diabetic retinopathy to approximately 85-95% and 80-90% respectively (Aldington et al., 1995). As the majority of the cognitive and intelligence tests performed in the present series of studies

were presented visually, only individuals who had no clinical evidence of diabetic retinopathy or who had minor degrees of diabetic retinopathy only (background diabetic retinopathy, Airlie House grades 1a-1c) which are not thought to interfere with visual function were selected for study. Greater degrees of diabetic retinopathy have the potential to interfere with visual function and may therefore confound the interpretation of tests of intelligence and cognitive ability.

Assessment of Diabetic Nephropathy

Each participant was screened for the presence of diabetic nephropathy. Screening was performed through the analysis of first morning specimen of urine. The concentration of albumin and creatinine in first morning specimens were determined and the ratio of urinary albumin to urinary creatinine calculated. Subjects who had urinary Albumin:Creatinine ratio (ACR) in excess of 3.5 were further examined for the presence of microalbuminuria, the earliest clinically detectable phase of diabetic nephropathy. Microalbuminuria was defined as albuminuria in excess of 30 mg/24 hours but less than 300 mg/24 hours. Macroproteinuria, or overt diabetic nephropathy, was defined as a 24 hour protein excretion in excess of 300 mg. Subjects in whom the initial urinary ACR was elevated supplied three further early morning specimens for confirmatory analysis. Subjects were requested not to consume alcohol of perform physical exercise on the day preceding the collection of these three further specimens, as alcohol and exercise both increase glomerular permeability and may transiently induce low-grade proteinuria within the microalbuminuric range. In those in whom the urinary ACR remained in excess of 2.5 (males) or 3.5 (females) despite the above precautions, and in whom a suspicion of persistent microalbuminuria remained, were not invited to participate. These exclusion criteria were applied as microalbuminuria and overt nephropathy are closely associated with the development of hypertension, a condition which is known to directly influence performance on cognitive ability tests and impact directly on the structure of the brain.

Assessment of Blood Pressure

Blood pressure was measured at the brachial artery using an appropriately sized cuff for the individual. A calibrated mercury sphygmomanometer was used for all measurements. Measurements were performed in the sitting position, after the subject had been seated for at least five minutes. An office blood pressure in excess of 140/90 mmHg was used to define the presence of hypertension.

Assessment of Peripheral Neuropathy

The presence of Peripheral Neuropathy was defined as an inability to detect the pressure applied by a 10g monofilament on the sole of the foot. Those with a history of previous foot ulceration, which infers the presence of peripheral neuropathy, peripheral vascular disease, or both processes, were not invited to participate. In addition, those with peripheral neuropathic symptoms were excluded from participation, even in the absence of objective clinical signs of peripheral neuropathy. Other more sensitive techniques, particularly the use of electrophysiological techniques, which can quantitatively measure nerve conduction velocity, were not employed due to restraints on time. However, despite this relative limitation it was unlikely that participants with significant degrees of diabetic peripheral neuropathy were recruited for study as a considerably greater cumulative glycaemic burden is required for the development of clinically apparent neuropathy than early degrees of diabetic retinopathy (Orchard et al, 1990).

6.4 ASSESSMENT OF INTELLIGENCE AND COGNITIVE ABILITY

A battery of tests was utilised to evaluate the intelligence and a range of cognitive abilities for each subject. Tests were administered by trained assessors and delivered in a standardised pre-determined manner. Assessors were blind to the diabetes-specific characteristics of those undergoing study to minimise investigator bias. Assessments included measures of pre-morbid intellectual ability, current intellectual performance, information processing ability and abilities spanning a number of other pre-specified cognitive domains. The battery of tests administered were selected on the basis of prior studies within the field (Langan et al., 1991;Perros and Frier, 1997) and weighted towards the assessment of pre-morbid intelligence, fluid intelligence and information processing ability. The specific tests utilised to measure these abilities are described in the following section.

6.4.1 National Adult Reading Test

The National Adult Reading Test (NART) is a test of word pronunciation ability and evaluates the ability of the individual to pronounce 50 irregularly spelled words (*e.g.* demesne, chord, prelate and campanile) (Nelson and Willison, 1991). Subjects are marked by trained raters on their ability to correctly pronounce the words, according to a standardised scoring system. NART scores are reliable and consistent provided the test is administered by pre-trained raters (Alcott et al., 1999;O'Carroll, 1987). NART scores remain stable over many decades of life in healthy ageing populations (Crawford et al., 2001) and in mild-to-moderate organic brain disease of differing aetiology. Scoring on the NART correlates closely with the lifetime peak intelligence quotient (IQ) of an individual and measures a type of intelligence known as "crystallised intelligence" due to its stability over time and resistance to the detrimental effects of ageing, neurological and psychiatric diseases. However, the reliability of NART scores as an estimate of pre-morbid IQ can be negatively affected by several factors. These include the technical training

of the assessor performing the test (Alcott et al., 1999) and the potential confounding effects of more severe degrees of organic brain disease (Cockburn et al., 2000) in which NART scores decline, providing a less robust estimate of pre-morbid IQ. NART scores can therefore be used to estimate the maximum intellectual ability of an individual prior to any deterioration in intellectual ability through the processes of ageing or organic disease, within the above caveats. As the series of studies presented in this thesis evaluated apparently healthy normal adults these specific deficiencies should not detract from the validity of NART scores as a reliable surrogate of pre-morbid IQ. The estimate of pre-morbid IQ from NART scores is superior to estimates derived from socio-economic factors, such as degree of education or measures of social deprivation, and for this reason the NART has been used as the estimate of 'best ever' intellectual ability in the present series of cross-sectional studies, within the limitations as summarised above.

6.4.2 Wechsler Adult Intelligence Scale - Revised

The Wechsler Adult Intelligence Scale-Revised (WAIS-R) is a tool for the assessment of current intellectual performance, validated across an age range spanning 16 years to 74 years and 11 months. The WAIS was first introduced in 1955 but subsequently modified and updated to the WAIS-R in 1981 to remove ambiguous tests and replace those with little or no discriminatory value. The full WAIS-R comprises eleven tests: six Verbal sub-tests (Information, Digit span, Vocabulary, Arithmetic, Comprehension and Similarities) and five non-verbal Performance sub-tests (Picture Completion, Picture Arrangement, Block Design, Object Assembly and Digit Symbol). Summation of individual test scores yields separate Verbal, Performance and combined Full-Scale scores which are converted to scaled-scores from which Verbal IQ, Performance IQ and Full-Scale IQ scores are derived (Wechsler, 1981). The IQ score for each sub-component can be compared with standardised reference populations, each of which exhibits a mean IQ score of 100 with a standard deviation of 15 points. In contrast to scoring on the NART, performance on the WAIS-R is extremely sensitive to the detrimental effects of ageing and can identify

deficits consequent upon subtle degrees of organic brain disease. Individual sub-tests which comprise the WAIS-R differ in their relative sensitivity to the detrimental effects of organic brain disease and normal human ageing. In general, performance on the Verbal sub-tests appears comparatively more resilient than on the Performance sub-tests. The WAIS-R provides a measure of the current intellectual performance of an individual, termed as their "fluid intelligence", due to its propensity to change with age and organic brain disease. For the series of studies presented in this thesis only the five Performance IQ sub-tests of the WAIS-R were utilised. These sub-tests were the Picture Completion, Block Design, Object Assembly and Digit Symbol tests. Performance on some of these specific tests has been previously demonstrated to be negatively affected by the development of the clinical complications of Type 1 Diabetes Mellitus.

Picture Completion

Subjects are shown a consecutive series of 20 picture cards of everyday objects or situations. From each picture an essential item is missing *e.g.* a car with missing door handles, a pair of spectacles where the nosepiece is missing. Subjects are given 20 seconds to identify the specific item missing from each test card, and the total number of correct responses is recorded from a potential maximum score of 20 points. The Picture Completion task is said to provide a measure of visual perception and long-term visual memory. Performance on the Picture Completion task is relatively resilient to ageing, certainly into the 8th decade of life in healthy populations, relatively unaffected by brain damage and is the least sensitive of the Performance IQ sub-tests (Lezak, 2004;Wechsler, 1981).

Block Design

Subjects are required to orientate a set of nine identical blocks (coloured red on two-sides, white on two-sides, with two-sides diagonal red/white) to exactly reproduce a series of ten two-

dimensional patterns displayed by the examiner on a printed card. The ten patterns presented are of ascending difficulty and subjects are required to complete each task in as short a time as possible. Marks are awarded on a structured scoring system which is dependent on the time taken to correctly complete each pattern. A maximum score of 51 may be attained. The Block design sub-test is a speed-based test which is said to provide a measure of visuo-spatial constructional ability and has been described by some researchers as providing a robust measure of current "fluid intelligence" (Lezak, 2004). Performance on the Block Design test is negatively affected by subtle degrees of brain damage, grossly affected by Alzheimer's disease and Alcohol-related brain injuries, and impaired in demyelination and other sub-cortical neuropathologies (Lezak, 2004). Scoring on the Block Design sub-test is most strongly affected by non-dominant hemisphere lesions, typically those involving the posterior aspects of the parietal lobe.

Object Assembly

Subjects are presented with a series of eccentrically cut jigsaw pieces which are presented in a pre-determined manner by the examiner and which are required to be assembled to create a recognisable object e.g. a profile, an elephant; a hand. For each of the four sets of jigsaw pieces presented, subjects are asked to assemble the object within a pre-defined time limit. Scores are allocated for the number of jigsaw pieces correctly aligned and perfect performance accrues a score of 41 points. The Object Assembly sub-test is said to measure visual constructional abilities. Scoring on this sub-test declines progressively with age and is particularly sensitive to lesions affecting the occipital lobes, particularly on the non-dominant side (Lezak, 2004).

Digit Symbol

The digits one through nine are represented by corresponding symbols. Subjects are required to write down the symbol corresponding to the given number for each in a standardised pre-determined array of 93 numbers. A maximum of 93 points can be attained according to the number of accurate responses, completed in sequential order, within the 90 seconds allocated for the task. The Digit Symbol test measures psychomotor speed, visual-motor speed and short-term visual memory(Lezak, 2004). Scoring on the Digit Symbol test declines with age, from the age of 30 years onwards, with scores exhibiting a more rapid decline after the age of sixty. Scoring is sensitive to subtle degrees of brain damage and is impaired by a broad variety of pathological processes affecting the central nervous system (Lezak, 2004).

WAIS-R Performance Score

The raw test scores for each of the WAIS-R Performance sub-tests were added together with total test scores converted into 'scaled scores' according to the criteria of the WAIS-R test manual (Wechsler, 1981). Scaled scores were used to calculate the Performance IQ for each subject. The Performance IQ score is a representative measure of the current "fluid intelligence" of an individual (Lezak, 2004).

6.4.3 Assessment of Information Processing and Cognitive Ability

Psychometric tests were utilised to evaluate other aspects of cognitive ability not captured by the use of the WAIS-R. The psychometric test battery utilised was selected on the basis of previous observations from studies of patients with Type 1 Diabetes Mellitus (Langan et al., 1991;Perros et al., 1997), weighted towards the assessment of information processing abilities and designed to be capable of identifying subtle ability differences. Through limitations of time, the assessment did not encompass all aspects of cognitive ability. In particular, frontal and executive functions were

not assessed in any depth, and memory and learning ability were not evaluated to any extent in the series of studies presented in this thesis.

6.4.3.1 Assessment of Early Visual Information Processing Ability

Inspection Time (IT) was used to assess visual perceptual speed, a component of information processing ability representing the early stages of visual information processing (McCrimmon et al., 1996). Participants discriminated between the spatial position (left or right) of two briefly-presented vertical lines of different lengths. The stimuli were backward-masked and the duration of presentation altered according to an adaptive staircase algorithm, such that the presentation time (in milliseconds) was progressively shortened until the threshold required by each subject to achieve an 85% response accuracy was reached. The duration of stimulus presentation required to reliably distinguish the stimulus (85% correct) was termed the subjects 'Inspection Time'.

6.4.3.2 Assessment of Psychomotor Speed: Choice Reaction Time

A Hick-type reaction time device was utilised to measure reaction time (Jensen, 1987). Reaction time provides a measure of the speed of information processing at the cognitive-experimental level. Reaction times consist of two separate components; termed 'decision time' and 'movement time' respectively. The device used to measure reaction time consists of a 'home' button and eight 'response' buttons equidistantly spaced from the home button. The subject is asked to maintain the 'home' button depressed with the dominant hand until one of the 'response' buttons is lit; the *decision time* is calculated from the time that the 'response' button is lit until the subject's hand leaves the home button. *Movement time* represents the time between the subject's hand leaving the 'home' button and depressing the correct 'response' button. In the present studies, measures were made of simple reaction time and 2-, 4- and 8-choice reaction times and thereafter 8- , 4-, and 2-choice reaction time and simple reaction time. Twenty trials were attempted at each level and the median Decision Time (DT) and median Movement Time (MT) in

milliseconds was recorded. Median values of the components of reaction time were recorded because multiple reaction time trials typically demonstrate a skewed distribution (Jensen, 1987).

Decision Time

Decision times lengthen with increasing complexity of the choice reaction task as additional 'response' buttons are added. This effect is known as the Hick Paradigm. The 'decision time' provides a measure of "psychomotor speed" or speed of information processing by the brain (Jensen, 1987).

Movement Time

Movement times are influenced by increasing the complexity of the task to a lesser extent than decision times and are more representative of motor speed rather than being a measure of cerebral information processing (psychomotor speed).

6.4.4 Borkowski Verbal Fluency Task

The Borkowski Verbal Fluency task is thought to assess frontal lobe and executive functions and scoring on the Borkowski Verbal Fluency task has been demonstrated to be sensitive to disruption by mild head injury (Borkowski and Benton, 1967). During the Verbal Fluency Task subjects are given one minute to state as many words as possible, beginning with a letter of the alphabet specified by the examiner. In the present series of studies the letters of the alphabet 'J', 'S', 'U' and 'M' were used. The total number of novel words stated for each letter was noted and a summation of all four recorded as the Verbal Fluency Score. Verbal fluency scoring remains stable into the 8th decade of life in healthy individuals and is sensitive to decrements in frontal lobe pathology. Verbal fluency performance is said to provide a measure of frontal lobe and executive functions (Lezak, 2004).

6.4.5 Paced Auditory Serial Addition Test (PASAT)

Performance on the PASAT provides an assessment of the ability to sustain attention and concentration and provides a measure of auditory information processing speed and flexibility (Deary I.J. et al., 1991). Subjects listen to a list of numbers which they are required to add together according to a given rule: 'add each number that you hear, to the number that preceded it'. After practice, two trials of 61 consecutive numbers are performed, the first with an interval of 4-seconds between numbers and the second with 2-seconds between successive digits, respectively. Performance on the 4-second test is associated with short-term memory ability, whereas performance on the 2-second test more closely relates to perceptual processing speed. The total number of correct responses for each test is recorded from a maximum score of 60.

6.5 ASSESSMENT OF MOOD AND ANXIETY

The Hospital Anxiety and Depression Scale (HAD) is a self-rating scale which provides a validated measure of mood and anxiety states for a subject in the week prior to testing (Zigmond and Snaith, 1983). Subjects are requested to answer 14 statements relating to mood and anxiety (*e.g.* 'I feel tense or wound up') and are instructed to complete the statements, on the basis of their mood state in the preceding week, from four potential alternative statements which best describe their present mood state. Individual responses are scored on a four-point scale from zero (not present) to three (considerable), and maximum summated anxiety and depression scores of 21 are obtainable (the minimum score is 0). Seven statements assess anxiety levels, and seven assess depression levels. Higher scores are indicative of greater levels of anxiety or depression. To determine whether a significant neurotic disorder is likely to be present, scores on the seven statements are each summated to provide sub-scale scores on the HADS-A and the HADS-D from zero to 21. In this study valid HADS subscale scores were defined as having answered at least five of seven items on both the HADS-A and the HADS-D. In order to be valid in patients with somatic problems, the HADS items were based on the psychological aspects of anxiety and

depression. The anxiety items were concentrated on general anxiety, with five of the items close to the diagnostic criteria of Generalised Anxiety Disorder. The depression items included in the questionnaire are based on anhedonia, which is considered to an essential criterion of depression (Lezak, 2004).

6.6 MAGNETIC RESONANCE NEUROIMAGING PROTOCOL

Brain magnetic resonance imaging was performed at the Department of Medical Radiology, University of Edinburgh and subsequent analysis of data was performed at the Department of Clinical Neurosciences, University of Edinburgh.

6.6.1 Magnetic Resonance Neuroimaging Protocol

Fundamentals of Magnetic Resonance Imaging

Magnetic resonance (MR) imaging generates images by applying a varying magnetic field to the body, which aligns the hydrogen atoms of the tissue under evaluation in accordance with the polarity of the applied magnetic field. When the field is released, radio waves are generated as the polarised hydrogen atoms return to their resting state. The frequency of the emitted radio waves is related to the chemical environment of the atoms as well as to their location. With computer analysis of these data, MR images can be generated.

Magnetic Resonance imaging systems are formed from several key components. These include a large magnet supplemented by shim coils to generate a sufficiently uniform powerful magnetic field. A radiofrequency (RF) coil is used to transmit controlled radio signals into the body tissue under evaluation. The characteristics of the transmitted radio signals are altered as they penetrate tissues, according to the properties of each specific tissue, and such alterations are detected by the receiver coil. Additional gradient coils assist in delineating the spatial localisation of the

returning radio signals, which are then computer integrated to generate reconstructed images for interpretation by a radiologist. A major advantage of MR techniques is the ability to alter radio signal settings to selectively enhance and delineate intra-cranial tissues. With the notable exception of bony structures most tissues within the skull vault can be outlined. This allows the accurate definition of white matter, gray matter, cerebrospinal fluid, adipose tissue and their interfaces, which facilitates volumetric analysis of specific tissue types. Alteration of the plane of imaging can also be manipulated to the specific anatomical brain area being studied, allowing regional brain areas to be outlined with a greater degree of accuracy than that permitted by previous neuroimaging techniques.

Alteration of Radio Signal Intensity and MR Images

Different image contrasts can be achieved by using specific RF pulse sequences and imaging parameters. The basic signal intensity parameters that can influence imaging are:

(1) Proton Density

The proton density (PD) of a tissue reflects the concentration of protons in the tissue, in the form of water and contained within macromolecules.

(2) T1 Relaxation Time

The T1 relaxation time reflects the longitudinal relaxation time taken by protons to revert back to their resting states after exposure to an RF pulse.

(3) T2 Relaxation Time

The T2 relaxation time reflects the transverse relaxation time taken by protons to revert back to their resting states after exposure to an RF pulse.

(4) Blood Flow

Blood flow can affect signal acquisition as rapidly flowing arterial blood produces deficits in signal. Exploitation of this characteristic underlies the development of specific sequences that facilitate MR angiography techniques.

The contrast on MR images can be manipulated by changing the parameters which characterise a pulse sequence. A pulse sequence defines the specific strength, number, timing and gradient of the RF pulses applied to the body tissue under evaluation. The two most important parameters are the repetition time (TR) and the echo time (TE). The TR is the time between consecutive 90 degree RF pulses. The TE is the time between the initial 90 degree RF pulse and the echo. T1-weighted sequences use a short TR and short TE (TR < 1000msec, TE < 30msec). T2-weighted sequences use a long TR and long TE (TR > 2000msec, TE > 80msec). T2-weighted sequences can be employed as a dual echo sequence to further enhance the contrast between tissues of interest. The first or shorter echo (TE < 30msec) is proton density-weighted, or a mixture of T1 and T2-weighted images. This image is very helpful for evaluating changes in the peri-ventricular white matter, such as those observed in demyelination or cerebral microangiopathy, because hyperintense white matter lesions are contrasted against the lower signal observed in the CSF. FLAIR (Fluid Attenuated Inversion Recovery) sequences are a further enhancement of the basic PD imaging sequence. FLAIR images are a special inversion recovery sequence in which the TI time is adjusted to match the relaxation time of the tissue whose image should be suppressed. This sequence is an important technique used to differentiate lesion type within the central nervous system.

Magnetic resonance (MR) imaging is an ideal modality for the imaging of brain structure, and has several advantages over X-ray dependent technologies such as CT scanning. The advantages of MR imaging include:

1. MR imaging does not involve the delivery of a dose of ionising radiation;
2. The imaging study can be tailored to the brain region of interest and to the disease process being studied. Radio signal intensities observed on T-1, T-2, and proton density-weighted images relate to specific characteristics of the tissues being evaluated.

Due to its high sensitivity for brain water, MR is generally more sensitive for detecting subtle brain abnormalities, during the early stages of disease, than CT based techniques. In addition, MR imaging is exquisitely sensitive for the detection of white matter changes, such as those caused by demyelination and cerebral microvascular disease. Patients with substantial white matter abnormalities on MR imaging may have an apparently normal CT scan.

6.6.2 MRI Protocol

Neuroimaging was performed using a 1.0T SPE Magnetom scanner (Siemens, Erlangen, Germany). Following midline localization, two sequences were used to image the entire brain (Lawrie et al., 1999). The first scan was a double spin-echo sequence giving simultaneous proton-density and T2-weighted images (TR=3565ms, TE=20ms and 90ms, 31 contiguous 5mm slices acquired in the Talairach plane, FOV 250mm) that were used to calculate whole brain and cerebrospinal fluid volumes using a supervised cluster analysis package (ANALYZE, Mayo Foundation, Rochester, MN, USA). The second scan, for the regional volumetric analysis, was a three dimensional magnetization prepared for rapid-acquisition gradient echo sequence consisting of an 180 inversion pulse followed by a fast low-angle shot collection (Flip angle 12, TR=10 ms, TE=4 ms, TI=200 ms, relaxation time delay time 500 ms, FOV 250mm) giving 128 contiguous 1.88mm thick slices in the coronal plane orthogonal to the Talairach plane. Inhomogeneity corrections were performed using a flood phantom.

6.7 OBJECTIVE ASSESSMENT OF IMAGING ABNORMALITIES

The MRI brain scans of all participants were reviewed by an experienced neuroradiologist (Professor Joanna Wardlaw, Department of Clinical Neurosciences, University of Edinburgh) who scored each scan for the presence of abnormalities, in particular the presence of high-intensity white matter lesions (Leukoaraiosis) (Breteler et al., 1994a;Breteler et al.,

1994b;Longstreth, Jr. et al., 1996;Mirsen TR et al., 1991;Shimada et al., 1990;Van Swieten et al., 1990;Wahlund et al., 1990) and cerebral atrophy(Breteler et al., 1994a;Breteler et al., 1994b;Longstreth, Jr. et al., 1996;Mirsen TR et al., 1991;Van Swieten et al., 1990;Wahlund et al., 1990). Professor Wardlaw assessed scans independently, and was blinded to the diabetes-specific factors of participants.

6.7.1 Cerebral Atrophy

Cerebral atrophy was defined on the basis of which area of the brain appeared to have undergone a relative loss of tissue volume. In the present series of studies scans were rated for the presence of two distinct types of cerebral atrophy: "Ventricular atrophy" (*i.e.* with enlargement of the ventricles) and "Sulcal atrophy" (*i.e.* with enlargement of the sulci). Professor Joanna Wardlaw independently assessed all of the MRI neuroimaging studies included in this thesis and was blinded to the diabetes-specific characteristics of each participant. The degree of cerebral atrophy observed within each brain area was rated on a subjective rating scale of 0-3, with the grading indicating the severity of atrophy:

Grade 0.	No atrophy
Grade 1.	Mild atrophy
Grade 2.	Moderate atrophy
Grade 3.	Severe atrophy

Using the above rating scale, the degree of cerebral atrophy observed on each MRI scan was scored separately for the presence of Ventricular atrophy and Cortical atrophy.

6.7.2 High-Intensity White Matter Lesions (Leukoaraiosis)

High-intensity white-matter lesions (Leukoaraiosis) are abnormalities detected within the cerebral-white matter on T2-weighted magnetic resonance images. White matter lesions (WML) are thought to represent focal areas of increased water content, gliosis and demyelination within

the white-matter, and are thought to signify foci of ischaemia, perhaps relating to microvascular disease of the cerebral circulation (Pantoni and Garcia, 1995). The presence and degree of Leukoaraiosis was independently evaluated by an experienced neuroradiologist (Professor Joanna Wardlaw) who was unaware of the clinical and diabetes-specific characteristics of participants. Several validated scoring systems were used to capture the intensity, distribution and appearance of WML as no single scale was judged by the rater to be an adequate summary of the type and distribution of the white matter abnormalities observed in those evaluated. Rating scales used to determine the degree of white matter changes differ in their relative sensitivity in scoring the presence of WML (Mantyla et al., 1997) and the majority of rating scales have been developed to assess WML load in elderly subjects in whom changes were often marked and advanced. Relatively few validated methodologies exist for the assessment of younger patient groups in whom the white matter pathology observed could reasonably be expected to be more subtle. The rating scales used to objectively determine the presence of white matter changes in the present series of studies were:

(1) Fazekas *et al* (Fazekas et al., 1987)

White matter lesions within the peri-ventricular white matter (PVH) are scored separately, on a scale of 0-3:

0. Absent
1. Caps or pencil-thin lining around ventricles
2. Smooth halo around ventricles
3. Irregular PVH extending into the deep white matter.

Lesions located within the deep white matter (DWMH) are scored on a scale of 0-3:

0. Absent
1. One punctate focal lesion
2. Early confluence of DWMH
3. Large areas of confluent DWMH.

(2) Shimada *et al* (Shimada et al., 1990)

The presence of WML was evaluated from three pre-determined slices and PVH and DWMH were considered together. The slices utilised were through the (a) Foramen of Munro; (b) body of lateral ventricle; (c) superior slice through the lateral ventricle. Scores were allocated on a scale of 1-4:

1. Absent or minimal PVH
2. Anterior and posterior caps or ≥ 1 focal PVH
3. Multiple PVH and early confluence of foci
4. Multiple confluent patches.

(3) Wahlund et al (Wahlund et al., 1990)

PVH and DWMH are considered together and rated on a scale of 0-3:

1. Absent
1.5 Small solitary white matter changes
2 Multiple discrete or large solitary white matter changes
2.5 Multiple partly confluent white matter changes
3 Multiple large confluent white matter changes.

(4) Van Swieten et al (Van Swieten et al., 1990)

White matter changes are assessed from three predetermined slices and PVH and DWMH are considered together. Anterior and posterior changes are assessed separately and rated on a scale of 0-3:

0. PVH absent or single lesion
1. Multiple focal lesions
2. Multiple confluent lesions throughout white matter.

(5) Mirsen et al (Mirsen TR et al., 1991)

PVH and DWMH are considered separately. PVH are rated as either absent or present. DWMH are rated on a scale of 0-4:

0. PVH absent
1. One or two focal lesions
2. Three to five lesions
3. >5 lesions
4. Confluent lesions.

(6) Breteler et al (Breteler et al., 1994a)

PVH and DWMH are considered together and rated on a scale of 0-3:

1. PVH absent or mild - small caps or lining, <5 lesions
2. Moderate PVH - regular shaped caps on both anterior and posterior of horns of the lateral ventricles; ≥ 5 punctate lesions; no confluence of lesions
3. Severe PVH – irregular outlines penetrating the deep matter at the horns of the lateral ventricles, or marked areas of WML surrounding the ventricles.

(7) Longstreth et al (Longstreth, Jr. et al., 1996)

PVH and DWMH are considered together and scored from a single slice through the lateral ventricles on a scale of 0-8:

0. Absent
1. Discontinuous PVH rim with minimal dots of sub-cortical disease
2. Thin continuous PVH rim with a few patches of sub-cortical disease
3. Thicker PVH with scattered patches of sub-cortical disease
4. More irregular PVH rim with mild sub-cortical disease
5. Mild PVH confluence surrounding the frontal and occipital horns
6. Moderate PVH confluence surrounding the frontal and occipital horns
7. Peri-ventricular confluence with moderate involvement of the centrum semi-ovale
8. Peri-ventricular confluence involving most of the centrum semi-ovale.

6.7.3 Small Punctate White Matter Lesions (SPWML)

MRI scans were also objectively scored for the presence of small punctate white-matter lesions (SPWML), representing enlarged peri-vascular spaces (typically < 1mm diameter), that were frequently observed in subjects but not accounted for by the rating scales described above that were used to evaluate white matter changes. Using the most affected hemisphere, small punctuate white matter lesions were rated on a scale of 0-3:

0. Absent
1. Mild - <10 lesions
2. Moderate - 10-20 lesions
3. Severe - >20 lesions.

The presence of SPWML were quantified in three brain regions:

1. Hippocampus
2. Basal ganglia
3. Centrum semi-ovale.

6.8 VOLUMETRIC ANALYSIS OF MAGNETIC RESONANCE IMAGES

Volumetric analysis was performed by an experienced technician (Annette Blain) from the Department of Medical Radiology, University of Edinburgh. The technician performing the volumetric analysis was blind to the clinical and diabetes-specific characteristics of all participants. Regions of interest analysis was performed on a Sun Microsystem (Sun Microsystems, CA, USA) workstation using a supervised cluster analysis package (ANALYZE®, MAYO Foundation, Rochester, USA). Regions of interest were outlined and their volumes calculated by summing voxels. The following regional brain volumes were measured:

1. Intra-cranial cavity
2. Whole brain
3. Lateral ventricles
4. Temporal lobes
5. Amygdala-hippocampal complexes.

6.9 DETERMINATION OF APOE GENOTYPE

DNA was extracted from venous blood from all participants in the sub-study which explored the effects of APOE Genotype on cognitive ability in Type 1 diabetes. The APOE genotype was determined using the standard methodology developed by Wenham et al (Wenham et al., 1991). This technique utilises a single stage PCR-RFLP assay to accurately determine the APOE genotype and provides a greater degree of accuracy when compared to previous methodologies which were reliant upon isoelectric focusing of plasma APOE isoforms.

CHAPTER 7

INFLUENCE OF DIABETES-SPECIFIC FACTORS ON CEREBRAL STRUCTURE AND FUNCTION IN TYPE 1 DIABETES

7. INFLUENCE OF DIABETES-SPECIFIC FACTORS ON CEREBRAL STRUCTURE AND FUNCTION IN TYPE 1 DIABETES

7.1 INTRODUCTION

Protracted severe hypoglycaemia is an uncommon complication of Type 1 diabetes but anecdotal reports have described permanent neurological and cognitive deficits following protracted severe hypoglycaemia that were associated with localised neuro-imaging abnormalities (Bakshi et al., 2000;Boeve et al., 1995;Chalmers et al., 1991;Chan et al., 2003;Finelli, 2001;Gold and Marshall, 1996;Holemans et al., 2001;Koppel and Daras, 1993;Maekawa et al., 2006;Perros et al., 1994;Rajbhandari et al., 1998;Richardson et al., 1981;Yoneda and Yamamoto, 2005). The neuro-radiological abnormalities described in these case reports predominantly affected the cortical gray matter and sub-cortical gray matter structures, particularly the hippocampus and basal ganglia, which appear more susceptible to hypoglycaemia-induced damage (Auer and Siesjo, 1993), but abnormalities of white matter have also been reported. It remains unresolved whether recurrent episodes of severe hypoglycaemia, in the absence of protracted coma, can have long-term deleterious effects on intellectual function in adults. Intensified insulin therapy, which can achieve strict glycaemic control and can substantially reduce the risk of developing diabetic microangiopathy and its associated morbidity, is associated with a three-fold higher incidence of severe hypoglycaemia (1997). Current treatment goals for those with Type 1 diabetes advocate the attainment of strict glycaemic control to reduce future microvascular morbidity and as such will increase the cumulative exposure to severe hypoglycaemia. The human brain depends on a continuous glucose supply and rapidly malfunctions during hypoglycaemia, but appears to recover rapidly; a single acute episode of hypoglycaemic coma temporarily impairs intellectual function but no permanent effects on cognitive ability are evident 36 hours later (Strachan et al., 2000). Prospective studies have found no adverse effect of recurrent episodes of severe hypoglycaemia on cognitive function in young people with type 1 diabetes (1996;Austin and

Deary, 1999;Reichard et al., 1991). However, cross-sectional studies in older adults in whom type 1 diabetes had commenced after adolescence, and in whom the duration of diabetes was longer, have demonstrated a modest, but significant cognitive decrement associated with the frequency of preceding severe hypoglycaemia (Langan et al., 1991;Lincoln et al., 1996;Sachon et al., 1992;Wredling et al., 1990). A study of a small sub-group of the subjects previously examined by Langan et al (Langan et al., 1991) were re-examined by Perros et al who used magnetic resonance imaging (MRI) of the brain to delineate the neuroimaging associations of severe hypoglycaemia exposure in this cohort. Perros et al reported that cortical atrophy was more prevalent in those with a history of frequent exposure to severe hypoglycaemia (n=11) relative to those who were severe hypoglycaemia naïve (n=11) (Perros et al., 1997). This small study is the only to date to examine the neuro-radiological associations of severe hypoglycaemia exposure in a structured manner.

The incidence of cerebral macrovascular disease is increased in Type 1 diabetes (Grunnet, 1963) and increases with age (Laing et al., 2003) but is rare below the age of 45 years (The DCCT Investigators, 1995;Klein et al., 2004;Nathan et al., 2005;Stettler et al., 2006). Multiple neuropathological abnormalities have been described in post-mortem brains of individuals with type 1 diabetes who had end-stage microangiopathy (Reske-Nielsen and Lundbaek, 1963), although changes could not be attributed exclusively to diabetic microangiopathy due to the confounding presence of co-existent macrovascular disease, hypertension and uremia. It remains uncertain whether diabetes *per se* is associated with the development of cerebral microangiopathy remains speculative. The concept of a "diabetic encephalopathy" has been mooted for in excess of 50 years and was revisited more recently by Dejgaard and colleagues (Dejgaard et al., 1991) who suggested along with others (McCall, 1992) that multiple factors would be likely contributors to the development of a diabetic microangiopathy, including metabolic derangements such as severe hypoglycaemia and diabetic keto-acidosis as well as hypertension. The potential

relationships between diabetic microangiopathy, cognitive performance and brain structure have only been explored in a limited manner: a few small underpowered studies confounded by co-morbidity (Dejgaard et al., 1991;Lunetta et al., 1994;Yousem et al., 1991) and limited by the deficiencies and relative insensitivity of the neuroimaging technology available at the time have been published. Results to date have been conflicting. High-intensity lesions of the cerebral white matter (Leukoaraiosis), abnormalities that are thought to represent foci of ischemia and gliosis (Pantoni and Garcia, 1995), were observed more frequently in a small cohort with long duration type 1 diabetes who had microangiopathy, hypertension and uremia (Dejgaard et al., 1991), whereas no abnormalities were evident on cerebral MRI in another study of subjects who had advanced diabetic retinopathy (Yousem et al., 1991). A further neuroimaging study observed cerebral atrophy more frequently in people with type 1 diabetes but was too small to give clues as to causality and utilised questionable techniques to determine the presence of cerebral atrophy (Lunetta et al., 1994).

Optimal intellectual development may be compromised by the onset of type 1 diabetes during early childhood. The diabetes-related factor most consistently related to measurements of cognitive ability and intelligence in later life is the onset age of type 1 diabetes; children developing the disorder in early childhood are more likely to score relatively poorly on cognitive tests, independently of their diabetes duration. Ack et al first identified an association between an early childhood onset of Type 1 diabetes and comparatively lower scores on a test of general intelligence; children whose onset of diabetes was before their 5th birthday scored on average 10 IQ points lower than their siblings (Ack et al., 1961). Subsequent studies identified small-to-moderate permanent differences in non-verbal intelligence (fluid intelligence) (Holmes and Richman, 1985;Northam et al., 1999;Rovet et al., 1988;Ryan et al., 1985a;Ryan et al., 1985a), verbal intelligence (Alvarez and Rovet, 1997;Ryan et al., 1985a;Schoenle et al., 2002), information processing (Bjorgaas et al., 1997;Northam et al., 2001;Ryan et al., 1985a;Northam et

al., 2001;Northam et al., 2001), visuo-spatial ability (Northam et al., 1999;Rovet et al., 1988;Ryan et al., 1985a;Northam et al., 1999), attention (Alvarez and Rovet, 1997;Bjorgaas et al., 1997;Chabriat et al., 1994;Northam et al., 2001;Service, 1995;Wysocki et al., 2003;Alvarez and Rovet, 1997), executive function (Northam et al., 2001;Service, 1995), and learning and memory ability (Hershey et al., 2003;Hershey et al., 2005;Holmes and Richman, 1985;Ryan et al., 1985a;Service, 1995;Holmes and Richman, 1985). Prospective evaluation has confirmed that an early childhood onset of T1DM independently influences verbal (Northam et al., 2001;Rovet and Ehrlich, 1999) and non-verbal intelligence (Schoenle et al., 2002), attention (Northam et al., 2001;Rovet and Ehrlich, 1999), psychomotor speed (Northam et al., 1998) and executive functions (Northam et al., 2001;Schoenle et al., 2002). Experts have suggested that the differences in ability observed in association with an early childhood onset of T1DM may reflect chronic hyperglycemia, severe hypoglycaemia or other diabetes-related organic factors, whereas others have proposed psychosocial factors, relating to school attendance (Ryan et al., 1985b) and behaviour (Rovet et al., 1993) as causative. However, the pathogenesis of differences in intellectual ability associated with an early childhood onset of T1DM remains uncertain.

7.2 AIMS

The present study examined a group of young people with type 1 diabetes of relatively long duration, who had developed the disorder during childhood or adolescence, and as a consequence may therefore be more vulnerable to the development of cognitive impairment (Northam et al., 2001;Ryan and Becker, 1999) than those in whom the onset of diabetes occurred after the full development and maturation of the central nervous system. The present study was designed to ascertain the neuroimaging and cognitive associations of the clinical complications and disease-specific factors unique to Type 1 diabetes. In particular, the neuroimaging correlates of preceding exposure to recurrent severe hypoglycaemia, the presence of microvascular disease (as retinopathy), the duration of diabetes and the age of onset of diabetes were of particular interest.

The present study was not designed to examine the effects of diabetes *per se* on cognitive function, rather was focused on determining the consequences of diabetes-specific disease factors on the neuropsychological and neuroimaging outcomes of interest.

7.3 SUBJECTS AND METHODS

7.3.1 Study Design

The study was cross-sectional in design and the study protocol was completed in sequential order:

1. detailed Magnetic Resonance Imaging (MRI) of the brain;
2. neuropsychological test battery;
3. retinal examination;
4. assessment of preceding severe hypoglycaemia;

Each component was evaluated and scored blind to all other information.

7.3.2 Subjects

Recruitment criteria were selected with the explicit aim of obtaining a cohort with a long duration of diabetes (> 10 years), sufficient to ensure exposure to the metabolic consequences of interest (severe hypoglycaemia, diabetic retinopathy, disease duration), but without other significant co-morbidity which may confound the interpretation of findings. Subjects were recruited who had developed type 1 diabetes in childhood or adolescence (age at diagnosis < 18 years) and who had attained full intellectual development (age > 20 years) at the time of study. An upper age limit of 45 years was applied to minimise the potential confounding effects of ageing on cognitive ability and brain structure, but also to minimise the likelihood of recruiting subjects with silent macrovascular disease of the cerebral circulation. Those invited to participate either had no clinical evidence of diabetic microvascular complications or had early microangiopathy, manifest as background retinopathy alone. As many of the cognitive ability tests utilised were highly dependent on visual function, those with more advanced degrees of diabetic retinopathy (Airlie

House grading ≥ 2) including diabetic maculopathy, pre-proliferative retinopathy, proliferative retinopathy or those who had previously received laser treatment for diabetic retinopathy were excluded from participation (The Diabetes Control and Complications Trial Group, 1991). The presence of any clinically apparent degree of diabetic neuropathy, the presence of microalbuminuria, or overt proteinuria were also exclusion criteria. Other exclusions included essential hypertension (defined as BP > 140/90 mmHg), any previous central nervous system pathology, previous alcohol or drug misuse, or other multi-system disease known to affect the central nervous system. The aim was to recruit only those in whom diabetes-related factors were likely to account for any differences observed in their cognitive performance or brain structure. The application of the above exclusion and inclusion criteria resulted in the recruitment of a group of people who had Type 1 diabetes of long duration, yet had a comparatively lower microvascular disease burden relative to the general population of individuals with similar disease duration. This caveat should be borne in mind when interpreting the study findings.

Seventy-four individuals with type 1 diabetes were recruited. Seventy-one participants completed the study protocol, with three participants failing to return to complete the cognitive assessment. The data from the 71 participants who completed the full study protocol are presented. The clinical characteristics of those studied are summarised in Table 1. Twenty-five subjects (35%) had background diabetic retinopathy, identified by digital retinal imaging and direct ophthalmoscopy. Background diabetic retinopathy was defined by the presence of at least two or more microaneurysms in one eye (Airlie House Gradings 1a-1c). Exposure to severe hypoglycaemia was assessed retrospectively using a validated formatted hypoglycaemia questionnaire (Deary et al., 1993). Participants were requested to discuss their severe hypoglycaemia history with relatives or friends before completing questionnaires, to improve the accuracy of estimates. Details were recorded of the lifetime total number of episodes, the frequency of occurrence of episodes, and the total number of episodes of convulsions, coma, and

other episodes requiring medical assistance to treat the hypoglycaemia. Severe hypoglycaemia was defined as any episode of hypoglycaemia sufficient in severity to require external assistance to facilitate recovery, similar to the definition used in the Diabetes Control and Complications Trial criteria [(The Diabetes Control and Complications Trial Group, 1997). Using this definition, twenty subjects (28%) had never experienced severe hypoglycaemia, while 21 (30%) had experienced ten or more previous episodes. Subdivision of the cohort by the presence of background retinopathy showed a similar level of exposure to severe hypoglycaemia in the two subgroups, with a slight excess of preceding severe hypoglycaemia in the sub-group who had no diabetic retinopathy. The participants who had established retinopathy were slightly older and tended to have had diabetes of longer duration, although the onset of diabetes occurred at a similar age. The subgroups with or without retinopathy were very similar in their duration of education and estimated pre-morbid cognitive ability.

Table 7.1. Characteristics of participants defined by Diabetic Retinopathy status.

	No Retinopathy (n=46)	Background retinopathy (n=25)	p-value
Age (years)	26.4 ± 4.6 (26, 20 to 36)	31.5 ± 6.0 (32, 21 to 44)	0.23
Sex (male/female)	23:23	14:11	0.63
Secondary Education (years)	6.9 ± 2.3 (6, 4 to 11)	7.3 ± 2.4 (8, 4 to 11)	0.95
NART (pre-morbid ability)	31.9 ± 6.0 (32, 15 to 42)	32.5 ± 6.6 (33, 16 to 41)	0.94
HbA1c at time of Study (5.0 - 6.5%)	8.4 ± 1.2	8.9 ± 1.3	0.83
Blood Pressure (mmHg)	123/73 ± 14/10	121/74 ± 12/11	0.56
Age at Diagnosis of T1DM (years)	9.5 ± 4.4 (9, 1 to 16)	9.9 ± 4.0 (10, 1 to 17)	0.67
Duration of Diabetes (years)	17.0 ± 4.6 (16, 10 to 30)	21.6 ± 5.9 (22, 10 to 31)	0.052
Severe Hypoglycaemia			
Total lifetime episodes (median, range)	6.0 (0 to 200)	4.0 (0 to 45)	0.86
No previous episodes (% participants)	26.1	32.0	-
1-4 episodes (% participants)	19.6	28.0	-
5-10 episodes (% participants)	23.9	12.0	-
> 10 episodes (% participants)	30.4	28.0	-

Data are means ± SD with median and range bracketed.

NART = National Adult Reading Test.

HbA_{1c} = Glycated Haemoglobin, non-diabetic range 5.0-6.5% (by HPLC).

Table 7.2. Characteristics of participants grouped by Diabetes Onset Age.

	Onset ≤ 7 Years (n=26)	Onset > 7 Years (n=45)	p-value
Age (years)	25.2 ± 4.6 (23, 20-36)	29.9 ± 5.6 (30, 20-44)	<0.0001
Sex (male/female)	13:13	24:21	0.79
Secondary Education (years)	7.0 ± 2.0 (6, 4-10)	7.1 ± 2.5 (6, 4-11)	0.82
NART (pre-morbid ability)	32.8 ± 5.4 (33, 21-41)	31.8 ± 6.6 (32, 15-42)	0.52
HbA1c at time of study (%)	8.3 ± 1.1	8.7 ± 1.3	0.20
Blood Pressure (mmHg)	122/72 ± 11/10	122/74 ± 14/10	0.94
Age at Diagnosis of T1DM (years)	5.0 ± 2.3 (5.5, 1-7)	12.2 ± 2.4 (12, 8-17)	<0.0001
Duration of Diabetes (years)	20.1 ± 5.2 (18.5, 13-31)	17.7 ± 5.5 (16, 10-29)	0.07
Background Retinopathy (%)	23%	42%	0.10
Severe Hypoglycaemia			
Median Total Episodes (% exposed, range)	7.0 (77%, 0 to 200)	4.0 (69%, 0 to 100)	0.48
Median Total Coma (% exposed, range)	2.0 (65%, 0 to 40)	0.0 (49%, 0 to 9)	0.26
Median Total Seizures (% exposed, range)	0.0 (46%, 0 to 30)	0.0 (33%, 0 to 6)	0.25

Data are means ± SD with median and range bracketed.

NART = National Adult Reading Test.

HbA_{1c} = Glycated Haemoglobin, non-diabetic range 5.0-6.5% (by HPLC).

7.3.3 Methods

An outline of the methods utilised for neuro-imaging, the assessment of intelligence and cognitive ability are provided in this section. Detailed descriptions of the neuro-imaging techniques, the characteristics of the neuropsychological test battery and the manner in which the clinical complications of Type 1 diabetes were evaluated are provided in the Methods Chapter.

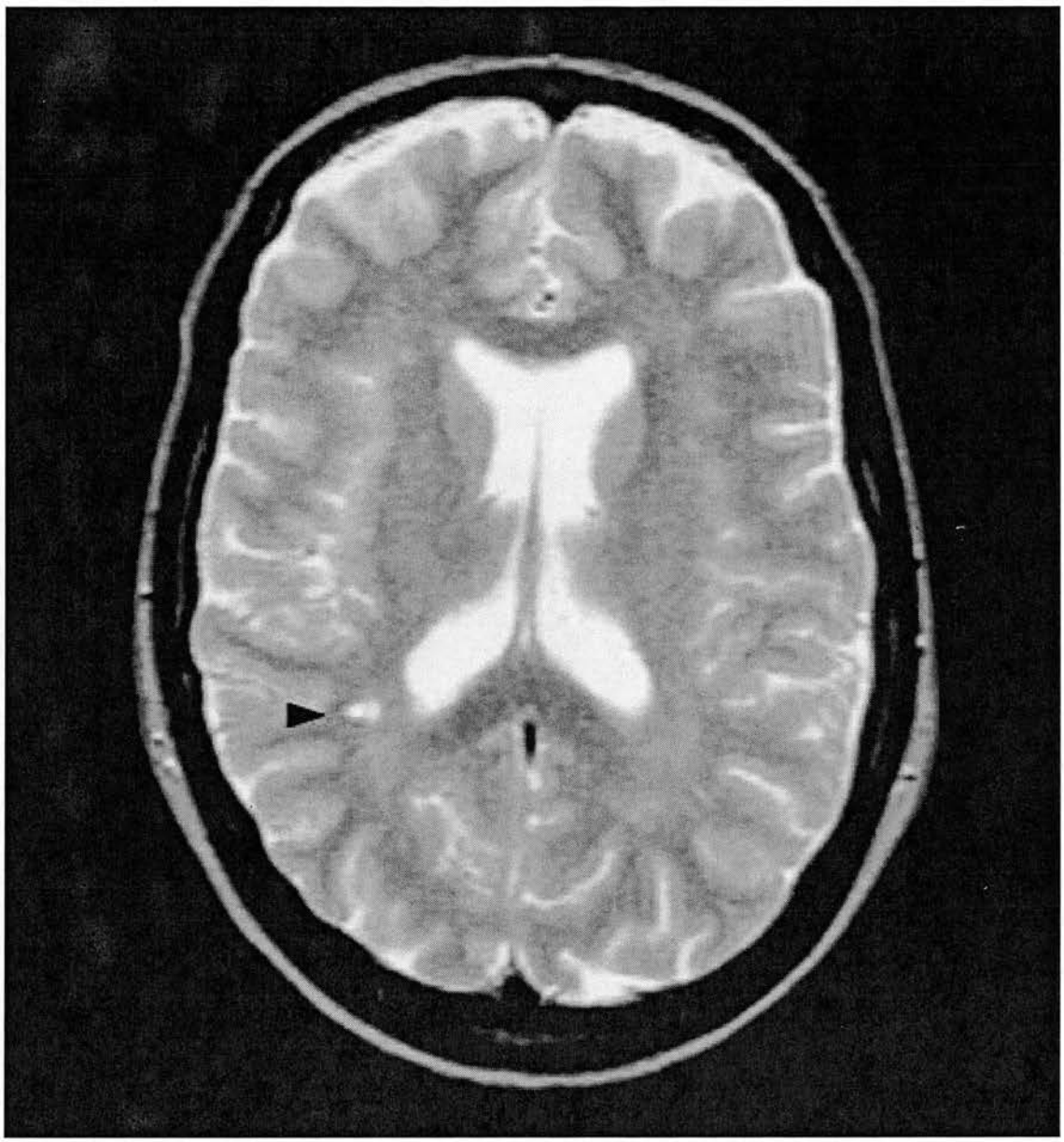
Magnetic Resonance Imaging Protocol

MRI examinations of the brain were performed using a 1.0T SPE Magnetom scanner (Siemens, Erlangen, Germany). Following midline localization, two sequences were used to image the whole brain (Lawrie et al., 1999). The first scan provided simultaneous proton-density and T2-weighted images (TR=3565ms, TE=20ms and 90ms, 31 contiguous 5mm slices acquired in the Talairach plane, FOV 250mm) that were used to calculate whole brain and cerebrospinal fluid volumes using a supervised cluster analysis package (ANALYZE, Mayo Foundation, Rochester, MN, USA). The second scan, for the regional volumetric analysis, was a three dimensional magnetization prepared for rapid-acquisition gradient echo sequence consisting of an 180 inversion pulse followed by a fast low-angle shot collection (Flip angle 12, TR=10 ms, TE=4 ms, TI=200 ms, relaxation time delay time 500 ms, FOV 250mm) giving 128 contiguous 1.88mm thick slices in the coronal plane orthogonal to the Talairach plane. Inhomogeneity corrections were performed on Sun Microsystem workstations using ANALYZE to outline neuroanatomical structures. The temporal lobe and amygdala-hippocampal complex were identified and their areas outlined and volumes calculated by summing voxels in the regions of interest.

Assessment of High-Intensity White Matter Lesion Burden

Each MRI scan was reviewed by an experienced Neuroradiologist (JW) and scored independently, and blinded to clinical factors, for the presence of high-intensity white matter lesions (leukoaraiosis) (Breteler et al., 1994a;Breteler et al., 1994b;Fazekas et al., 1987;Longstreth, Jr. et al., 1996;Mirsen TR et al., 1991;Shimada et al., 1990;Van Swieten et al., 1990;Breteler et al., 1994a). Several scoring systems were used to capture the intensity, distribution and appearance of white-matter lesions as no single scale was judged to be an adequate summary (Mantyla et al., 1997). An example of a high-intensity white matter lesion (Leukoaraiosis) from a participant of the present study is illustrated in Figure 7.1.

Figure 7.1. High-Intensity White Matter Lesion in deep white matter (arrowhead).



MRI scans were also scored for the presence of a distinctly different type of white matter abnormality, the small punctate white-matter lesion (SPWML), which was frequently observed but not accounted for by any of the above rating scales (Figure 7.2). A scale of 0-3 was utilised to quantify the presence of SPWML:

0. No SPWML;

1. Mild SPWML with total number not exceeding 10 lesions,

2. Moderate SPWML with total number of 10-20 lesions,

3. Severe SPWML with greater than 20 lesions observed.

This scale was used to quantify the presence of SPWML, indicating enlarged peri-vascular spaces, in three distinct brain regions (hippocampus, basal ganglia, centrum semi-ovale) using the most affected hemisphere. Examples of an MRI scan where small punctuate white matter lesions were observed in participant of the present study is illustrated in Figures 7.2(a) and 2(b).

Figure 7.2 (a) Small Punctate White Matter Lesions (arrowheads) in the region of the Hippocampus.

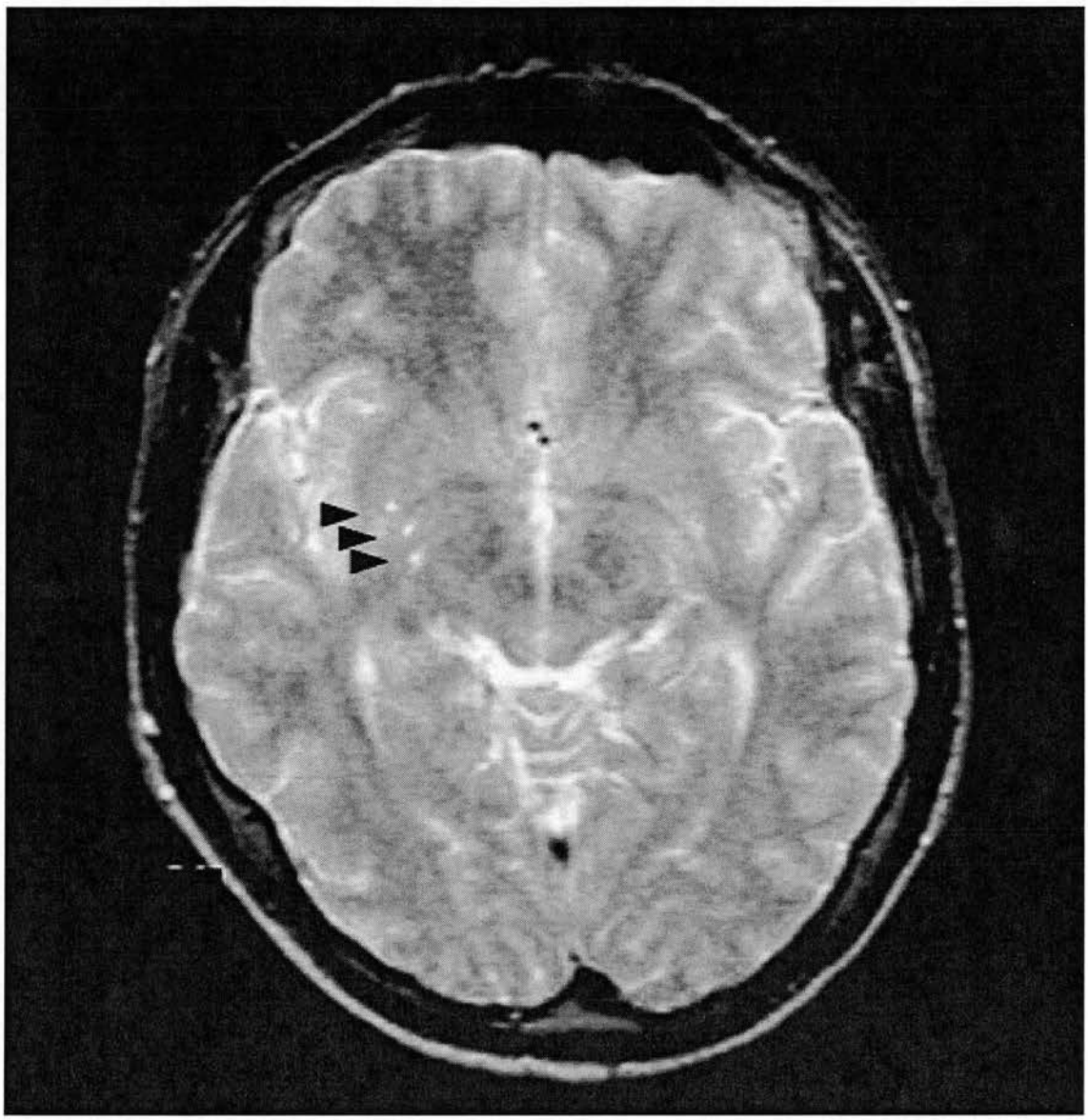
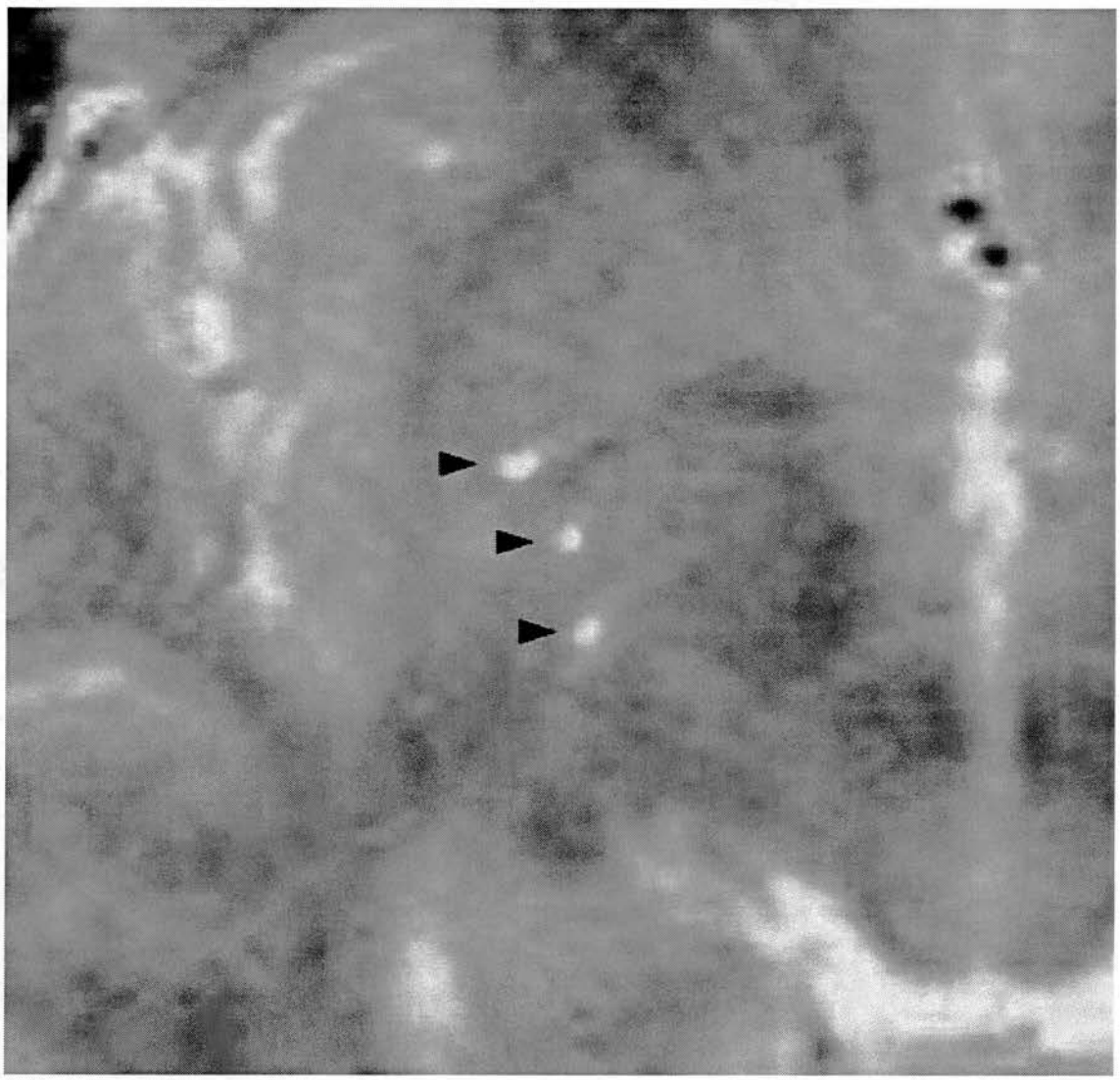


Figure 7.2 (b) Magnified version of the same Hippocampal SPWML (arrowheads).



Assessment of the Presence and Degree of Cerebral Atrophy

The presence or absence of cerebral atrophy was subjectively determined by an experienced Neuroradiologist. The severity of atrophy was rated on a subjective scale of 0-3:

0. No cerebral atrophy;

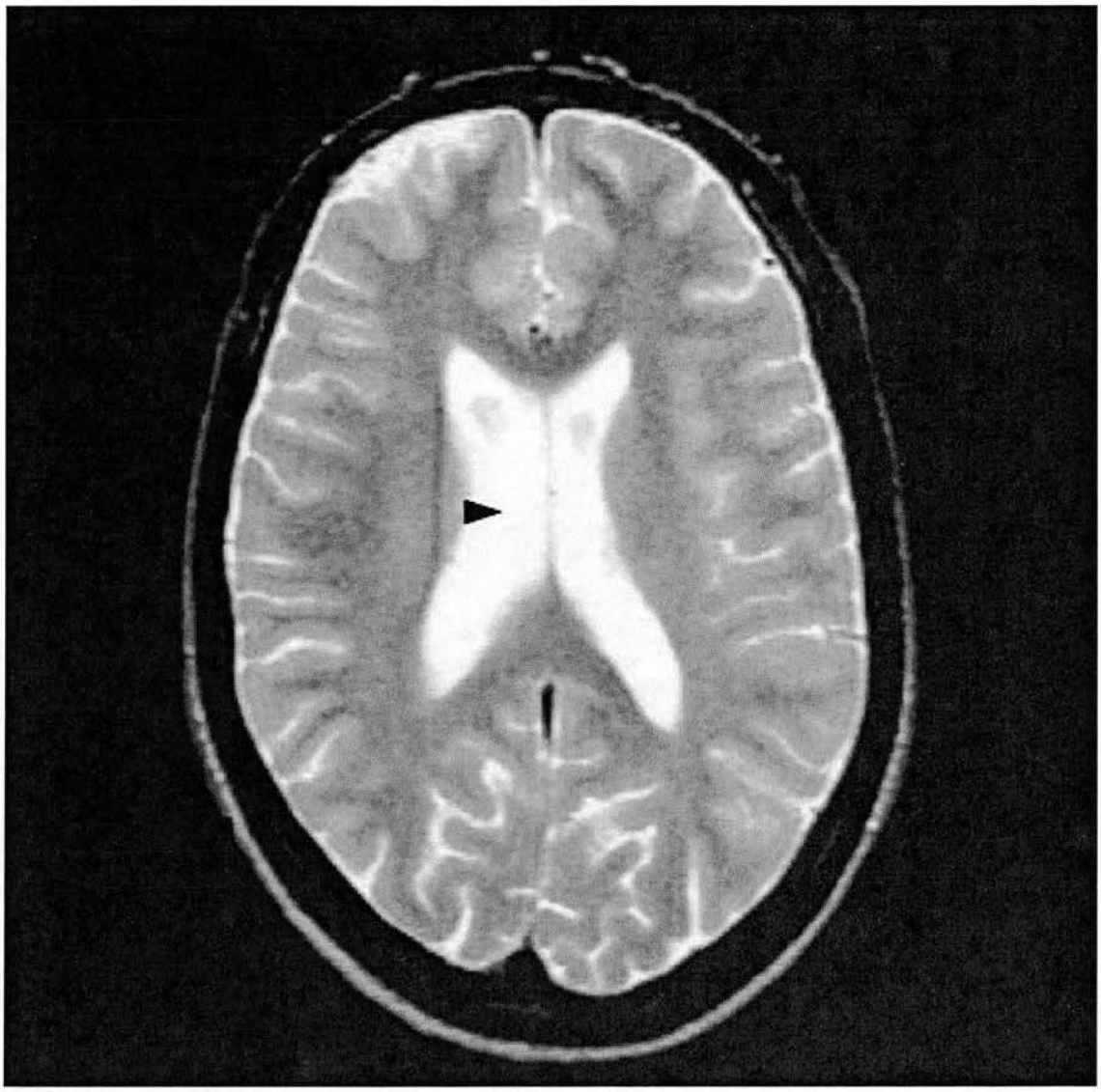
1. Mild cerebral atrophy;

2. Moderate cerebral atrophy;

3. Severe cerebral atrophy.

When present, cerebral atrophy was defined as being either "Ventricular" or "Sulcal". Ventricular atrophy, also known as central brain atrophy, reflects enlargement of the cerebral ventricles. Sulcal atrophy, also known as gyral or cortical atrophy, reflects enlargement of the cerebral sulci and infers loss of cortical gray matter. An example of ventricular atrophy from the MRI scan of a participant of the present study is illustrated in Figure 7.3. An example of sulcal atrophy has not been provided due to the scarcity and mild nature of this neuroimaging abnormality that was observed in the present cohort.

Figure 7.3. Ventricular Atrophy from a participant of the present study (arrowheads). The arrowhead indicates enlargement of the cavity of the body of the Lateral Ventricle.



Assessment of Neuropsychological Function

The neuropsychological test battery was chosen to be sensitive to mild-to-moderate cognitive decrements and to provide an assessment of some major cognitive domains. Trained assessors, blinded to the diabetes characteristics of participants, administered neuropsychological tests in a standardised manner. The capillary glucose concentration was measured before the cognitive assessment to exclude prevailing hypoglycaemia. The neuropsychological session was rescheduled if hypoglycaemia was present or if antecedent hypoglycaemia had occurred within the preceding 24 hours.

The neuropsychological tests utilised were:

Hospital Anxiety and Depression Scale (Zigmond and Snaith, 1983)

The potential confounding effects that affective disorders can have on cognitive ability were assessed.

Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Wechsler, 1981)

The Performance IQ subtests of the WAIS-R are sensitive to disruption by organic brain disease and measure current intellectual performance (fluid intelligence). The Picture Completion, Object Assembly, Block Design, Digit Symbol tests were utilised.

National Adult Reading Test (NART) (Nelson and Willison, 1991)

Performance on the NART is relatively resistant to the effects of organic brain disease and performance on this test correlates more closely with pre-morbid IQ than estimates obtained from socio-economic or demographic variables. NART performance is representative of 'best ever' global cognitive performance (irrespective of age, disease or time) and in the present study was used as the estimate of pre-morbid intellectual ability (pre-morbid IQ, crystallized intelligence) of

each participant. Pre-morbid ability is generally the most powerful determinant of performance on many neuropsychological tests.

Inspection Time (IT) (McCrimmon et al., 1996)

Inspection Time, a sub-component of information processing ability, was used to provide a measure of early visual information processing ability. Participants were asked to discriminate between the spatial position (left or right) of two briefly-presented vertical lines of different lengths. The stimuli were backward-masked, the presentation duration was varied, and the duration of time required to reliably distinguish the stimulus (85% correct) was termed the participant's 'inspection time'.

Choice Reaction Time (CRT) (Jensen, 1987)

Choice reaction time was utilised as a measure of psychomotor speed and complemented inspection time in assessing information processing ability. Tests measuring 1,2,4,8 and 8,4,2,1-choice reaction times were used. Decision and movement times were measured.

Borkowski Verbal Fluency Test (controlled association) (Borkowski and Benton, 1967)

The Borkowski Verbal Fluency Test is thought to provide an assessment of frontal lobe and executive functions. In this test participants have 60 seconds to state as many words as possible, beginning with letters of the alphabet pre-specified by the tester.

Paced Auditory Serial Addition Task (PASAT) (Deary I.J. et al., 1991)

The PASAT was used to assess the ability of participants to sustain attention and concentration. Participants listened to a numbers list, which they were required to add together according to a given rule. After practice, two consecutive trials of 61 numbers were performed with 4s and 2s between successive digits respectively.

7.3.4 Statistical Analyses

Statistical analysis was performed using SPSS v10.0 (®SPSS Inc, Chicago, USA). The relationship between demographic variables, neuropsychological performance, brain volumes and MRI outcomes (cerebral atrophy, high-intensity white matter lesions, coded numerically) was examined initially using bi-variate correlation (Spearman's rho). Neuropsychological performance was analysed by domain to reduce the possibility of Type 1 error, before sub-analysis of individual tests was attempted. Sub-analysis was not performed if the multivariate statistic (F-value) was non-significant. Parameters were entered into multivariate linear models (MANCOVA) on the basis of prior hypotheses and correlates between demographic variables, neuropsychological performance and brain volumes. This technique was utilised to determine whether Diabetes Onset Age, Diabetic Retinopathy status, Severe hypoglycaemia exposure, or Duration of Diabetes influenced neuropsychological performance or brain volumes and to estimate the magnitude of their influence (Eta^2). Specific models were constructed for the analysis of neuropsychological performance and brain volumes. The neuropsychological model included Gender, Diabetic Retinopathy status (coded as present/absent) and the Diabetes Onset Age (coded as EOD $\leq$ 7 years, LOD > 7 years) as between-subjects variables. Pre-morbid IQ (NART), Age, Duration of Diabetes and Severe Hypoglycaemia exposure (as log transformed total number of lifetime episodes due to skewed distribution) were entered as co-variates. The brain volume model included Gender, Diabetic Retinopathy status (coded as present/absent) and Diabetes Onset Age (coded as EOD $\leq$ 7 years, or LOD > 7 years) as between-subjects factors, with Age, Diabetes Duration, Severe Hypoglycaemia exposure (as log transformed total number of lifetime episodes) and Intracranial Volume as co-variates. The relationship between the diabetes variables of interest and subjectively-rated cerebral atrophy (coded as present/absent) or the presence of white-matter lesions (coded as present/absent) was analysed by multiple logistic regression, using Gender (male/female), Diabetes Onset Age (coded as EOD/LOD) and Diabetic

Retinopathy status (coded as present/absent), Severe Hypoglycaemia exposure (coded as naïve/exposed) as factors. Age and Diabetes Duration were included as co-variates. Co-variates and factors were entered into the multiple logistic regression models using a forward conditional stepwise approach, such that non-significant variables were ejected from the final model.

7.4 RESULTS

7.4.1 Independent Predictors of Cognitive Ability (MANCOVA)

General linear modeling (MANCOVA) identified the following variables, listed in descending order of magnitude of influence, as statistically significant independent predictors of performance on the entire battery of neuropsychological tests:

1. Pre-morbid intellectual ability (NART, $F=7.9$, $p<0.0001$, $Eta^2=0.38$);
2. Diabetic Retinopathy ($F=6.3$, $p<0.0001$, $Eta^2=0.33$);
3. Gender ($F=3.8$, $p<0.01$, $Eta^2=0.23$);
4. Diabetes Onset Age Group ($F=3.0$, $p=0.03$, $Eta^2=0.19$).

Other factors of interest that were included in the multi-variate model but which did not appear to exert a statistically significant influence on neuropsychological performance included:

1. Severe Hypoglycaemia exposure ($F=0.64$, $p=0.64$, $Eta^2=0.05$);
2. Duration of Diabetes ($F=1.0$, $p=0.41$, $Eta^2=0.07$).
3. Age ($F=1.34$, $p=0.26$, $Eta^2=0.13$).

7.4.2 Severe Hypoglycaemia and Neuropsychological Performance

A wide range of previous exposure to severe hypoglycaemia was recorded within the cohort, allowing examination of possible neuropsychological consequences associated with cumulative exposure to severe hypoglycaemia (Table 7.1). Approximately one-third of participants were

naïve to severe hypoglycaemia with a further third having experienced in excess of ten lifetime episodes. Despite a wide spread of cumulative exposure to severe hypoglycaemia within the study cohort no summary measure of preceding severe hypoglycaemia was significantly correlated with neuropsychological performance. The correlation co-efficients (Spearman's Rho) between estimates of preceding severe hypoglycaemia and neuropsychological performance are shown in Table 7.3.

Table 7.3. Correlation co-efficients between measures of exposure to severe hypoglycaemia and neuropsychological function (Spearman's rho).

	Performance IQ (WAIS-R)	**Frontal & Executive Function** (Borkowski)	**Visual Information Processing** (IT)	**Psychomotor Speed** (Choice Reaction Time)	**Attention & Concentration** (PASAT)
Lifetime episodes	-0.009	0.068	-0.068	0.012	-0.129
Frequency of episodes	-0.013	0.063	-0.048	0.011	-0.085
Episodes requiring glucagon	-0.061	0.069	-0.121	0.032	-0.038
Episodes of coma	0.021	0.121	-0.066	-0.044	-0.089
Episodes of convulsion	-0.023	0.008	-0.086	-0.080	0.044

All correlations $p>0.05$.

7.4.3 Background Retinopathy and Neuropsychological Performance

The relative neuropsychological performance of those with and without Background Diabetic Retinopathy is summarised in Table 7.4, along with the estimated effect size (Eta^2) of Background Retinopathy and pre-morbid IQ (NART) on cognitive ability. Any cognitive difference observed between the two sub-groups formed by the presence/absence of Diabetic Retinopathy is not consequent upon differences in the degree of education or prior intellectual

ability, firstly because the two groups are very similar on these variables (Table 7.1) and secondly as pre-morbid IQ was entered as a co-variate in the analyses.

Those subjects who had background retinopathy performed less well across most, but not all, of the cognitive domains examined. The presence of Diabetic Retinopathy was associated with a relative disadvantage in current intellectual performance (WAIS-R performance IQ, $p=0.008$) of moderate effect size ($Eta^2=0.14$). Information processing ability (sum of Z-scores for Choice Reaction Time and Inspection Time) was poorer in those with Retinopathy ($p=0.001$) and the effect size was moderate-to-large ($Eta^2=0.22$). Both components of the tests which comprised the information processing domain were affected: performance disadvantage was observed in Choice Reaction times (4 Choice Reaction Time decision time component, $p=0.003$, $Eta^2=0.17$) and early visual information processing ($p=0.03$, $Eta^2=0.09$). The ability to sustain attention and concentration (PASAT) was also poorer in those who had Retinopathy ($p=0.03$, $Eta^2=0.09$). However, the presence of Diabetic Retinopathy was not associated with a performance disadvantage on the Borkowski Verbal Fluency Test, a test that is thought to examine frontal lobe and executive functions.

Table 7.4. Influence of Diabetic Retinopathy and Pre-Morbid Intellectual Ability (NART) on neuropsychological performance.

Neuropsychological Domain	No Retinopathy Mean Score (SD)	Retinopathy Mean Score (SD)	No Retinopathy Z-score (SD)	Retinopathy Z-Score (SD)	Effect of Retinopathy	Effect of NART
Performance IQ	150.7	132.9	0.22	-0.41	p=0.008	p<0.0001
(WAIS-R raw score)	(21.8)	(24.9)	(0.8)	(1.0)	Eta^2=0.14	Eta^2=0.32
Frontal & executive functions	43.3	46.4	-0.04	0.07	p=0.72	p=0.01
(Verbal Fluency score)	(13.0)	(11.7)	(1.0)	(0.75)	Eta^2=<0.01	Eta^2=0.13
Information processing						
Inspection Time (ms)	46.8 (17.9)	56.2 (17.5)	-0.15 (0.95)	0.29 (0.86)	p=0.03, Eta^2=0.09	p=0.78, Eta^2=<0.01
Median 4-Choice Reaction Time (ms)	308.2 (43.1)	346.2 (60.2)	-0.23 (0.63)	0.44 (1.2)	p=0.003, Eta^2=0.17	p=0.13, Eta^2=0.05
Attention and concentration	23.3	28.0	-0.16	0.30	p=0.03	p=0.009
(PASAT 2s errors)	(10.1)	(14.2)	(0.55)	(1.4)	Eta^2=0.09	Eta^2=0.14

7.4.4 Interaction Between Diabetic Retinopathy and Severe Hypoglycaemia

The co-existence of Diabetic Retinopathy and preceding exposure to Severe Hypoglycaemia did not result in any additional cognitive disadvantage over and above that associated with the presence of Diabetic Retinopathy alone: Retinopathy × Severe Hypoglycaemia interaction $F=0.52$, $p=0.76$, $Eta^2=0.05$. However, the size of the sub-groups for this sub-analysis were small [Diabetic Retinopathy alone (n=8) vs. Severe Hypoglycaemia and Retinopathy (n=17)], which makes definitive conclusions inappropriate through limitations in statistical power. However, this analysis does infer that if any detrimental interaction does exist between Retinopathy and Severe Hypoglycaemia it is likely to be of small magnitude and may be of little practical consequence.

7.4.5 Early Onset Age of Diabetes and Neuropsychological Performance

The neuropsychological performance of those with an Early Onset Diabetes (EOD ≤ 7 years of age) relative to those with a Later Onset Diabetes (LOD > 7 years of age) is summarised in Table 7.5. The statistical significance and estimated magnitude of effect (Eta^2) of the Diabetes Onset Age grouping are provided. An EOD was associated with a performance disadvantage across a number of ability domains. Those with an EOD exhibited poorer fluid intelligence ability (WAIS-R performance IQ, $p=0.03$) of small-to-moderate magnitude ($Eta^2=0.09$), a difference largely accounted for by performance on the Block Design and Digit Symbol Substitution subtests, tests which assess spatial ability and psychomotor speed respectively. Information processing ability (sum of Z-scores for Choice Reaction Time and Inspection Time) was significantly poorer in those with an EOD ($p=0.006$) and the disadvantage was of moderate magnitude ($Eta^2=0.13$). The disadvantage in information processing performance associated with EOD was limited to performance on tests of Simple Reaction Time ($p=0.04$, $Eta^2=0.07$, decision time component) and Choice Reaction Time (2, 4 and 8-Choice Reaction Time decision time component ($p=0.006$-0.06, $Eta^2=0.06$-0.12). An EOD was not associated with poorer

performance on tests of early visual information processing ability (Inspection time), sustained attention and concentration (PASAT) or frontal lobe and executive functions (Borkowski Verbal Fluency Test), indicating that the cognitive disadvantage associated with EOD was selective. No statistically significant interaction was observed between an EOD and other diabetes-specific factors. In particular, the presence of Diabetic Retinopathy and an EOD did not interact to statistically influence cognitive ability on multi-variate testing ($F=0.71$, $p=0.59$, $Eta^2=0.05$), although those individuals in whom an EOD and Diabetic Retinopathy were co-existent performed the most poorly.

Table 7.5. Influence of Diabetes Onset Age group (EOD/LOD) on neuropsychological performance.

Neuropsychological Domain	Early Onset of Diabetes Z-Score (95% CI)	Later Onset of Diabetes Z-Score (95% CI)	Relative Performance of EOD Group (SD)	Effect of Onset of Diabetes Group EOD ≤ 7years LOD > 7years
Performance IQ (Fluid intelligence)	-0.61 (-1.1 to -0.07)	0.27 (-0.08 to 0.61)	-0.88	p=0.03 Eta^2=0.09
Frontal & executive function (Borkowski Verbal Fluency)	-0.07 (-0.80 to 0.66)	0.01 (-0.45 to 0.48)	-0.08	p=0.83 Eta^2=<0.01
Information processing				
Inspection Time	0.04 (-0.64 to 0.72)	0.15 (-0.28 to 0.59)	-0.09	p=0.61, Eta^2<0.01
4-Choice reaction time	-0.84 (-1.4 to -0.26)	0.31 (0.07 to 0.68)	-1.15	p=0.006, Eta^2=0.13
Attention & concentration (PASAT 2s Errors)	-0.09 (-0.76 to 0.56)	0.01 (-0.41 to 0.43)	-0.10	p=0.41 Eta^2=0.01

Data in columns 2 and 3 represent Z-scores with 95% Confidence Intervals (brackets).

Data in column 5 summarise the multi-variate statistics for the Diabetes Onset Age grouping.

7.4.6 Neuroimaging Abnormalities

Abnormalities detected on the MRI scans of participants were generally mild and subtle. High-intensity peri-ventricular white matter lesions particularly as peri-ventricular caps, or pencil-thin rims, were common and present in one-third of scans. Deep white matter lesions were observed infrequently. Small punctuate white matter lesions were frequently observed.

Severe Hypoglycaemia and Brain Structure

No significant correlation was identified between any summary measure of previous exposure to severe hypoglycaemia and the presence of any structural MRI neuroimaging abnormalities. In particular, no correlation was observed between the presence of high-intensity lesions in the cerebral white matter (ρ=0.018, p=0.90), or cerebral atrophy (ρ=-0.026, p=0.84) and any summary measure of previous severe hypoglycaemia exposure.

Diabetic Retinopathy and Structural Brain Abnormalities

No associations were observed between Diabetic Retinopathy and the presence of cerebral atrophy, as either ventricular or sulcal atrophy, or regional brain volumes (Table 7.8). However, the presence of Diabetic Retinopathy was associated with more frequent small punctate high-intensity peri-ventricular white matter lesions (SPWML) in the region of the basal ganglia. One third (33.3%) of those with Diabetic Retinopathy had a degree of peri-ventricular SPWMLs in the basal ganglia, which were usually mild (<10 lesions), relative to 4.7% of those who did not have Diabetic Retinopathy. More advanced degrees of SPWML were not observed. The robustness of the association was evaluated using MANCOVA, which also considered the influence of age and gender. The relationship remained statistically significant (p=0.005), but Diabetic Retinopathy accounted for a small proportion of the variance in SPWMLs scores (Eta^2=0.012) and the presence of SPWML was not associated with neuropsychological performance. SPWML in the Hippocampus or Centrum Semi-Ovale, or a summation of SPWML in all three areas, were no

more frequent in those who had Diabetic Retinopathy. White matter lesions, as Leukoaraiosis, rated by a variety of published rating scales, (Breteler et al., 1994b;Breteler et al., 1994a;Fazekas et al., 1987;Longstreth, Jr. et al., 1996;Mirsen TR et al., 1991;Shimada et al., 1990;Van Swieten et al., 1990) were no more common in those who had Diabetic Retinopathy.

Table 7.8. Diabetic Retinopathy status, Age and Regional Brain Volumes. Data are presented after adjustment for Intra-Cranial Volume (MANCOVA).

Brain Volume (cm^3)	**No Retinopathy** Estimated Marginal Mean (95% CI))	**Retinopathy** Estimated Marginal Mean (95% CI)	**Effect of Retinopathy**	**Effect of Age**
Whole Brain	1259 (1238-1281)	1262 (1238-1287)	p=0.86 Eta^2<0.01	p=0.11 Eta^2=0.04
Lateral Ventricles	22.1 (19-25)	18.0 (15-21)	p=0.08 Eta^2=0.05	p=0.57 Eta^2<0.01
Right Temporal Lobe	78 (75-80)	75 (72-77)	p=0.12 Eta^2=0.04	p=0.78 Eta^2=<0.01
Left Temporal Lobe	73 (71-75)	72 (70-75)	p=0.09 Eta^2=0.06	p=0.91 Eta^2<0.01
Right Amygdala-Hippocampal Complex	4.4 (4.2-4.6)	4.3 (4.1-4.5)	p=0.47 Eta^2<0.01	p=0.004 Eta^2=0.14
Left Amygdala-Hippocampal Complex	4.4 (4.2-4.6)	4.3 (4.1-4.5)	p=0.57 Eta^2<0.01	p=0.007 Eta^2=0.12

Interaction: Diabetic Retinopathy, Severe Hypoglycaemia and Brain Structure

Cerebral atrophy or white matter lesions, in the form of small punctate lesions or leukoaraiosis, were no more prevalent in those who had background retinopathy and who had also experienced severe hypoglycaemia compared to those with only background retinopathy.

Diabetes Onset Age and Structural MRI Abnormalities

The relationship between Diabetes Onset Age and subjectively-rated MRI scan abnormalities is shown in Table 7.9. An EOD was associated with a significantly higher prevalence of ventricular atrophy (Figure 7.3). Ventricular atrophy was commonly observed, present in 60% of EOD subjects and 20% of LOD subjects [p=0.01, odds ratio=4.6 (95% CI=1.4-14.8), multiple logistic regression], and was generally mild-to-moderate (EOD 50% mild, 10% moderate; LOD 13% mild, 7% moderate). Subjectively-rated sulcal atrophy was uncommon and no more prevalent in those with an EOD [p=0.81, odds ratio=1.3 (95% CI 0.15-11.1), 9% EOD scans vs. 7% of LOD scans, multiple logistic regression]. MRI scans were also rated for the presence of small punctate white matter lesions (SPWML) (Figures 7.2a, 7.2b) and leukoaraiosis (Figure 7.1). An EOD was associated with a significantly higher frequency of SPWML in the region of the Hippocampus (14% EOD vs. 2% LOD) but the Diabetes Onset Age grouping (EOD/LOD) did not independently predict the presence of SPWML in the region of the Basal Ganglia, Centrum Semi-Ovale, or the total number of SPWML observed in all brain areas. The presence of leukoaraiosis was not consistently different between the EOD/LOD groups, although an EOD independently predicted the presence of leukoaraiosis when the scoring method of Wahlund et al (Wahlund et al., 1990) was used (EOD 59% vs. 24% LOD, odds ratio=4.5 (95% CI=1.5-13.3), p=0.007, logistic regression). However, given the multiple analyses and rating scales utilised this difference may reflect Type 1 statistical error, or the inherent attributes of the rating scales, rather than a true representative difference in white matter pathology associated with Diabetes Onset Age.

Table 7.9. Subjective ratings of MRI scan appearances and Diabetes Onset Age group.

	EOD n=22 (% of scans)	**LOD** n=45 (% of scans)	**Effect of Diabetes Onset Age Group** (Log Regression)
Ventricular Atrophy	60	20	OR=4.6, p=0.010
Sulcal Atrophy	9	7	NS
Leukoaraiosis			
Wahlund	59	24	OR=4.5, p=0.007
Longstreth	55	53	NS
Van Swieten (superficial/deep WML)	0/0	2/2	NS/NS
Breteler	18	27	NS
Fazekas (superficial/deep WML)	55/0	60/18	NS/NS
Shimada	0	11	NS
Mirsen (superficial/deep WML)	41/0	42/9	NS/NS
SPWML			
Hippocampal	14	2	0.03
Basal Ganglia	5	20	0.38
Centrum Semi-ovale	9	22	0.08
% with punctate WML	18	40	0.17

NS = non-significant EOD/LOD effect in the final multiple logistic regression model.

OR=odds ratio.

Diabetes Onset Age and MRI Brain Volumes

General linear modelling, which co-varied for head size (as Intracranial Volume) and Gender, identified Diabetes Onset Age group as a significant independent multi-variate determinant of those regional brain volumes that were assessed in the present study ($F=2.4$, $p=0.04$, $Eta^2=0.22$). The relationships between Diabetes Onset Age group and brain volumes are summarised in Table 7.10. An EOD was associated with a comparatively larger volume of the Lateral Ventricles ($F=11.1$, $p=0.002$, $Eta^2=0.16$), with an absolute difference in volume of 6.6 cm^3, equating to a 36.9% greater Ventricular volume than that observed in those in whom the onset of diabetes was later (LOD group), independently of Intra-Cranial Volume. The influence of an early onset age of Type 1 diabetes appeared specific as an EOD was not associated with any other significant differences in either the volume of the whole brain, temporal lobes or amygdala-hippocampal complex after considering co-variates (MANCOVA).

Table 7.10. Diabetes Onset Age group and Regional Brain Volumes (cm^3).

Brain Volume (cm^3)	**EOD** Estimated Marginal Mean (SEM)	**LOD** Estimated Marginal Mean (SEM)	**Effect of Diabetes Onset Age Group** (MANCOVA)	**Effect of Age** (MANCOVA)
Whole Brain	1270 (13.1)	1258 (8.9)	F=0.58 p=0.45 Eta^2=0.01	F=1.6 p=0.21 Eta^2=0.03
Lateral Ventricles	24.5 (1.6)	17.9 (1.1)	F=11.1 p=0.002 Eta^2=0.16	F=4.1 p=0.05 Eta^2=0.07
Right Temporal Lobe	76 (1.3)	76 (0.9)	F=0.01 p=0.92 Eta^2<0.01	F=0.19 p=0.66 Eta^2<0.01
Left Temporal Lobe	72 (1.3)	73 (0.9)	F=0.48 p=0.49 Eta^2=0.01	F=0.14 p=0.71 Eta^2<0.01
Right Amygdala-Hippocampal complex	4.3 (0.1)	4.4 (0.1)	F=0.87 p=0.35 Eta^2=0.02	F=4.4 p=0.04 Eta^2=0.07
Left Amygdala-Hippocampal complex	4.2 (0.1)	4.3 (0.1)	F=0.41 p=0.53 Eta^2=0.01	F=4.4 p=0.04 Eta^2=0.07

7.5 DISCUSSION

7.5.1 Severe Hypoglycaemia and Cognitive Ability

In the present study no significant relationship was identified between any summary measure of previous exposure to severe hypoglycaemia and cognitive disadvantage. This observation is entirely consistent with those reported by the prospective studies which have evaluated the relationship between severe hypoglycaemia exposure and subsequent neuropsychological performance, which have included the Diabetes Control and Complications Trial (DCCT) (The DCCT Investigators, 1996;Austin and Deary, 1999), the Stockholm Diabetes Intervention Study (SDIS) (Reichard et al., 1991), and the EDIC study (Jacobson et al., 2007). In each of these studies no evidence of impaired cognitive function was identified in young adults who had been exposed to recurrent severe hypoglycaemia. However, retrospective cross-sectional studies in adults with type 1 diabetes (Langan et al., 1991;Lincoln et al., 1996;Sachon et al., 1992;Wredling et al., 1990;Langan et al., 1991) have identified modest decrements in aspects of cognitive ability, directly related to the frequency of previous recurrent severe hypoglycaemia. These cross-sectional studies examined people who developed type 1 diabetes in adulthood and who were older than the participants of the present study. Their observations conflict with the findings of the present study. Others have suggested that this may reflect relative vulnerability of the aging brain to severe hypoglycaemia, but the recently published findings of the EDIC study (Jacobson et al., 2007) mitigate this interpretation, as participants were of a similar age to those evaluated by the cross-sectional studies. Other factors, such as selection bias and methodological weaknesses may offer some explanation for the divergent findings of the prospective and cross-sectional studies. However, no convincing evidence exists which definitively demonstrates that repeated exposure to severe hypoglycaemia, at least during adulthood, is detrimental to subsequent neuropsychological performance. Some caveats require to be applied to this statement. Firstly, the severe hypoglycaemia exposure data of the present study were collected retrospectively. This

does not allow examination of the timing of severe hypoglycaemia with respect to age, which may be of particular importance in early childhood as some have reported relative vulnerability of the childhood brain to a hypoglycaemic insult, although data are conflicting and inconsistent. However, structured retrospective assessments of preceding severe hypoglycaemia have been shown to be reproducible over a modest period (Deary et al., 1993) and are seldom sufficiently inaccurate to fail to delineate those naïve or heavily exposed to severe hypoglycaemia. Secondly, protracted severe hypoglycaemia is undoubtedly damaging to the human brain but is extremely uncommon in routine clinical practice in those with Type 1 diabetes, except in the setting of a deliberate attempt at self-harm or following alcohol ingestion (Arky et al., 1968). Such episodes of protracted severe hypoglycaemia do cause structural brain damage (Boeve et al., 1995;Chalmers et al., 1991;Chan et al., 2003;Finelli, 2001;Gold and Marshall, 1996;Holemans et al., 2001;Maekawa et al., 2006;Perros et al., 1994;Rajbhandari et al., 1998;Richardson et al., 1981;Yoneda and Yamamoto, 2005). Thirdly, the apparently reassuring findings with respect to exposure to severe hypoglycaemia from the present study do not exclude detrimental effects on those domains of cognitive ability which were not examined: the assessment of cognitive abilities in the present study was not all embracing; learning and memory ability, abstract reasoning and a detailed examination of executive functions were not performed. However, the findings of the EDIC study, which did examine all recognised domains of cognitive ability in detail would suggest that the present observations are representative: no detrimental effect of any prospective measure of severe hypoglycaemia exposure was associated with cognitive disadvantage (Jacobson et al., 2007).

7.5.2 Severe Hypoglycaemia and Structural Brain Abnormalities

In the present study, in addition to the absence of any discernible effect of severe hypoglycaemia on the aspects of cognitive ability that were assessed, structural abnormalities of the brain were not observed in association with a history of preceding severe hypoglycaemia. In particular, no

relationship was identified between the frequency of previous severe hypoglycaemia and cerebral atrophy, in contrast to the observations of an earlier study (Perros et al., 1997) which examined a small number of participants (n=22) recruited from a larger cohort (Deary et al., 1993;Langan et al., 1991), who were chronologically older and had developed Type 1 diabetes in adulthood. In addition, in analysing these earlier results Perros had not accounted for some potential confounders, most notably the difference between the incidence of microvascular disease between the study groups (82% in those previously exposed to severe hypoglycaemia vs. 55% in those who were not exposed to severe hypoglycaemia). These differences may underlie the apparent disparity between the observations of the present study, which suggest that recurrent severe hypoglycaemia may not be detrimental to the brain, at least in young adults below the age of 45 years who do not have advanced or end-stage microangiopathy. This reassurance cannot at present be applied to older people with Type 1 diabetes, those with a longer duration of the disorder, or those in whom advanced microangiopathy is present.

7.5.3 Diabetic Retinopathy and Neuropsychological Performance

A significant independent association was observed between the presence of Background Diabetic Retinopathy (early retinopathy) and cognitive ability, which remained robust when between-subjects factors and covariates (age, gender, pre-morbid IQ, severe hypoglycaemia exposure and duration of diabetes) were considered. The subgroup that had Background Diabetic Retinopathy performed less well on cognitive tests of fluid intelligence, information processing speed, and the ability to maintain attention and concentration. The magnitude of the relative disadvantage in cognitive performance was moderate (Eta^2=0.09-0.17, 0.4-0.7SD), *i.e.* approximately equivalent to 6-10 IQ points, and similar to that observed by other studies that have examined the relationship between microangiopathy and cognition (Dejgaard et al., 1991;Ryan et al., 1992;Ryan et al., 1993). However, the differences observed in the present study may be evident at a lower cumulative degree of glycaemic exposure than that associated with the

development of Diabetic Peripheral Neuropathy (Orchard et al., 1997). The present observations differ from those reported by Dejgaard and colleagues (Dejgaard et al., 1991) in terms of the domains of ability compromised by microangiopathy, but are remarkably consistent with the larger studies performed by Ryan et al: psychomotor speed, sustained attention and visuo-spatial ability (WAIS-R Block Design Sub-test) were detrimentally affected by microangiopathy. The mechanism by which the development of Background Diabetic Retinopathy may confer cognitive disadvantage is unknown. Differences in visual function were considered as being potentially responsible for the disadvantage observed, yet the visual component of the information processing pathway (as measured by P100 latency) is known to be unaffected by Background Retinopathy (Ewing et al., 1998). It is more likely that the presence of Diabetic Retinopathy, may be a surrogate marker for the presence of microvascular disease elsewhere within the brain. The relationship between retinal abnormalities and the development of subsequent cerebral macrovascular episodes or cognitive impairment is established in middle-aged and elderly individuals (Ikram et al., 2006;Longstreth, Jr. et al., 2007;Mitchell et al., 2005;Wong et al., 2002). In the setting of Type 1 diabetes in the younger population studied in the present study it is unlikely that the presence of Diabetic Retinopathy is a surrogate marker of cerebral macrovascular disease, indeed no neuroimaging evidence of silent cerebrovascular disease was observed. Rather, the presence of Background Diabetic Retinopathy is more likely to be a surrogate for preceding chronic hyperglycemia, accompanying sub-optimal control of diabetes. The present observations infer that the cerebral circulation may not be as immune to the development of microangiopathy as previously thought, although neuropathological study would be required to prove this assumption beyond doubt. Such neuropathological data is not presently available.

High-intensity white-matter lesions (leukoaraiosis) are abnormalities detected on T2-weighted magnetic resonance images representing areas of increased water content, gliosis and

demyelination within white-matter (Pantoni and Garcia, 1995) and are thought to signify foci of microvascular ischemia (Englund, 2002). The small punctate white matter lesions (SPWML), of the type that were associated with Background Retinopathy in this study, are thought to correspond to enlarged peri-vascular spaces, in keeping with the structural alterations observed in association with diabetic microangiopathy in other tissues. The intra-cranial circulation, with the exception of the ophthalmic artery, was previously considered to be spared from the development of typical diabetic microangiopathy. The correlation observed in the present study between Background Retinopathy and SPWML in the region of the Basal Ganglia has not been previously reported and would suggest that microangiopathy can affect cerebral microvessels beyond the confines of the ophthalmic artery. Basal Ganglia abnormalities have been observed in young people with type 1 diabetes: basal ganglia infarction has been observed in association with microvascular complications (Bellassoued et al., 2001) and Basal Ganglia pathology in the setting of keto-acidosis (Ertl-Wagner et al., 1999;Roe et al., 1996;Rogers et al., 1990). The apparent vulnerability of the Basal Ganglia may relate to microvascular disease of the Lenticulo-Striatal Arteries, penetrating arteries which supply a threshold watershed zone in the deep gray matter.

The association observed between Background Retinopathy and white matter lesions is consistent with other observations associating retinal vascular abnormalities with white matter lesions on cranial MRI scans. Data from studies of subjects with Type 2 diabetes have previously described independent associations between the presence of Diabetic Retinopathy , Hypertension and white-matter abnormalities (Inoue et al., 1996). Hypertensive retinopathy is associated with cerebral high-intensity white matter lesions (Wong and Mitchell, 2007)1740(Ikram et al., 2006;Longstreth, Jr. et al., 2007) implying that the presence of retinal microangiopathy is a surrogate marker for more generalised cerebral microangiopathy at least in those with hypertension. Despite the association observed in the present study between Background Retinopathy and small punctuate white matter lesions (enlarged peri-vascular spaces), the

presence of these white matter lesions was not associated with cognitive ability. This may imply that Diabetic Retinopathy and enlarged peri-vascular spaces do not in fact represent an identical histopathological abnormality. Alternatively, the pathogenesis of Diabetic Retinopathy and enlarged peri-vascular spaces may be similar, with non-exudative retinopathy representing an early manifestation and enlarged peri-vascular spaces indicating more advanced damage. A further explanation is offered by the difference in sensitivity for the detection of microangiopathy provided by cerebral magnetic resonance imaging when compared with ophthalmoscopy and digital retinal imaging. The pathognomonic lesion of background diabetic retinopathy, the microaneurysm, is a few microns in size, whereas the voxel size utilised in MRI neuroimaging protocols is several orders of magnitude greater.

Irrespective of the association between background retinopathy and structural abnormalities of the brain, the pathogenesis of the cognitive disadvantage in the subgroup with established background diabetic retinopathy is likely to have a vascular aetiology. Functional MRI studies suggest that cerebrovascular responsiveness may be important during normal cognitive activity, as discrete changes in regional cerebral blood flow occur during cognitive tasks (Rosenthal et al., 2001). Long duration type 1 diabetes complicated by Diabetic Retinopathy has been shown to be associated with impaired cerebrovascular reactivity in response to Acetazolamide (Fulesdi et al., 1997) and it is possible that impairment of vascular reactivity could in part explain the cognitive disadvantage observed in the present study. In addition, the results of the present study support the concept of a "microvascular-mediated" effect on cognitive function in people with diabetes of long duration, as a significant contributory factor in the pathogenesis of a "diabetic encephalopathy". The exclusion and inclusion criteria resulted in the recruitment of a group of people who had Type 1 diabetes of long duration, yet had a low microvascular disease burden relative to the general population of individuals with similar disease duration. In view of this, the findings of the present study, with respect to the apparently detrimental effects of

microangiopathy on the brain, could be reasonably expected to under-estimate the magnitude of disadvantage which is likely in the general population of people with Type 1 diabetes of similar disease duration.

An interaction between multiple complications of diabetes and cognitive performance has been reported previously. Ryan and colleagues identified an association between poorer cognitive performance and Peripheral Neuropathy in adults who had developed type 1 diabetes in childhood or adolescence, while no direct detrimental effect of severe hypoglycaemia alone was detected (Ryan et al., 1992). A statistically greater degree of cognitive dysfunction was observed when Peripheral Neuropathy co-existed with a history of preceding exposure to recurrent severe hypoglycaemia, although the magnitude of additional variance accounted for by this interaction was small and perhaps of academic interest rather than a clinically meaningful affect. The present study did not identify a greater degree of cognitive disadvantage in those who had Background Retinopathy and a history of exposure to recurrent severe hypoglycaemia when compared to those who had Background Retinopathy alone. However, the sub-groups (n=8 vs. n=17) for this analysis were small, and no firm conclusions regarding the presence or absence of an interaction between microangiopathy, severe hypoglycaemia and cognitive ability can be drawn from the present study which was underpowered to address this issue.

7.5.4 Problems with the Definition of "Early Onset Diabetes"

In the present study an early onset age of Type 1 diabetes (EOD) was defined as an age at the diagnosis of diabetes at, or below, seven years. Studies of cognitive ability in children with Type 1 diabetes have varied in their definition of an "early onset" and "later onset" of Type 1 diabetes, with definitions ranging between four and seven years for early onset diabetes (EOD) (Ack et al., 1961;Alvarez and Rovet, 1997;Bjorgaas et al., 1997;Chabriat et al., 1994;Hagen et al., 1990;Hershey et al., 1997;Holmes and Richman, 1985;Northam et al., 1999;Northam et al.,

2001;Rovet et al., 1988a;Ryan et al., 1985a;Sarac et al., 2005;Schoenle et al., 2002;Service, 1995;Wolters et al., 1996;Wysocki et al., 2003;Ack et al., 1961;Ack et al., 1961). MRI neurodevelopmental studies have demonstrated that human brain volume increases rapidly in the first two to three years of life and by 5-10 years adult whole brain volumes are attained (Durston et al., 2001;Lenroot and Giedd, 2006). Subsequent neurodevelopment involves proportional changes in the ratio of cortical gray matter to cerebral white-matter: cortical gray matter decreases from a maximal volume at 4 years of age through a remodelling process known as "pruning" to approach adult volume by 10-17 years, with the age at maturation being gender and regionally-specific (Lenroot and Giedd, 2006), whilst cerebral white matter volumes progressively increase until the 3rd decade of life through the process of myelination (Durston et al., 2001;Lenroot and Giedd, 2006). An EOD of seven years or less was selected as a compromise between the cognitive and neuro-developmental studies, so as to provide some degree of comparability between the findings of the present study and the body of literature describing cognitive ability in children with Type 1 diabetes whilst providing a sufficiently mature cut-off age such that an adult whole brain volume would have been largely attained in those defined as LOD.

7.5.5 Cognitive Ability and Early Onset Diabetes

Before interpreting the observations of the present study specific methodological issues require consideration. Firstly, the cognitive assessment utilised in the present study was not all encompassing; frontal and executive functions were not assessed in any depth, and memory and learning abilities were not evaluated. Instead, an emphasis was placed upon the assessment of information processing and fluid intelligence, as deficits in these abilities had been identified in previous studies of people with Type 1 diabetes at our centre (Deary et al., 1993;Langan et al., 1991). Secondly, the cross-sectional design of the present study can merely infer associations rather than causality, which requires detailed and careful prospective assessment. Finally, the National Adult Reading Test (NART) was used to estimate the maximum intellectual attainment

(crystallised intelligence) of participants (Nelson and Willison, 1991). NART scores are relatively resistant to organic brain disease throughout adult life and predict pre-morbid intellectual ability (crystallised intelligence) with a greater degree of accuracy than estimates derived from socio-economic demographics. Whilst the NART is a valid estimate of crystallised intelligence in healthy adults who have completed intellectual development in the absence of any significant childhood pathology, its validity remains relatively untested in the setting of childhood disease. The early childhood development of Type 1 diabetes could theoretically interfere with the normal attainment of crystallised intelligence, particularly if diabetes developed during the critical period of rapid brain growth evident during early childhood neurodevelopment. It is therefore possible that the use of the NART in the present study may have underestimated the magnitude of any detrimental effect that an EOD had on cognitive ability. However, to have not included a marker of pre-morbid intellectual ability in the analysis of cognitive ability would confound any meaningful interpretation of results.

Accepting the above caveats, the present study identified an association between the diagnosis of Type 1 diabetes at, or below the age of seven years (EOD), and relatively poorer fluid intelligence ability (WAIS-R performance IQ) and slower psychomotor speed (Simple and Choice reaction time), an association that was independent of all other factors identified as being significant determinants of cognitive performance (pre-morbid intellectual ability, the presence of background retinopathy and gender) and other factors of interest that appeared to have no statistically significant effect on cognitive ability (diabetes duration and severe hypoglycaemia exposure). The specific cognitive abilities that appeared to have been compromised by the development of diabetes in early childhood were generally consistent with previous observations in children who had developed Type 1 diabetes at an early age, although direct comparisons are hampered by the inconsistency of the definition of an EOD between individual studies; comparatively lower fluid intelligence scores (WAIS-R performance IQ) (Holmes and Richman,

1985;Northam et al., 1999;Rovet et al., 1988a;Rovet et al., 1988b;Ryan et al., 1985a;Rovet et al., 1988a) and slower information processing speed (Bjorgaas et al., 1997;Hagen et al., 1990;Northam et al., 2001;Ryan et al., 1985a;Service, 1995;Bjorgaas et al., 1997;Ryan et al., 1985a) have been previously observed, although no effect on information processing ability has also been reported (Kail et al., 2000). The other cognitive abilities examined by the present study (early visual information processing, frontal and executive functions and the ability to sustain attention and concentration) did not appear to be disadvantaged by the development of diabetes at an early age. This is not entirely consistent with published observations: poorer performance has been described in association with an EOD in attention ability (Alvarez and Rovet, 1997;Bjorgaas et al., 1997;Hagen et al., 1990;Northam et al., 2001;Rovet and Ehrlich, 1999;Ryan et al., 1985a;Wolters et al., 1996;Rovet and Ehrlich, 1999). Differences in executive function, relative to healthy controls, have also been reported in children developing Type 1 diabetes at a young age (Hagen et al., 1990;Hershey et al., 1997;Northam et al., 2001;Sarac et al., 2005;Service, 1995). The disparities between the present study findings and published literature highlights difficulties within this research area; relative deficits in cognitive abilities are generally identified in those with an EOD but inconsistencies in the specific cognitive abilities affected are common. These inconsistencies may reflect the heterogeneity of the clinical characteristics of the study samples and control group selection, sample size issues, the varied psychometric instruments used to evaluate intellectual performance and diversity in the definitions used to characterise early onset diabetes.

The present study was not designed to determine the pathogenesis of the cognitive disadvantage observed in those with an EOD but may offer some clues as to causality. As an EOD influenced cognitive ability independently of the presence of Background Retinopathy, a surrogate marker of lifetime glycaemic exposure, and duration of diabetes this implies that the pathogenesis is unlikely to simply reflect cumulative glycaemic exposure. Experts have hypothesised that the

cognitive disadvantage associated with an EOD may relate to severe hypoglycaemic exposure during early childhood neurodevelopment (Ryan, 1988), a hypothesis based upon retrospective reports of a higher frequency of hypoglycaemic-seizures in children who developed diabetes at a very young age (Daneman et al., 1989;Davis and Jones, 1998;Golden et al., 1985;Ternand et al., 1982). Subsequent evaluation has largely refuted this hypothesis as the prospective studies within this field have determined that the diagnosis of Type 1 diabetes in early childhood is associated with poorer neuropsychological performance across a broad range of cognitive domains (Northam et al., 2001), whereas the detrimental effects of early life exposure to severe hypoglycaemia appear more specific and modest in effect size (Northam et al., 2001;Rovet and Ehrlich, 1999;Schoenle et al., 2002). The assessment of severe hypoglycaemia exposure in the present study was retrospective and, as such, insufficient to accurately delineate the precise age at which exposure to severe hypoglycaemia, or hypoglycaemia-associated seizures had occurred. This relative weakness of the present study means that it cannot be definitively concluded that severe hypoglycaemia is not injurious to the developing brain and the acquisition of intellectual abilities, despite the lack of statistical associations between any summary measure of severe hypoglycaemia exposure and intellectual performance. The unfavourable effects of an EOD on intellectual ability therefore appear to be independent of cumulative glycaemic load, microvascular complications and duration of diabetes, and have previously been shown to be independent of severe hypoglycaemia exposure (Northam et al., 2001;Schoenle et al., 2002). The pathogenesis of these cognitive consequences remains uncertain and alternative explanations may be required. Alternative hypotheses have suggested that school attendance problems (Hagen et al., 1990;McCarthy et al., 2002;McCarthy et al., 2003;Parker et al., 1994;Rovet et al., 1987;Ryan et al., 1985b), behavioural difficulties (McCarthy et al., 2002;Rovet et al., 1987) and perhaps abnormal myelination as a consequence of diabetes *per se* (Vlassara H et al., 1983) may be potential factors. The relative-deficiency of insulin during childhood neurodevelopment could also potentially contribute. Insulin has anabolic effects in many tissues, receptor expression in

specific brain areas which are involved in cognition, particularly the neocortex and medial temporal lobe, and may influence cognition through effects on neurotransmitter metabolism and synaptic plasticity (Biessels et al., 1994). However, it is not known whether insulin has neurotrophic effects during childhood and adolescence which contribute significantly to central nervous system maturation and intellectual development.

7.5.6 Brain Structure and Early Onset Diabetes

The present study compared measures of brain structure in two groups of subjects who had developed Type 1 diabetes during childhood or adolescence. A significantly higher frequency of structural brain abnormalities was observed in those with an onset of diabetes ≤ 7 years of age (EOD). Abnormalities were present in 60% of EOD subjects and included macroscopic abnormalities, apparent on routine clinical inspection of MRI brain scans, and sub-clinical differences in regional brain volumes, elicited through detailed volumetric analysis. General linear modelling (MANCOVA) was used to determine the independent effect of the age of onset of diabetes (EOD/LOD) on regional brain volumes. Intra-cranial volume is gender and gene dependent, such that > 90% of whole brain volume and sub-regional volumes are inherited (White T et al., 2002): males and those of greater somatic size have proportionally larger brains, although gender-specific differences also significantly influence regional brain volumes such that the absolute volume of the Hippocampus is comparable between genders, whereas the volume of the Caudate is proportionally greater and the Amygdala smaller in females (Lenroot and Giedd, 2006). In order to facilitate the detection of brain volume changes caused by an early onset age of diabetes (EOD), intra-cranial volume and age were entered as co-variates and gender as a between-subjects factor for all volumetric analyses. It could be argued that co-varying for intra-cranial volume could potentially mask any reduction in cerebral volume consequent upon the development of diabetes at an early age, under the premise that early onset diabetes could impair brain growth and hence the volume of the skull (intra-cranial volume). However, as no

correlation between intra-cranial volume and age at diagnosis of diabetes was observed, co-variance for intra-cranial volume within the volumetric analysis appeared appropriate. The lack of correlation between an early childhood onset of diabetes and head size was unsurprising as the development of microcephaly is generally associated with a major *in utero* or early life brain insult.

The comparatively higher frequency of ventricular atrophy (odds ratio=4.6), which was in general mild, and the relatively greater lateral ventricular volumes, consistent with a sub-clinical degree of central brain atrophy, in those with an EOD are consistent with the study hypothesis *i.e.* that the development of Type 1 diabetes at an early age would detrimentally affect brain structure. Ventricular atrophy with enlargement of the lateral ventricles are characteristically observed as a feature of normal brain aging and the co-existence of these features in the EOD group could be reflect sub-optimal brain development, or alternatively, an advanced state of brain aging relative to chronological age. During normal childhood neurodevelopment the volume of the Lateral Ventricles increases, being greater in males than in females (Lenroot and Giedd, 2006). The process underlying this increase in Ventricular cavity size is thought to represent "pruning" or selective programmed neuronal and glial cell death and it is unclear whether the development of diabetes during childhood modulates this process, favouring a greater degree of cell loss. The cross-sectional design of the present study does not permit clarification of which process is responsible, but as the higher frequency of ventricular atrophy and greater lateral ventricular volumes in the EOD group were observed independently of diabetes duration and chronological age, this implies that sub-optimal neurodevelopment rather than a state of advanced brain aging may be more likely. No direct measurements of gray or white matter volumes were obtained in the present study and it remains uncertain whether the differences in ventricular atrophy and lateral ventricular volume observed in the EOD group represent a reduction in the volume of gray matter, white matter or a combination of both.

In addition to structural changes consistent with mild brain atrophy, differences in the frequency of high-intensity white-matter lesions were inconsistently observed between EOD and LOD groups. MRI scans were rated for the presence of two different types of white-matter lesion; leukoaraiosis, high intensity white matter lesions observed on T2-weighted images thought to represent foci of microvascular ischaemia (Englund, 2002;Fazekas et al., 1991), and small punctuate white-matter lesions (SPWML) thought to represent enlarged peri-vascular spaces. Rating scales for leukoaraiosis differ in their sensitivity in scoring WML, with some being relatively insensitive to minor degrees of leukoaraiosis (Fazekas et al., 2002;Mantyla et al., 1997;Wardlaw et al., 2004). Certain rating scales appeared relatively insensitive to the leukoaraiosis observed in the present study and attempts at analysis were inappropriate due to small numbers of positively scored scans(Shimada et al., 1990), whereas others appeared more sensitive in scoring the type of leukoaraiosis observed (Fazekas et al., 1987;Longstreth, Jr. et al., 1996;Mirsen TR et al., 1991;Wahlund et al., 1990;Wahlund et al., 1990). Of those scales which scored the type of leukoaraiosis observed in the present study, an EOD was not consistently identified as an independent predictor of the presence of leukoaraiosis: a significantly higher frequency of leukoaraiosis was observed when the Wahlund rating scale was used (Wahlund et al., 1990) but not with any other rating scale(Fazekas et al., 1987;Mirsen TR et al., 1991). This inconsistency may reflect differences in sensitivity and scoring criteria inherent in the rating scales, or alternatively Type 1 statistical error. Alternatively, if the difference in leukoaraiosis determined by the method of Wahlund et al is considered significant, this may add corroboration to animal work suggesting that abnormal myelination occurs as a consequence of diabetes (Vlassara H et al., 1983), although the independence from surrogates of glycaemic exposure (Background Retinopathy) may mitigate this interpretation. Hippocampal small punctate white-matter lesions (SPWML) were observed more frequently in those who developed diabetes at an early age (EOD). The hippocampus appears vulnerable in diabetes and mesial temporal lobe

sclerosis has been reported in up to 15% of IDDM subjects who developed diabetes at < 6 years of age (Davis et al., 2002). In the present study, the validity of the statistically higher frequency of Hippocampal SPWML should be viewed with considerable caution as the statistical analysis was based upon 3 cases of Hippocampal SPWML in EOD subjects vs. 1 case in LOD subjects, and no other significant differences were observed in other areas of white-matter. Therefore, from the present study it cannot be concluded with any degree of certainty that an EOD is associated with either the leukoaraiosis or SPWML. As white matter abnormalities are thought to be manifestations of cerebral microvascular disease (Englund, 2002;Fazekas et al., 1991;Pantoni and Garcia, 1995), and the structural brain abnormalities associated with an EOD were present independently of microangiopathy elsewhere (Diabetic Retinopathy) and diabetes duration, an alternative pathogenesis is implied. This is in keeping with the neuropsychological findings of the present study.

The present study did not account for other potential confounding factors related to Type 1 diabetes that could potentially account for at least some of the differences in cognitive ability and brain structure identified by the present study. The most prominent diabetes-related factor that was not explored was the effect of exposure to episodes of diabetic keto-acidosis (DKA) on brain structure and function. This specific factor was not examined as preceeding exposure to DKA could not be accurately determined as the patient group from which participants were recruited consisted of young adults, many of whom had developed Type 1 diabetes in early childhood and had only relocated to the Edinburgh area in their late-teens or early twenties from across the United Kingdom. It was therefore not possible to attain a preceeding DKA history with any degree of accuracy, particularly with respect to the timing of episodes, and as such no formal attempt was made to collect this data. As such, DKA must remain as a potential confounding factor, which could account for at least some of the variance in cognitive ability and brain structure not captured by the linear modelling techniques utilised in the present study.

In conclusion, the present study indicates that subtle structural abnormalities of the brain, consistent with a mild degree of central brain atrophy, and modest deficits in cognitive performance are common in adults who had developed Type 1 diabetes before their seventh birthday. The co-existence of structural brain abnormalities and cognitive disadvantage strongly implies that normal neurodevelopment may be adversely affected by the early childhood onset of Type 1 diabetes. The pathogenesis of the cognitive disadvantage and structural brain abnormalities observed in those who had developed Type 1 diabetes in early childhood is uncertain. Previous prospective reports have suggested that the cognitive disadvantage associated with an EOD is independent of that associated with severe hypoglycaemia exposure (Northam et al., 2001;Schoenle et al., 2002) and the present study infers that cognitive disadvantage and structural brain abnormalities are unlikely to be a consequence of chronic hyperglycaemia and the development of microangiopathy, or a manifestation of the duration of diabetes. Prospective evaluation of neurodevelopment in children with Type 1 diabetes is required to validate the above findings and further elucidate the pathogenesis of the negative effects that an early onset of Type 1 diabetes appears to confer upon the central nervous system.

CHAPTER 8

INFLUENCE OF DIABETES-SPECIFIC FACTORS ON OBJECTIVELY RATED STRUCTURAL BRAIN ABNORMALITIES IN TYPE 1 DIABETES

CHAPTER 8: INFLUENCE OF DIABETES-SPECIFIC FACTORS ON OBJECTIVELY RATED STRUCTURAL BRAIN ABNORMALITIES IN TYPE 1 DIABETES

8.1 INTRODUCTION

The neuroimaging associations of Type 1 diabetes remain under explored. Several diabetes-specific factors have been reported in the literature as being associated with the presence of structural brain abnormalities in case reports and small case-control studies. These diabetes-specific factors include exposure to protracted and profound degrees of severe hypoglycaemia, the development of Diabetic Keto-Acidosis in children and adolescents, and the development of microvascular complications as a consequence of chronic exposure to hyperglycaemia.

Anecdotal reports have reported permanent neuro-imaging abnormalities following exposure to protracted and profound degrees of hypoglycaemia in Type 1 diabetes (Bakshi et al., 2000;Boeve et al., 1995;Chalmers et al., 1991;Chan et al., 2003;Finelli, 2001;Gold and Marshall, 1996;Holemans et al., 2001;Koppel and Daras, 1993;Maekawa et al., 2006;Perros et al., 1994;Rajbhandari et al., 1998;Richardson et al., 1981;Yoneda and Yamamoto, 2005). The neuro-radiological abnormalities summarised by these case reports confirm that abnormalities consequent upon profound hypoglycaemia predominantly affect the cortical gray matter, sub-cortical gray matter structures, particularly the hippocampus and basal ganglia, but can also affect the brain stem if hypoglycaemia is particularly protracted. This pattern is consistent with that reported in rodent models of hypoglycaemic brain injury (Auer and Siesjo, 1993). It remains unresolved whether recurrent episodes of severe hypoglycaemia, in the absence of protracted coma, can have long-term deleterious effects on cerebral structure in Type 1 diabetes. A study of a small sub-group of the subjects previously examined by Langan et al (Langan et al., 1991) were

re-examined by Perros who reported that cortical atrophy was more prevalent in those with a history of frequent exposure to severe hypoglycaemia (n=11) relative to those who were naïve to severe hypoglycaemia (n=11) (Perros et al., 1997). This small study is the sole study published to date which has attempted to examine the neuro-radiological associations of severe hypoglycaemia exposure in a structured manner.

The effects of microangiopathy on cerebral structure in Type 1 diabetes has been examined in three cross-sectional studies. Dejgaard and colleagues examined sixteen patients with long-duration Type 1 diabetes, complicated by proliferative diabetic retinopathy and peripheral neuropathy, relative the appearances observed in non-diabetic healthy controls (n=40). High-intensity white matter lesions were observed in 69% of the diabetes group, of whom 31% were also considered to have a degree of subjectively-rated cerebral atrophy. The white matter lesions observed by Dejgaard were non-specific in their distribution, were small in size, infrequent in number and more common in the microangiopathic group (69%) relative to healthy age-matched controls (12%). Dejgaard et al interpreted the higher prevalence of white-matter lesions observed in the microangiopathic group as being indicative of the presence of intra-cranial microangiopathy, complicating Type 1 diabetes, or alternatively as being indicative of small foci of ischaemia, known as Leukoaraiosis (Pantoni and Garcia, 1995) as part of a subcortical arteriosclerotic encephalopathy (Dejgaard et al., 1991). Youssem and colleagues (Yousem et al., 1991) compared the frequency of white-matter lesions in 25 adult patients with Type 1 diabetes complicated by proliferative diabetic retinopathy (severe retinopathy) relative to 10 age-matched healthy control subjects and did not identify any structural brain abnormalities. Lunetta and co-workers examined ten patients with Type 1 diabetes, who had a relatively short median duration of diabetes, variable hypoglycaemia exposure and differing degrees of microangiopathy (Lunetta et al., 1994) and reported an increased Cerebro-Ventricular Index, a surrogate of central brain atrophy, in 70% of the diabetes group, and a degree of subjectively-rated cortical atrophy in some

participants (Lunetta et al., 1994). The small studies summarised above appear to provide conflicting insights into the structural brain associations of microangiopathy, each utilising differing MRI methodologies and studying diverse patient groups. The relationship between Type 1 diabetes and white matter lesions remains under evaluated at present with the limited data inferring that the prevalence of white matter change and perhaps cerebral atrophy may be greater in those with T1DM complicated by microangiopathy.

The onset of Type 1 diabetes in early childhood is associated with a degree of cognitive disadvantage in prospective studies (Northam et al., 2001;Rovet and Ehrlich, 1999;Schoenle et al., 2002). Experts have hypothesised that the association may be explained by diabetes-specific organic factors (Ryan et al., 1985a), such as exposure to severe hypoglycaemia or diabetic keto-acidosis in early life, whereas others have suggested that socio-behavioural factors could explain the differences observed (Fallstrom, 1974;Rovet et al., 1987b). Despite an extensive body of literature exploring the neuropsychological consequences of early life exposure to severe hypoglycaemia, there is an extremely limited body of evidence relating to brain structure (Christiaens et al., 2003), with no case reports of neuroimaging sequelae of severe hypoglycaemia published in children with Type 1 diabetes. In contrast, diabetic keto-acidosis remains the leading cause of death and disability in children with Type 1 diabetes (Edge et al., 1999) and an extensive body of case reports and case-series have identified neuroimaging abnormalities consequent upon diabetic keto-acidosis (Greene et al., 1990;Hoffman et al., 1999;Iwai et al., 1987;Keane et al., 2002;McAloon et al., 1990;Roe et al., 1996;Rogers et al., 1990;Shrier et al., 1999). DKA can be complicated by neurological deterioration and permanent neuroradiological abnormalities, most commonly as cerebral infarction in severe cases. The pattern of brain injury differs significantly from that appears to be associated with hypoglycaemic brain damage. However, no studies to date have examined the neuroimaging associations of an early life onset of Type 1 diabetes.

8.2 AIMS

The present study examined a large group of adults with type 1 diabetes of relatively long duration: a subgroup had developed the disorder during childhood or adolescence, and as a consequence were exposed to the potential negative consequences of diabetes during neurodevelopment and a second subgroup in which the onset of diabetes had occurred after neurodevelopment was largely complete. The present study was designed to achieve a number of aims:

1. To determine the prevalence of white matter abnormalities and cerebral atrophy in a population of young adults with T1DM.
2. To determine whether structural brain abnormalities, such as white matter lesions or cerebral atrophy, were disadvantageous to aspects of cognitive ability in T1DM.
3. To explore whether diabetes-specific factors independently influenced the presence of either white matter abnormalities or cerebral atrophy.

As no healthy age-matched control group was included, the present study was not designed to examine the effects of Type 1 diabetes *per se* on brain structural outcome measures, rather concentrated on determining the consequences of the diabetes-specific disease factors of interest on macroscopic neuroimaging outcomes of interest.

8.3 SUBJECTS AND METHODS

8.3.1 Study Design

The study was cross-sectional in design and the study protocol was completed in sequential order:

1. detailed Magnetic Resonance Imaging (MRI) of the brain;

2. neuropsychological test battery;

3. retinal examination;

4. assessment of preceding severe hypoglycaemia;

Each component was evaluated and scored blind to all other information.

8.3.2 Subjects

Recruitment criteria were selected with the explicit aim of obtaining a cohort with a long duration of diabetes (> 10 years), sufficient to ensure some exposure to the metabolic consequences of interest (severe hypoglycaemia, diabetic retinopathy, disease duration), but without accruing other significant co-morbidity which may confound the interpretation of neuroimaging findings. One hundred and thirty three subjects were recruited of whom 71 had developed Type 1 diabetes in childhood or adolescence (age at diagnosis < 18 years) with the remaining 62 having developed T1DM after their 18th birthday and had therefore largely completed neurodevelopment before the onset of diabetes. For the total cohort of 133 individuals, an upper age limit of 45 years was applied to minimise the potential confounding effects of ageing on brain structure and cognitive ability, but also to minimise the likelihood of recruiting subjects with silent macrovascular disease of the cerebral circulation (Klein et al., 2004;Nathan et al., 2005;Stettler et al., 2006). Those invited to participate either had no clinical evidence of diabetic microvascular complications or had early microangiopathy, manifest as background diabetic retinopathy alone. As many of the cognitive ability tests utilised were highly dependent on visual function, those

with more advanced degrees of diabetic retinopathy (Airlie House grading ≥ 2) (1991b) including diabetic maculopathy, pre-proliferative retinopathy, proliferative retinopathy or those who had previously received laser treatment for diabetic retinopathy were excluded from participation. The presence of any clinically apparent degree of diabetic neuropathy, the presence of microalbuminuria, or overt proteinuria were also exclusion criteria. Other exclusions included essential hypertension (defined as BP > 140/90 mmHg), any previous central nervous system pathology, previous alcohol or drug misuse, or other multi-system disease known to affect the central nervous system. The aim was to recruit only those in whom diabetes-related factors were likely to account for any differences observed in their cognitive performance or brain structure. The clinical characteristics of those studied are summarised in Table 8.1.

Table 8.1. Clinical characteristics of participants.

	Mean (± SD) n=133	**Median (Range)** n=133
Age (years)	32.3 (± 7.0)	32 (20-45)
Sex (male:female)	68:65	51.1% male
NART (pre-morbid ability)	33.2 (± 6.2)	34 (15-46)
HbA1c at time of study (%)	8.5 (± 1.3)	8.4 (5.5-12.5)
Blood Pressure (mmHg)	123/74 (± 13/9)	120/74 (88/50-140/88)
Age at Diagnosis of T1DM (years)	16.1 (± 8.4)	15 (1-36)
Duration of Diabetes (years)	16.1 (± 5.7)	15 (9-31)
Background Retinopathy (naïve:retinopathy)	84:49	36.8% Retinopathy
Severe Hypoglycaemia		
Median Total Episodes (% exposed, range)	13.8 (± 29.5)	4 (0-200), 67% exposed
Median Total Coma (% exposed, range)	3.0 (± 6.9)	1 (0-50), 53% exposed
Median Total Seizures (% exposed, range)	1.9 (± 4.9)	0 (0-29), 33% exposed

HbA_{1c} normal reference range 5.0-6.5%.

8.3.3 Methods

An outline of the methods utilised for neuro-imaging and the evaluation of structural brain abnormalities, and a list of the battery of tests used to assess intelligence and cognitive ability are provided in this section. Detailed descriptions of the neuro-imaging techniques, the characteristics of the neuropsychological test battery and the manner in which the clinical complications of Type 1 diabetes were evaluated are provided in the Methods Chapter, Section 6.4.

Assessment of the Clinical Complications of Type 1 Diabetes

Background diabetic retinopathy was defined by the presence of at least two or more microaneurysms in one eye (Airlie House Gradings 1a-1c) (1991b) and assessed using direct ophthalmoscopy and digital retinal imaging. Exposure to severe hypoglycaemia was assessed retrospectively using a validated formatted hypoglycaemia questionnaire (Deary et al., 1993). Participants were requested to discuss their severe hypoglycaemia history with relatives or friends before completing questionnaires, to improve the accuracy of estimates. The following parameters were recorded: the total lifetime number of episodes, the frequency of episodes, the total number of episodes of convulsions, coma, and other episodes requiring medical assistance to treat the hypoglycaemia. Severe hypoglycaemia was defined as any episode of hypoglycaemia sufficient in severity to require external assistance to facilitate recovery, similar to the definition used in the Diabetes Control and Complications Trial criteria (1997). The retrospective ascertainment of severe hypoglycaemia utilised in the series of studies comprising this thesis is a methodological weakness relative to the gold-standard methodology of prospective ascertainment, although is sufficient to determine with a degree of accuracy those naïve to and those previously exposed to severe hypoglycaemia. To overcome this relative methodological deficit would take decades of meticulous prospective evaluation, which is neither practicable nor easily funded.

Magnetic Resonance Imaging Protocol

MRI examinations of the brain were performed using a 1.0T SPE Magnetom scanner (Siemens, Erlangen, Germany). The first scan provided simultaneous proton-density and T2-weighted images that were used to calculate whole brain and cerebrospinal fluid volumes using a supervised cluster analysis package (ANALYZE, Mayo Foundation, Rochester, MN, USA). The second scan, for the regional volumetric analysis, was a three dimensional magnetization prepared for rapid-acquisition gradient echo sequence consisting of an 180 inversion pulse followed by a fast low-angle shot collection giving 128 contiguous 1.88mm thick slices in the coronal plane orthogonal to the Talairach plane. Inhomogeneity corrections were performed on Sun Microsystem workstations using ANALYZE to outline neuroanatomical structures. The regional brain volumes of interest were identified and their areas outlined and volumes calculated by summing voxels in the regions of interest. Additional detail of the MRI methods utilised are provided in the Methods Chapter, Section 6.6.

Assessment of High-Intensity White Matter Lesion Burden

Each MRI scan was reviewed by an experienced Neuroradiologist (Professor Wardlaw) and scored independently, and blinded to clinical factors, for the presence of high-intensity white matter lesions, also known as Leukoaraiosis (Breteler et al., 1994a;Breteler et al., 1994b;Fazekas et al., 1987;Longstreth, Jr. et al., 1996;Mirsen TR et al., 1991;Shimada et al., 1990;Van Swieten et al., 1990;Wahlund et al., 1990). Several scoring systems were used to capture the intensity, distribution (PVWH, DWMH) and appearance of white-matter lesions as no single scale was judged to be an adequate summary (Fazekas et al., 2002;Mantyla et al., 1997). The scoring of all MRI scans was performed by a single rater (Professor Wardlaw) to minimise the potential confounder of inter-observer variability (Wardlaw et al., 2004).

The presence of a distinctly different type of white matter abnormality, the small punctate white-matter lesion (SPWML), which was frequently observed but not accounted for by any of the above rating scales was also rated. A scale of 0-3 was utilised to quantify the severity of SPWML: 0=None; 1=Mild < 10 Lesions; 2=Moderate 10-20 Lesions; 3=Severe > 20 Lesions. This scale was used to quantify the presence of SPWML, indicating enlarged peri-vascular spaces, in three distinct brain regions (Hippocampus, Basal Ganglia, Centrum Semi-Ovale) using the most affected hemisphere.

Assessment of the Presence and Degree of Cerebral Atrophy

The presence or absence of cerebral atrophy was subjectively determined by an experienced Neuroradiologist (Professor Wardlaw)) and the severity rated on a subjective scale of 0-3:

0. No cerebral atrophy;
1. Mild cerebral atrophy;
2. Moderate cerebral atrophy;
3. Severe cerebral atrophy.

When present, cerebral atrophy was defined as being either "Ventricular" or "Sulcal".

Assessment of Neuropsychological Function and Intelligence

The neuropsychological test battery was delivered by a trained assessor blinded to the clinical characteristics of participants, provided an assessment of some major cognitive domains and was sensitive to mild-to-moderate cognitive decrements. The neuropsychological session was rescheduled if hypoglycaemia was present or if antecedent hypoglycaemia had occurred within the preceding 24 hours. Details of the neuropsychological test battery are provided in the Methods Chapter, Section 6.4.

The neuropsychological tests utilised were:

1. *Hospital Anxiety and Depression Scale* (Greenwood et al., 2000)
2. *Wechsler Adult Intelligence Scale-Revised* (WAIS-R) (Wechsler, 1981)
3. *National Adult Reading Test* (NART) (Nelson and Willison, 1991)
4. *Inspection Time* (IT) (McCrimmon et al., 1996)
5. *Choice Reaction Time* (CRT) (Jensen, 1987)
6. *Borkowski Verbal Fluency Test* (Borkowski and Benton, 1967)
7. *Paced Auditory Serial Addition Task* (PASAT) (Deary I.J. et al., 1991)

8.3.4 Statistical Analyses

All statistical analysis were performed using SPSS v10.0 (®SPSS Inc, Chicago, USA).

Structural Brain Outcome Measures

The relationship between the MRI outcomes of interest (cerebral atrophy, high-intensity white matter lesions, small punctate white matter lesions, each coded numerically), demographic variables and diabetes-specific factors was examined initially using bi-variate correlation (Spearman's rho). The relationships between the diabetes-specific variables of interest, demographic variables and subjectively-rated cerebral atrophy (coded as present/absent), the presence of white-matter lesions (coded as present/absent) and small punctate white matter lesions (coded as present/absent) were thereafter analysed by Multiple Logistic Regression. Logistic Regression Models were constructed on the basis of correlations (Spearman's rho) and prior hypotheses. Factors and co-variates were entered into each model in a stepwise forward conditional manner, such that non-significant parameters were ejected from the final model. Factors entered into the Multiple Logistic Regression Models included: Gender (male/female), Diabetes Onset Age (coded as Early Onset < 7^{th} birthday, Later Onset > 7^{th} birthday), Diabetic

Retinopathy status (coded as present/absent) and Severe Hypoglycaemia exposure (coded as naïve/exposed). The Multiple Logistic Regression Models also included Age, Systolic Blood Pressure and Diabetes Duration as co-variates.

Neuropsychological Performance

The relationships between the categorical MRI outcomes of interest (cerebral atrophy, high-intensity white matter lesions, coded numerically) and neuropsychological performance were examined using bi-variate correlation (Spearman's rho). On the basis of the results of simple correlation no further analyses were performed.

8.4 RESULTS

8.4.1 Frequency of Macroscopic Abnormalities on MRI

Table 8.2 summarises the prevalence of cerebral atrophy observed in the 133 participants. In general, the structural brain abnormalities observed in the cohort were subtle, with severe abnormalities very uncommon.

Frequency of Brain Atrophy

Ventricular atrophy was commonly observed, being present in 30.8% of subjects. 6.7% of the cohort had a moderate degree of ventricular atrophy, with no participant exhibiting severe atrophy. In contrast to ventricular atrophy, sulcal atrophy with atrophy of the cerebral cortex, was uncommon being present in only 10.5% of participants. When present, the degree of sulcal atrophy was mild: no participants had moderate to severe atrophy of the cerebral cortex (Table 8.2).

Table 8.2. Prevalence of cerebral atrophy in 133 participants with T1DM.

	Absent (% of scans)	Mild (% of scans)	Moderate/Severe (% of scans)	Prevalence (% of scans)
Ventricular Atrophy	69.2%	24.1%	6.7%	30.8%
Sulcal Atrophy	89.5%	10.5%	None	10.5%

Frequency of Leukoaraiosis and Small Punctate White Matter Lesions

The prevalence of Leukoaraiosis (PVWH, DWMH) and Small Punctate White Matter Lesions (SPWML) observed in the 133 participants are summarised in Table 8.3. In general, the white matter abnormalities observed in the cohort were subtle, with severe abnormalities being infrequently observed.

White matter lesions, as Leukoaraiosis, were commonly observed but were generally mild, focal rather than confluent, small in number and predominantly located in the peri-ventricular white matter, as PVWH. DWMH were less commonly observed and when present were also mild. As expected, the frequency of white matter lesions varied according to the rating scale utilised to score their presence (Fazekas et al., 2002;Mantyla et al., 1997). Many of the white matter rating scales, which were primarily developed to objectively rate white matter lesion burden in studies of the elderly, appeared insensitive to the type of white matter changes observed in this relatively young cohort of people with Type 1 diabetes. Three validated scales appeared to rate the relatively subtle degrees of Leukoaraiosis observed in the present study: the methods described by Fazekas (Fazekas et al., 1987), Longstreth (Longstreth, Jr. et al., 2000) and Wahlund (Wahlund et al., 1990) appeared more sensitive. Those methodologies described by Breteler (Breteler et al., 1994a), Shimada (Shimada et al., 1990), Mirsen (Mirsen TR et al., 1991) and Van Swieten (Van Swieten et al., 1990) appeared insensitive. The low frequency of white matter

changes scored by these latter methodologies rendered attempts at further analysis inappropriate (Table 8.3).

Table 8.3. Prevalence of Leukoaraiosis and SPWML in 133 subjects with T1DM.

	Absent (% of scans)	Mild (% of scans)	Moderate/Severe (% of scans)	Prevalence (% of scans)
LEUKOARAIOSIS				
Fazekas				
Peri-ventricular WML	39.8%	59.4%	0.8%	60.2%
Deep WML	85.7%	13.5%	0.8%	14.3%
Longstreth	44.3%	54.9%	0.8%	55.7%
Wahlund	69.9%	30.1%	None	30.1%
Breteler	87.2%	12%	0.8%	12.8%
Mirsen				
Peri-ventricular WML	93.4%	6.6%	None	6.6%
Deep WML	96%	3.2%	0.8%	4.0%
Shimada	96.2%	3.8%	None	3.8%
Van Swieten				
Anterior WML	99.2%	0.8%	None	0.8%
Posterior WML	98.4%	1.6%	None	1.6%
SPWML				
Total (all areas)	53.4%	31.6%	15.0%	46.6%
Hippocampus	78.2%	21.8%	None	21.8%
Basal Ganglia	69.2%	27.8%	3.0%	30.8%
Centrum Semi-Ovale	66.9%	21.1%	12.0%	33.1%

8.4.2 Structural Brain Outcome Measures and Diabetes-Specific Factors

The prevalence of cerebral atrophy and white matter lesions according to the presence of Diabetic Retinopathy and Diabetes Onset Age Group is summarised in Table 8.4.

Table 8.4. Prevalence of structural brain abnormalities by Retinopathy Status and Diabetes Onset Age Group in 133 participants.

	Background Retinopathy n=48 (% of scans)	**No Retinopathy** n=85 (% of scans)	**Chi²-test**	**Early Onset** n=26 (% of scans)	**Later Onset** n=107 (% of scans)	**Chi²-test**
Ventricular Atrophy	32.6%	35.1%	p=0.84	60%	29.6%	p=0.02*
Sulcal Atrophy	14.6%	8.2%	p=0.26	7.7%	11.4%	p=0.74
LEUKOARAIOSIS						
Fazekas (PVWH)	68.9%	58.3%	p=0.26	52.0%	63.7%	p=0.36
Longstreth	60.0%	55.9%	p=0.71	48.0%	58.8%	p=0.37
Wahlund	37.8%	27.4%	p=0.06	36.0%	29.4%	p=0.42
SPWML						
Total (all areas)	48.9%	45.2%	p=0.71	28.0%	50.9%	p=0.04*
Hippocampus	31.1%	15.5%	p=0.04*	4.0%	24.5%	p=0.02*
Basal Ganglia	37.8%	26.2%	p=0.24	8.0%	36.3%	p=0.007**
Centrum Semi-Ovale	40.0%	28.6%	p=0.24	20%	36.3%	p=0.16

Early Onset = diagnosis before 7th birthday; Later Onset = diagnosis > 7th birthday.

* = $p<0.05$. ** = $p<0.01$.

Background Diabetic Retinopathy

The presence of Background Diabetic Retinopathy did not appear to exert a major influence on the prevalence of macroscopic neuroradiological abnormalities. The sole exception to this was the observation of a two-fold greater prevalence of SPWML in the region of the Hippocampus in those with Retinopathy ($p=0.04$, Chi^2-test).

Diabetes Onset Age

The age at onset of diabetes appeared to exert a greater influence on brain structural outcomes: Ventricular Atrophy was twice as prevalent in those diagnosed before the age of 7 years ($p=0.02$), whereas the prevalence of SPWML appeared greater in the region of the Hippocampus ($p=0.02$), Basal Ganglia ($p=0.007$) and in total SPWML burden ($p=0.04$) in those in whom the onset of diabetes was in later childhood, adolescence or in adulthood.

Duration of Diabetes

The duration of diabetes appeared to weakly correlate with the total burden of SPWML in all areas ($r^2=-0.19$, $p=0.03$) and the presence of Basal Ganglia SPWML ($r^2=-0.20$, $p=0.03$), with a non-significant trend for Hippocampal and Centrum Semi-Ovale SPWML. Diabetes duration was also correlated with Leukoaraiosis scores using the Longstreth rating scale ($r^2=-0.24$, $p=0.007$) but not on any other scales. Diabetes duration did not correlate with either type of cerebral atrophy.

Severe Hypoglycaemia

No summary measure of preceding exposure to severe hypoglycaemia appeared to correlate with any of the structural brain outcomes of interest. In particular, the presence of cerebral atrophy, high intensity white matter lesions, or SPWML were not influenced by summary measures of severe hypoglycaemia (Table 8.5).

Table 8.5. Spearman's correlates between summary measures of preceding severe hypoglycaemia exposure and structural brain outcome measures.

	Ventricular Atrophy	Sulcal Atrophy	Leukoaraiosis (Fazekas)	SPWML (Total SPWML)
Total lifetime episodes	0.001	0.06	-0.04	0.03
	p=0.99	p=0.54	p=0.72	p=0.75
Total coma	0.04	-0.07	-0.07	0.06
	p=0.71	p=0.48	p=0.53	p=0.55
Total seizures	0.11	-0.12	-0.04	-0.07
	p=0.32	p=0.25	p=0.70	p=0.50

All correlations p>0.05.

8.4.3 Structural Brain Outcome Measures and Demographic Factors

With the exception of a borderline correlation observed between Systolic Blood Pressure and the presence of Sulcal Atrophy (r^2=-0.21, p=0.08) no other demographic factors appeared to be significantly correlated with the presence of Cerebral Atrophy. In particular, age and gender did not correlate with the presence of Ventricular or Sulcal Atrophy.

No demographic factors (age, gender, blood pressure) were significantly correlated with the presence of Leukoaraiosis, as either PVWH or DWMH.

Age was correlated with the presence of SPWML in all areas (r^2=0.29-0.33, p=0.0001-0.002) and a trend between Systolic Blood Pressure (r^2=-0.21, p=0.08). No other demographic factor correlated with the presence of SPWML.

8.4.4 Independent Predictors of Structural Brain Outcomes

Multiple Logistic Regression was used to determine whether the associations identified between diabetes-specific factors (Retinopathy Status; Diabetes Onset Age Group), demographics and the categorical structural outcomes were independent.

Ventricular Atrophy

The only independent predictor of the presence of Ventricular Atrophy, as determined by Multiple Logistic Regression, was the Diabetes Onset Age Group. An early onset of diabetes before the 7th birthday independently predicted the presence of Ventricular Atrophy [β=1.3, SE=0.51, EXP(β)=3.6, 95% CI of β=1.3-9.6, p=0.01, Log Regression]. An early onset of diabetes was independently associated with a significantly higher risk of Ventricular Atrophy (Estimated odds ratio=3.6).

Leukoaraiosis

The only variable to independently predict the presence of Leukoaraiosis was the duration of diabetes [Longstreth rating scale: β=-0.07, SE=0.03, EXP(β)=0.93, 95% CI of β=0.87-0.99, p=0.035, Log Regression]. A shorter duration of diabetes was associated with a modest increased prevalence of PVWH. Leukoaraiosis as determined by the Fazekas or Wahlund scales were unrelated to any identified diabetes-specific or demographic factors.

Small Punctate White Matter Lesions

Several factors were identified as being independent predictors of the presence of SPWML by Multiple Logistic Regression:

(a) Presence or Absence of SPWML

The presence of SPWML in any area brain was independently predicted by Diabetes Onset Age [β=-0.08, SE=0.03, EXP(β)=1.11, 95% CI of β=1.01-1.16, p=0.02, Log Regression]. SPWML were significantly less frequently observed in those in whom the onset of T1DM was before their 7th birthday.

(b) SPWML in the region of the Hippocampus

Two factors independently predicted the presence of SPWML in the region of the Hippocampus. Diabetes Onset Age [β=0.15, SE=0.05, EXP(β)=1.17, 95% CI of β=1.05-1.29, p=0.004, Log Regression] and Retinopathy status [β=-1.22, SE=0.55, EXP(β)=0.30, 95% CI of β=0.10-0.86, p=0.03, Log Regression]. Hippocampal SPWML were significantly more prevalent in those with a later onset of diabetes, with disease onset after their 7th birthday, and in those who had Background Diabetic Retinopathy.

(c) SPWML in the region of the Basal Ganglia

Following Logistic Regression no factor emerged which independently predicted the presence of SPWML in the region of the Basal Ganglia.

(d) SPWML in the region of the Centrum Semi-Ovale

The presence of SPWML in the region of the Centrum Semi-Ovale was independently predicted by Diabetes Onset Age [β=0.10, SE=0.04, EXP(β)=1.10, 95% CI of β=1.02-1.19, p=0.01, Log

Regression]. No other factors were independent predictors. SPWML were significantly less prevalent in the region of the Centrum Semi-Ovale in those with an early onset of T1DM.

8.4.5 Structural Brain Outcomes and Neuropsychological Performance

The Spearman's correlates observed between the structural brain outcome measures of interest (Cerebral Atrophy, White Matter Lesions and Small Punctate White Matter Lesions) and performance on the tests of intelligence and neuropsychological performance are summarised in Table 8.6.

Table 8.6. Spearman's correlates between structural brain outcome measures and neuropsychological performance.

	Ventricular Atrophy	**Sulcal Atrophy**	**Leukoaraiosis**			**SPWML** (Total SPWML)
			Fazekas (PVWH)	**Longstreth**	**Wahlund**	
Performance IQ (WAIS-R)	0.14 p=0.90	-0.01 p=0.92	-0.15 p=0.16	-0.01 p=0.96	0.07 p=0.52	-0.14 p=0.19
Inspection Time (PEST Score)	-0.07 p=0.52	-0.03 p=0.76	0.09 p=0.39	0.09 p=0.40	-0.19 p=0.08	0.11 p=0.30
Reaction Time (4-Choice Decision Time)	0.08 p=0.50	0.11 p=0.29	-0.14 p=0.89	0.01 p=0.88	0.03 p=0.78	0.16 p=0.13
Attention/Concentration (PASAT)	0.10 p=0.38	-0.02 p=0.83	-0.03 p=0.76	0.01 p=0.94	0.01 p=0.94	0.15 p=0.16
Verbal Fluency (Borkowski VFT)	-0.07 p=0.53	-0.06 p=0.56	-0.18 p=0.09	-0.05 p=0.63	0.01 p=0.96	-0.12 p=0.24
Pre-Morbid IQ (NART)	-0.004 p=0.97	0.04 p=0.72	-0.01 p=0.94	-0.05 p=0.66	-0.04 p=0.71	-0.07 p=0.49

All correlations p>0.05.

No significant correlations were observed between the presence of Cerebral Atrophy and neuropsychological performance. No significant correlations were observed between the presence of white matter lesions, as Leukoaraiosis or SPWML, and neuropsychological performance.

8.5 DISCUSSION

8.5.1 Prevalence of Structural Brain Abnormalities

Structural abnormalities which are readily apparent on MRI imaging of the brain are commonly present in adults with Type 1 diabetes. The majority of participants in the present study had structural abnormalities of the brain, most commonly manifest as a mild degree of high intensity white matter lesions (60.2%), although central brain atrophy (30.8%) and punctate white-matter lesions (46.6%) were also common. The presence of some of these structural brain abnormalities appeared, at least in part, to be determined by factors specific to diabetes, the two most prominent of which were the onset of diabetes in early childhood and the presence of microvascular complications. However, other structural abnormalities, such as the frequency of Sulcal Atrophy and Leukoaraiosis did not appear to relate to the clinical complications of diabetes examined by the present study, suggesting other factors may be of importance to their development. As a healthy control group was not included in the present study it is not possible to evaluate whether the prevalence of structural brain abnormalities observed in this group of adults with Type 1 diabetes differs from that which would be expected in age-matched non-diabetics. From the limited epidemiological evidence which has examined white matter abnormalities in younger adult populations of similar age to those evaluated in the present study, the CAMERA study identified a point prevalence of Leukoaraiosis of 42% in healthy adults aged 30-45 years (Kruit et al., 2004), suggesting that Leukoaraiosis is probably at least as prevalent as in healthy adults.

8.5.2 Macrovascular Disease

The structural abnormalities reported in this chapter are unlikely to be as a consequence of macrovascular disease of the cerebral circulation: no participant had MRI evidence of cerebrovascular disease. In particular, no neuroradiological evidence of Cerebral Infarction, Lacunar Infarction or previous Intra-Cerebral Haemorrhage was observed in any participant. This suggests that the strict application of exclusion criteria was effective, consistent with the epidemiological evidence base which identified the risk factors for Stroke in T1DM (Fuller et al., 2001;Klein et al., 2004;Nathan et al., 2005;Stettler et al., 2006).

8.5.3 Leukoaraiosis

High intensity white matter lesions, present as Leukoaraiosis, were commonly observed in the present cohort of young adults with Type 1 diabetes. Leukoaraiosis was present in 30-60% of participants in the present study, when those rating scales which appeared more sensitive to subtle degrees of white matter abnormalities were used to quantify their presence (Fazekas et al., 1987;Longstreth, Jr. et al., 2000;Wahlund et al., 1990). However, some rating scales appeared insensitive to the subtle degree of white matter abnormalities (Breteler et al., 1994a;Mirsen TR et al., 1991;Shimada et al., 1990;Van Swieten et al., 1990). The white matter lesions observed in the present study were at the mild end of the spectrum of severity. Lesions were typically located in the peri-ventricular white matter (PVWH) and consisted of peri-ventricular rims, caps, or focal PVWH. Confluent lesions, or lesions extending into the deep white matter were very uncommon. Two previous studies have reported the prevalence of Leukoaraiosis in T1DM. Dejgaard identified a five-fold increased frequency of Leukoaraiosis, relative to age-matched controls (n=40), in a small group (n=16) with T1DM complicated by advanced degrees of diabetic microangiopathy (Dejgaard et al., 1991). The prevalence of Leukoaraiosis (69%) reported by

Dejgaard was similar to that observed in the present study (60.2%), however, direct comparisons are hampered by methodological differences, particularly with respect to the sensitivity to white matter changes of the MRI sequences utilised and the rating of Leukoaraiosis. Youssem compared the frequency of white-matter lesions in 25 adult patients with Type 1 diabetes with advanced diabetic retinopathy relative to 10 age-matched healthy control subjects using MRI techniques consisting of T1-weighted and Proton Density images (Yousem et al., 1991). In contrast to the current observations and those of Dejgaard (Dejgaard et al., 1991), no white-matter lesions were identified and Youssem concluded that white-matter pathology was uncommon in T1DM. Subsequent advances in MRI technology in the intervening years since this publication have since revealed that the imaging sequences utilised by Youssem are unsuited for the quantification of white-matter changes, being unlikely to detect subtle differences.

The neuropathological appearances of mild to moderate PVWH, of the type that were observed in 60% of participants in the present study, are those of demyelination, reactive gliosis without substantial degenerative changes in blood vessels (Englund, 2002). Severe PVWH and DWMH, both of which are associated with advancing age, hypertension and increased macrovascular events [REFS Fazekas ASPS; de Leeuw RSS], were uncommonly observed in the present cohort: 14.3% had DWMH using the Fazekas rating scale (Fazekas et al., 1987) whereas only 4.0% were judged to have DWMH when the Mirsen scale was used (Mirsen TR et al., 1991). The exclusion criteria of the present study, which limited age to a maximum of 45 years and which excluded those with hypertension are likely explanations for the comparatively low prevalence of DWMH and more severe degrees of PVWH. More severe PVWH and DWMH exhibit similar pathological appearances described above for mild to moderate PVWH but with additional features of microvascular ischaemia indicated by irregularity of blood vessel luminal cross-sectional diameter, peri-vascular hyalinosis and loss of oligodendrocytes and marked damage to myelin (Englund, 2002). Despite the histopathological evidence for a microvascular basis for

severe PVWH and DWMH, no association between the presence of Diabetic Retinopathy and such white matter lesions was observed. The low prevalence of severe PVWH and DWMH, as a consequence of the study exclusion criteria, may make the present cohort less than ideal to examine the association between Retinopathy and Leukoaraiosis.

8.5.4 Small Punctate White Matter Lesions

Histologically, SPWML consist of areas of peri-vascular hyalinosis of small arterioles and focal demyelination (Fazekas et al., 1991) and the peri-vascular features of SPWML share significant histological commonality with the limited histological data from study of the brain in Type 1 diabetes in which hyalinosis of cerebral microvessels was also a prominent feature (Reske-Nielsen and Lundbaek, 1963). In the present study, Diabetes Onset Age group and the presence of Diabetic Retinopathy independently influenced the frequency of SPWML in specific brain areas. In those in whom diabetes was diagnosed in later childhood (after their 7th birthday), adolescence or adulthood, SPWML were more prevalent in the region of the Hippocampus, Centrum Semi-Ovale and in terms of the cumulative total in all brain areas. Contrary to expectations that an early onset of Type 1 diabetes would disadvantage normal neurodevelopment with neuroradiological evidence supportive of this concept, those in whom the onset of diabetes was in early childhood may be relatively more resistant to the development white matter pathology, in the form of Small Punctate White Matter Lesions. This relationship was independent of age, diabetes duration, the presence of Diabetic Retinopathy and other factors. If a microvascular pathogenesis for the development of SPWML in T1DM is to be assumed, as described in the middle-aged and elderly (Fazekas et al., 1991), then the present findings are in keeping with previous observations that those with an early life onset of T1DM may be exhibit relative resistance to the development of microangiopathy: the median duration of diabetes required for the development of microvascular complications of diabetes, notably as Diabetic

Retinopathy, is substantially longer in those with an onset of diabetes before puberty (Holl et al., 1998;Kostraba et al., 1989;Kullberg et al., 2002;Olsen et al., 2004), independently of disease duration and degree of glycaemic control (Kullberg et al., 2002). In the present study Diabetic Retinopathy was independently associated with the presence of SPWML in the region of the Hippocampus, which could be interpreted as being supportive evidence in favour of microangiopathy affecting the brain. Why this association was only observed for the region of the Hippocampus remains unclear. The Hippocampus is known to be rich in insulin receptors (Craft and Watson, 2004) and glucose transporters (McEwen and Reagan, 2004) but structural changes in association with hyperglycaemia have not been reported, whereas hypoglycaemia-related damage has been described (Boeve et al., 1995;Chalmers et al., 1991;Finelli, 2001;Holemans et al., 2001). As Retinopathy selectively affected this single specific brain area the alternative interpretation is that the association could represent a Type 1 error.

The clinical relevance of SPWML remains unclear at present, largely through limited epidemiological data. Available data from the Austrian Stroke Prevention Study suggest that SPWML may be a benign white matter abnormality in the elderly (Schmidt et al., 2003), at least relative to the adverse macrovascular prognosis associated with more severe degrees of PVWH and DWMH (Iwai et al., 1987;Schmidt et al., 2003). The factors that are associated with the development and progression of SPWML are unclear, although the Austrian Stroke Prevention Study suggests that progression may be limited over a 6 year period (Schmidt et al., 2003). In the setting of Type 1 diabetes, prospective studies are required to evaluate the natural history of these lesions, to determine factors associated with their progression or regression, and to evaluate whether these lesions are the forebears of further structural brain abnormalities or the development of cognitive disadvantage. If the hypothesis that the presence of SPWML are related to cerebral microangiopathy, then it would seem reasonable to assume that the progression

of Diabetic Retinopathy, which can be objectively determined, would be associated with an increasing SPWML burden. This hypothesis has not yet been tested.

8.5.5 White Matter Lesions and Neuropsychological Performance

No relationship was observed between the presence of any type of white-matter abnormality, as Leukoaraiosis or SPWML, and poorer performance on any of the cognitive abilities assessed, although some cognitive domains were not examined. Given the relatively mild degree of white matter change observed in the present study this lack of association is perhaps unsurprising. Studies in older adults and the elderly infer that extensive white matter disease may be necessary before differences in cognitive abilities become measurable, leading to the suggestion that a "threshold" of cumulative white matter burden is necessary before cognition becomes impaired (Desmond, 2002). Correlations between white matter lesion burden have been most consistently described between psychomotor and information processing speed, with attention & executive function and global performance becoming impaired in the presence of severe disease (Boone et al., 1992;Breteler et al., 1994b;De Groot et al., 2000;DeCarli et al., 1995;Junque et al., 1990;Longstreth, Jr. et al., 1996;Schmidt et al., 2003;Ylikoski et al., 1993). It is unclear whether the presence of white matter abnormalities in the present study is a marker for the future risk of developing cognitive impairment and prospective assessment would be required to ascertain this.

8.5.6 Cerebral Atrophy

Mild degrees of Ventricular Atrophy and Sulcal Atrophy were observed in the present study, of which only Ventricular Atrophy was frequent (30.8%). Epidemiological studies of brain development and ageing in adults and the elderly have identified chronological age, diastolic blood pressure and smoking as the major risk factors for the development of brain atrophy (Ikram et al., 2007). The exclusion criteria of the current study ensured that only a younger adult

normotensive group were studied, making these macrovascular risk factors unlikely to be contributors to the presence of atrophy observed in those studied. No specific factors were identified, other than the presence of Ventricular Atrophy (r^2=0.34, p<0.001, Spearman's correlate), which correlated with the presence of Sulcal Atrophy. This correlation infers commonality for the pathogenesis of both types of brain atrophy. In contrast, Ventricular Atrophy was significantly more prevalent in those in whom diabetes had developed during early childhood, relative to those who developed the disorder at a later age. However, relatively little is known about the processes which underlie the development of ventricular atrophy in younger adults. Neuropathological studies have identified that programmed necrosis of neurones and their supporting glial cells, a process known as "pruning", occurs from early childhood into adulthood. This process is thought to contribute, at least in part, to the reduction in total gray matter volume and the 60-150% increase in the volume of the lateral ventricles that has been observed across the same timeframe (Durston et al., 2001). Whether the onset of T1DM during early childhood neurodevelopment influences the process of "pruning" is unknown. Alternatively, exposure to the metabolic decompensations associated with the onset of T1DM in early life could contribute to the development of central brain atrophy. The neuroimaging sequelae of DKA in children and adolescents have been associated with ventricular dilatation (Hoffman et al., 1988;Hoffman et al., 1999;Krane et al., 1985) whereas no case reports have linked early childhood exposure to severe hypoglycaemia with ventricular dilatation, although such a relationship has been reported following protracted severe hypoglycaemia in neonates (Vannucci and Vannucci, 2001) and adults (Gold and Marshall, 1996;Maekawa et al., 2006).

The presence of mild Ventricular Atrophy did not correlate with performance on any test of the neuropsychological battery and therefore the clinical relevance remains unclear. From the present data it is not possible to determine what underlies the increased lateral ventricle volumes observed in those with an "early onset" of Type 1 diabetes. Longitudinal studies of human

neurodevelopment suggest that changes in gray matter volume through "pruning" are responsible for the increase in lateral ventricle cavity size during normal neurodevelopment (Durston et al., 2001;Lenroot and Giedd, 2006). Prospective evaluation of the neuroimaging associations of Type 1 diabetes across neurodevelopment would be necessary to provide further clarity.

8.5.7 Severe Hypoglycaemia and Structural Brain Outcomes

No summary of exposure to severe hypoglycaemia was associated with measurable differences in brain structure in the present study. The literature summarising the structural brain associations of severe hypoglycaemia is limited. Case studies following exposure to protracted and profound severe hypoglycaemia have identified a distinct pattern of permanent brain injury where structural abnormalities preferentially affect the neocortex, hippocampus and basal ganglia (Bakshi et al., 2000;Boeve et al., 1995;Chalmers et al., 1991;Chan et al., 2003;Finelli, 2001;Gold and Marshall, 1996;Holemans et al., 2001;Maekawa et al., 2006;Meer et al., 1988;Richardson et al., 1981;Yoneda and Yamamoto, 2005) and less commonly the brain stem (Perros et al., 1994;Rajbhandari et al., 1998). It is beyond doubt that protracted and profound severe hypoglycaemia can produce permanent structural brain abnormalities. However, it remains debatable whether recurrent exposure to less profound episodes of severe hypoglycaemia, of the type that more commonly present in clinical practice, is permanently injurious to the brain. Perros examined 22 participants of a previously published cohort (Langan et al., 1991), in whom the preceding frequency of severe hypoglycaemia was associated with poorer current intellectual performance (WAIS-R Performance IQ). Perros identified a greater frequency of cortical atrophy (45.5%) in the 11 subjects with a previous history of exposure to severe hypoglycaemia relative to the remaining 11 participants who naïve to severe hypoglycaemia in whom cortical atrophy was not observed (Perros et al., 1997). The reasons underlying the disparity between present observations and those of Perros are not clear, but may reflect the smaller sample size and

perhaps recruitment bias as those studied were selected from a larger cohort on the basis of their cognitive ability differences. The present study, in which no neuropsychological or neuroradiological differences were associated with severe hypoglycaemia exposure, is consistent with the findings of the EDIC study (Jacobson et al., 2007). Both studies infer that repeated exposure to severe hypoglycaemia may have no major measurable negative consequences in adults who have T1DM. However, unlike the EDIC study the present study examined only some cognitive abilities and did not examine regional differences in gray or white matter density or volume. Musen and colleagues recently reported subtle differences in gray matter density in association with a preceding exposure to severe hypoglycaemia, notably in the cerebellum (Musen et al., 2006). However, the practical meaning of this observation remains unclear. Therefore, subtle differences in brain structure or function may indeed be related to severe hypoglycaemia exposure, the clinical meaning of which remains unclear at present.

8.5.8 Conclusions

The present study has identified that structural abnormalities of the brain, manifest as different types of white matter abnormalities and ventricular atrophy, are common in young adults who have Type 1 diabetes. In general, these abnormalities are subtle and do not appear to be associated with measurable differences of brain function, within the limitations inherent in the methods utilised to measure cognitive abilities in the present study. The clinical relevance of these abnormalities of brain structure are not presently apparent and prospective study will be required to determine whether these abnormalities progress, regress or remain unaltered with time. The use of more detailed and novel neuroradiological techniques, such as Voxel Based Morphometry (VBM) for the delineation of subtle regional gray matter differences, and Diffusion Tensor Imaging (DTI) for the analysis of white matter tractography, may assist in clarifying which processes underlie the present observations.

CHAPTER 9

APOE GENOTYPE AND COGNITIVE FUNCTION IN TYPE 1 DIABETES

9. APOE GENOTYPE AND COGNITIVE FUNCTION IN TYPE 1 DIABETES

9.1 Introduction

Permanent cognitive impairment is a rare consequence of insulin-induced hypoglycaemia and in many of the cases reported in the literature appears consequent upon protracted severe hypoglycaemia, often precipitated by a deliberate attempt at self-harm, or in association with alcohol consumption (Arky et al., 1968). In insulin-treated diabetes episodic severe hypoglycaemia is common, with an annual prevalence of 30% in Type 1 diabetes and a higher incidence in people with impaired awareness of hypoglycaemia and strict glycaemic control (1991a). Whether recurrent exposure to severe hypoglycaemia promotes the development of long-term cognitive sequelae is unresolved and remains debatable. Retrospective cross-sectional studies have indicated that in at least some people with type 1 diabetes a modest cognitive decrement may accrue following repeated exposure severe hypoglycaemia (Langan et al., 1991;Lincoln et al., 1996;Sachon et al., 1992;Wredling et al., 1990). This observation has not been replicated by prospective studies over an evaluation period extending for up to 10 years (1996;Austin and Deary, 1999;Jacobson et al., 2007;Reichard et al., 1996). Cross-sectional studies have reported a range of individual differences in cognitive decrements in those exposed to severe hypoglycaemia (Langan et al., 1991), suggesting that factors, other than neuroglycopenia *per se*, may influence the subsequent risk of developing cognitive impairment. Chronological age, diabetes duration and the presence of co-existent microvascular and macrovascular complications have all been proposed as potential mediators of increased susceptibility to hypoglycaemia-induced cognitive impairment (Deary et al., 1993;Langan et al., 1991). Genetic factors have been identified as significant modulators of outcome in a variety of neurological diseases (Corder et al., 1993;Wright, 2005) and could partly mediate the susceptibility to severe hypoglycaemia-induced cognitive impairment. The role of genetic factors

as mediators of cognitive dysfunction following severe hypoglycaemia has not been previously examined.

9.1.2 Polymorphism of the Apoliprotein-E Gene

Polymorphism of the gene for Apolipoprotein-E (APOE) is the most important single genetic determinant of late-onset Alzheimer's disease (Corder et al., 1993). The APOE gene has three common alleles (ε2, ε3, ε4) coding for three corresponding protein isoforms, designated as E2, E3 and E4 respectively, each of which vary in their biological activity. The APOE E4 isoform has the least biological activity and the bioactivity of the protein isoform appears to be of clinical importance. Apolipoprotein-E (APOE) mediates central nervous system cholesterol transport in an isoform-specific manner and is an important determinant of neuronal repair and cytoskeletal maintenance: the likelihood of neuronal recovery and survival following brain injury appears to be mediated in an isoform-specific manner with the least active E4 isoform conferring a disadvantage (Maclullich AMJ et al., 1998).

9.1.3 APOE Polymorphisms and the Brain in Healthy Individuals

The APOE ε4 allele appears to influence aspects of cognitive ageing, even in apparently healthy individuals, although no negative consequences have been reported as yet in childhood or adulthood. This lack of effect in early life infers that the disadvantage conferred through the ε4 allele is not neurodevelopmental. Children and young adults with an ε4 allele appear not to exhibit any cognitive disadvantage on tests of general intelligence (Turic et al., 2001) and the relative frequencies of APOE genotypes appears not to differ between high and low-IQ children (Plomin et al., 1994). In another study of young Chinese women superior fluid intelligence was observed in association with ε4 (Yu et al., 2000). However, by middle-age possession of the ε4 allele appears to confer disadvantage. Apparently healthy middle-aged adults possessing an ε4

allele exhibit subtle deficits in learning and memory abilities relative to those with other genotypes (Flory et al., 2000). APOE genotypes also appear to influence the rate at which certain cognitive abilities decline with normal ageing: healthy elderly subjects with an ε4 allele have poorer information processing speed and memory abilities (Staehelin et al., 1999) and may undergo a more rapid rate of loss of cognitive ability than that observed in those with no ε4 allele (Pendleton et al., 2002;Staehelin et al., 1999).

9.1.4 APOE Polymorphisms and Brain Injury

The APOE ε4 allele has been associated with poorer cognitive outcomes following exposure to a wide variety of cerebral insults [Smith JD, Ann Med 2000]. Associations have been reported for head injury (Crawford et al., 2002;Teasdale et al., 1997), demyelinating disease (Pinholt et al., 2006), cerebro-vascular accidents (Louko et al., 2006;Sudlow et al., 2006), ex-professional Boxers (Jordan et al., 1997;Jordan, 2000) and US Football players (Kutner et al., 2000).

9.1.5 APOE Polymorphisms and Cerebral Glucose Metabolism

Polymorphism of the APOE gene appears to influence regional cerebral glucose utilisation. Healthy middle-aged relatives of patients with Alzheimer's disease exhibit reduced parietal lobe glucose metabolism and increased right-left hemisphere asymmetry if they carry the ε4 allele (Small et al., 2000). More widespread abnormalities of regional cerebral glucose utilisation, affecting the posterior cingulate, parietal, temporal, and prefrontal cortex, have also been reported in apparently cognitively healthy ε4 heterozygotes. The reduction observed in glucose utilisation was apparent decades before any clinical presentation of Alzheimer's disease could reasonably be expected and was detectable in the absence of any measurable difference in cognitive ability, as determined by a neuropsychological test battery (Reiman et al., 2001;Reiman et al., 2004;Reiman et al., 2005).

9.1.1 Aims

The hypothesis of the present study was to examine whether possession of an APOE ε4 allele was associated with measurable cognitive disadvantage in an adult group of people with type 1 diabetes, but also to determine whether any differences in cognitive ability observed between the APOE groups was additionally modified by the subjects' preceding exposure to severe hypoglycaemia. The present study was under-powered to accurately determine this secondary aim and the data presented with respect to this aim are exploratory.

9.3 SUBJECTS AND METHODS

9.3.1 Study Design

Ninety-six people with type 1 diabetes were recruited for study and all completed the cross-sectional study protocol. The study protocol consisted of a battery of neuropsychological assessments, determination of the APOE genotype, and collection of diabetes related data, with a particular focus on previous exposure to severe hypoglycaemia. Participants were randomly selected from two pre-existing study cohorts (63 patients (Deary et al., 1993), 33 patients (Ferguson et al., 2003) from whom extensive epidemiological data and diabetes-specific data had been collected. Both cohorts had completed an identical neuropsychological assessment battery, the description of which is outlined below. Those selected from the cohort examined by Deary (Deary et al., 1993) included those still living in the Edinburgh area who were still alive and physically able to attend for blood sampling. A significant number of this 1993 cohort were deceased at the time of the present project. The remaining 33 participants were recruited from those examined by Ferguson (Ferguson et al., 2003) and participated in the studies reported in

Chapters 7 and 8 of this thesis. The relative advantages and disadvantages of the battery of ability tests that were utilised are discussed in greater detail in the Methods Chapter (Section 6.4). To minimise the influence of potential confounders in neuropsychological performance, strict exclusion criteria were applied to those selected for study. These included the presence of Hypertension (defined as BP > 140/90 mmHg); any previous central nervous system pathology; any previous history of, or current psychiatric disease; alcoholism; drug misuse; any multi-system disease known to affect the central nervous system.

9.3.2 Assessment of Neuropsychological Function

The neuropsychological test battery was chosen to be sensitive to cognitive decrements across diverse cognitive abilities but was weighted towards the assessment of information processing and current intellectual performance. The assessments were performed by a pre-trained assessor who remained blinded to the diabetes-specific characteristics and APOE genotype of participants. The neuropsychological test battery was administered in a standardised pre-determined order. Incipient hypoglycaemia, or hypoglycaemia within the preceding 24-hours, was excluded before neuropsychological assessment. Evidence of biochemical (capillary glucose concentration < 3.5 mmol/L) or symptomatic hypoglycaemia resulted in rescheduling of the neuropsychological session. The psychometric instruments used to evaluate the performance of participants were:

Hospital Anxiety and Depression Scale (Zigmond and Snaith, 1983)

The potential confounding effects of low mood and anxiety were evaluated. Further detail is given in the Methods Chapter (6.5).

Wechsler Adult Intelligence Scale Revised (WAIS-R) (Wechsler, 1981)

The performance sub-tests measure current intellectual performance (fluid intelligence, non-verbal intelligence) and are very sensitive to disruption by organic brain disease and ageing. The four performance sub-tests utilised included the Picture Completion, Object Assembly, Block Design and Digit Symbol tests. Further detail is given in the Methods Chapter (6.4).

National Adult Reading Test (NART) (Nelson and Willison, 1991)

Performance on the NART is relatively resistant to the detrimental effects of age and most types of organic brain disease. NART performance correlates more closely with pre-morbid IQ than demographic variables and was used to control for the confounding effects of prior intellectual ability (pre-morbid IQ, crystallised intelligence) in the present study. Further detail is given in the Methods Chapter (6.4).

Inspection Time (McCrimmon et al., 1996)

Inspection Time provides an assessment of visual perceptual speed, a component of information processing ability. Participants discriminated between the spatial position (left or right) of the longer of two briefly presented vertical lines. The stimuli were backward-masked, the presentation duration was varied, and the duration of time required to reliably distinguish the stimulus (85% correct) was termed the 'inspection time'. Further detail is given in the Methods Chapter (6.4).

Choice Reaction Time (Jensen, 1987)

Choice reaction time was used to assess psychomotor speed and completed the tests of information processing ability. Simple reaction time and 2, 4, 8-Choice reaction time were firstly assessed in ascending order, and provided a pre-practice element to the assessment. Thereafter 8, 4 ,2-Choice reaction time and simple reaction time were examined in descending order and

utilised for subsequent analyses. Further detail is given in the Methods Chapter (6.4).

Borkowski Verbal Fluency Test (controlled association) (Borkowski and Benton, 1967)

The Borkowski Verbal Fluency Test is thought to assess frontal lobe and executive function. Participants have 60 seconds to state as many words as possible, beginning with letters of the alphabet specified by the assessor. The letters J, S, U and M were utilised. Further detail is given in the Methods Chapter (6.4).

Paced Auditory Serial Addition Task (PASAT) (Deary I.J. et al., 1991)

The PASAT was used to assess the ability to sustain attention and concentration. Participants listened to a number list, which they were required to add together according to a given rule. After a practice period, two consecutive 61-number trials were administered with 4s and 2s between successive digits respectively. Further detail is given in the Methods Chapter (6.4).

9.3.3 Assessment of Severe Hypoglycaemia Exposure

Severe hypoglycaemia was defined as an episode requiring external assistance to facilitate recovery (The DCCT Research Group, 1997) and exposure was assessed retrospectively using a validated and formatted hypoglycaemia questionnaire (Deary et al., 1993). Participants were asked to discuss their history of severe hypoglycaemia with family members, partners or friends before completing questionnaires to improve the accuracy of estimates. The total number of lifetime episodes, frequency of episodes, and total numbers of hypoglycaemic seizures, coma, and episodes requiring medical intervention were recorded for each subject. The retrospective ascertainment of severe hypoglycaemia utilised in the series of studies comprising this thesis is a methodological weakness relative to the gold-standard methodology of prospective ascertainment, although is sufficient to determine with a degree of accuracy those naïve to and

those previously exposed to severe hypoglycaemia. To overcome this relative methodological deficit would take decades of meticulous prospective evaluation, which is neither practical or easily funded.

9.3.4 Determination of Apolipoprotein-E Genotype

DNA was extracted from venous blood from all subjects using standard methods. APOE genotyping was performed using a one stage PCR-RFLP assay as described by Wenham et al (Wenham et al., 1991). Further detail is given in the Methods Chapter (6.9).

9.3.5 Statistical Analysis

SPSS v12.0 (®SPSS Inc, Chicago, USA) was used for all statistical analysis. Bi-variate correlation was used to identify demographic factors which significantly influenced neuropsychological performance. From the correlations observed a multivariate linear model (MANCOVA) was constructed which included age, duration of diabetes and pre-morbid intellectual ability (NART) as co-variates and gender, severe hypoglycaemia category and APOE genotype as between-subjects factors. Severe hypoglycaemia was dichotomised into two groups based upon the total number of previous episodes of severe hypoglycaemia experienced by the subject: (1) naïve to severe hypoglycaemia; (2) previously exposed to severe hypoglycaemia. For the purposes of analysis APOE genotype was categorised into two groups on the basis of the presence of the ε4 allele: (1) The ε4- group included those subjects with an ε2/2, ε2/3, or ε3/3 genotype; (2) the ε4+ group included those with one or more ε4 allele. i.e. ε2/4, ε3/4, or ε4/4 genotypes. MANCOVA was used to determine the independent significance (p) and the magnitude of effect size (Eta^2) that each variable of interest contributed towards performance on the battery of psychometric tests.

9.3.6 Subjects

The clinical characteristics of participants are shown in Table 9.1. Participants were randomly recruited from two pre-existing patient cohorts. Sixty-three participants were recruited from the surviviors of the cohort examined by Deary (Deary et al., 1993) with a further 33 patients recruited from the cohort examined by Ferguson (Ferguson et al., 2003). Data on glycaemic control have not been included as the laboratory reference ranges and the methodologies utilised (HbA_1, HbA_{1c}) for estimating Glycated Haemoglobin changed across the two time frames during which data from participants were collated.

Sub-division of participants by APOE ε4 produced two groups, ε4+ (n=21) and ε4- (n=75). The clinical characteristics of these groups are summarised in Table 9.1. Each subgroup had similar pre-morbid intellectual ability (NART), degree of education and had experienced a similar cumulative exposure to severe hypoglycaemia. Those possessing ε4 were significantly older (p=0.033, t-test) by a mean of 3.7 years and had tended to have had longer duration diabetes by a mean of 4.1 years (p=0.059, t-test). The differences observed between these demographic factors in the ε4+ and ε4- subgroups were controlled for in subsequent multivariate analyses.

Table 1. Characteristics of participants subdivided by the presence of the APOE ε4 allele.

	Entire Cohort (n=96)	ε4- (n=75)	ε4+ (n=21)	p-value
Age (years)	38.9 ± 7.2 (40, 22 to 54)	38.1 ± 7.4 (39, 22 to 52)	41.8 ± 5.7 (42, 31 to 54)	0.033*
Sex (M/F)	53:43	44:31	9:12	0.20
NART (pre-morbid IQ score)	32.7 ± 8.6 (3 to 50)	32.8 ± 8.8 (3 to 50)	32.1 ± 8.2 (18 to 45)	0.74
Age at Diagnosis of Diabetes (years)	24.3 ± 8.2 (25, 4 to 45)	24.3 ± 8.4 (25, 4 to 45)	24.1 ± 7.3 (26, 4 to 34)	0.93
Duration of Diabetes (years)	14.5 ± 7.3 (12, 5 to 40)	13.6 ± 6.7 (12, 5 to 32)	17.7 ± 8.8 (14, 8 to 40)	0.059
Severe Hypoglycaemia				
Total number of episodes (median)	2.0 (0 to 100)	3.0 (0 to 100)	5.0 (0 to 46)	0.43
No previous episodes (% patients)	30.2	32.0	23.8	-
1-10 episodes (% patients)	40.6	42.6	33.3	-
> 10 episodes (% patients)	29.2	25.4	42.9	-

Data are means ± SD with median and range in brackets.

9.4 RESULTS

9.4.1 Multivariate Predictors of Cognitive Ability

The F-statistic for each variable that was included in the MANCOVA model in descending order of their influence on cognitive ability were: Pre-morbid IQ (NART) $F=7.9$, $p<0.0001$; Age $F=3.5$, $p=0.01$; APOE group $F=2.5$, $p=0.04$; Gender × APOE group interaction $F=2.5$, $p=0.04$; Severe Hypoglycaemia group $F=1.1$, $p=0.35$; APOE group × Severe Hypoglycaemia group interaction $F=0.88$, $p=0.50$; Duration of diabetes $F=0.60$, $p=0.70$; Gender $F=0.25$, $p=0.94$.

9.4.2 Severe Hypoglycaemia and Neuropsychological Performance

The range of exposure to severe hypoglycaemia of those studied was wide, from those naïve to severe hypoglycaemia (30%) to those who had experienced in excess of ten episodes (29%) (Table 9.1). No significant correlation (Spearman's rho) was observed between preceding exposure to severe hypoglycaemia (dichotomised as naïve or exposed) and performance on any cognitive ability test: WAIS-R Performance IQ $r^2=-0.14$, $p=0.18$; Borkowski Verbal Fluency $r^2=0.014$, $p=0.89$; PASAT $r^2=-0.013$, $p=0.90$; PEST Score $r^2=0.033$, $p=0.76$; Median 4-Choice Decision Time $r^2=0.098$, $p=0.37$. The differences in estimated marginal means, following MANCOVA, for those with and without a history of severe hypoglycaemia are summarised in Table 9.2.

Table 9.2. Effect of severe hypoglycaemia on cognitive ability and interaction with APOE.

Neuropsychological Test	SH- n=29 Estimated Marginal Mean (SEM)	SH+ n=67 Estimated Marginal Mean (SEM)	Effect of Hypoglycaemia Exposure (n=96)	ε4 + SH- n=6 Estimated Marginal Mean (SEM)	ε4 + SH+ n=15 Estimated Marginal Mean (SEM)	APOE × Hypoglycaemia Interaction (n=96)
Performance IQ	126.7	128.1	p=0.80	118.4	124.4	p=0.31
(WAIS-R performance subtests)	(4.4)	(2.7)	Eta^2=0.001	(7.6)	(4.8)	Eta^2=0.01
Frontal & Executive Function	37.3	41.3	p=0.28	33.9	38.7	p=0.44
Function (Borkowski VFT)	(3.1)	(1.9)	Eta^2=0.002	(5.4)	(3.3)	Eta^2<0.01
Early Visual Processing	77.8	66.8	p=0.34	84.0	55.1	p=0.25
(Inspection Time PEST Score)	(9.8)	(6.1)	Eta^2=0.04	(17.2)	(10.7)	Eta^2=0.04
Psychomotor Speed	351.9	352.8	p=0.95	354.3	353.4	p=0.70
(Median 4-Choice Reaction Time)	(12.9)	(8.0)	Eta^2<0.001	(22.5)	(14.1)	Eta^2<0.01
Attention and Concentration	38.1	34.8	p=0.29	39.7	34.9	p=0.22
(PASAT 2s)	(2.6)	(1.6)	Eta^2=0.02	(4.6)	(2.8)	Eta^2=0.04

SH- = no previous severe hypoglycaemia. SH+ = previous exposure to severe hypoglycaemia. ε4- = no ε4 allele. ε4+ = one or more ε4 allele. Multivariate linear model [age, gender, NART, severe hypoglycaemia category (naïve/exposed), APOE category (ε4+ or ε4-)].

9.4.3 APOE ε4 and Neuropsychological Performance

On initial multivariate testing APOE genotype, coded by the presence or absence of the ε4 allele, was a significant independent predictor of performance on the battery of neuropsychological tests. The APOE ε4 allele was independently associated with a disadvantage in current intellectual performance (WAIS-R performance IQ, $p=0.037$, $Eta^2=0.072$) and a trend towards poorer frontal lobe and executive functions (Borkowski Verbal Fluency, $p=0.063$, $Eta^2=0.057$) after consideration of the potential confounding effects of age, gender, duration of diabetes, preceding severe hypoglycaemia and pre-morbid intellectual ability (NART). The relative disadvantage in cognitive ability observed in association with the APOE ε4 allele appeared to be gender-specific (APOE × gender interaction: $F=2.5$, $p=0.04$, $Eta^2=0.15$). Taking account of the gender-specific influence of APOE genotype on cognitive performance, multivariate analysis (MANCOVA) was performed thereafter on a gender-specific basis, using a similar multivariate model but excluding gender as a between-subjects factor. This gender-specific sub-analysis demonstrated that the APOE genotype was only a significant determinant of cognitive performance in female participants, with no statistically significant differences observed in male participants (Table 9.3). The significant differences in cognitive ability observed in women related to poorer performance of APOE ε4 carriers on specific neuropsychological tests. Poorer performance was observed in current intellectual performance (WAIS-R performance test score, $p=0.005$, $Eta^2=0.37$) and frontal lobe and executive function (Borkowski VFT, $p=0.029$, $Eta^2=0.13$). The magnitude of the difference observed in ability in association with possession of the APOE ε4 allele in female participants was moderate-to-large (Eta^2). No differences were observed in early visual information processing ability, psychomotor speed or in the ability to sustain attention and concentration. Performance subtests of the WAIS-R each measure different aspects of current intellectual performance. A further multivariate analysis with the WAIS-R subtests (Picture Completion, Block Design, Object Assembly and Digit Symbol tests) as

dependent variables was performed to clarify which particular intellectual abilities were compromised in those with the APOE ε4 allele. An identical model to that employed in the gender-specific analysis was utilised for this aim. The performance of participants on the WAIS-R subtests is summarised in Table 9.4. Significantly poorer performance was observed in association with the ε4 allele in female participants on the Object Assembly, Block Design and Digit Symbol performance subtests.

Table 9.3. Effect of APOE (ε4- and ε4+), age and pre-morbid intellectual ability (NART) on cognitive performance.

Neuropsychological Test	ε4 – n=75 Estimated Marginal Mean (SEM)	ε4 + n=21 Estimated Marginal Mean (SEM)	Effect of ε4	Effect of Age	Effect of NART
Performance IQ (WAIS-R)	133.4 (2.6)	121.4 (4.6)	p=0.037 Eta^2=0.07	p<0.001 Eta^2=0.19	p<0.001 Eta^2=0.29
Frontal & Executive Function (Borkowski)	42.2 (1.8)	36.3 (3.2)	p=0.063 Eta^2=0.06	p=0.53 Eta^2=0.007	p<0.001 Eta^2=0.19
Early Visual Processing (Inspection Time)	75.1 (5.8)	69.6 (10.2)	p=0.37 Eta^2=0.01	p=0.022 Eta^2=0.08	p=0.041 Eta^2=0.07
Psychomotor Speed (4-Choice Reaction Time)	349.9 (7.6)	354.9 (13.4)	p=0.67 Eta^2=0.003	p=0.12 Eta^2=0.04	p=0.10 Eta^2=0.04
Attention and Concentration (PASAT)	35.6 (1.5)	37.3 (2.7)	p=0.75 Eta^2=0.002	p=0.82 Eta^2=0.001	p<0.001 Eta^2=0.19

Data are means ± SD. Multivariate linear model [age, gender, NART, severe hypoglycaemia category (naive/previous exposure), APOE category (ε4+ or ε4-), duration of diabetes].

Table 9.4. APOE alleles, estimated marginal means (MANCOVA) of cognitive ability tests and gender.

Neuropsychological Test	Male					Female				
	ε2 / ε3 (n=44)	ε4 (n=9)	F	p-value	Eta²	ε2 / ε3 (n=31)	ε4 (n=12)	F	p-value	Eta²
Performance Score (WAIS-R)	124.4	124.8	0.14	0.97	<0.01	140.0	113.1	4.6	0.005	0.37
Picture Completion	15.6	15.6	0.02	0.98	<0.01	15.5	13.8	1.4	0.24	0.04
Block Design	32.0	30.8	0.04	0.85	<0.01	33.2	26.1	4.7	0.04	0.12
Object Assembly	27.7	28.2	0.11	0.74	<0.01	30.8	24.2	17.7	0.0002	0.34
Digit Symbol Substitution	49.0	50.2	0.30	0.59	<0.01	60.5	48.9	4.6	0.04	0.12
Frontal & Executive Function (Borkowski Verbal Fluency)	38.3	37.9	0.006	0.94	<0.01	45.7	32.6	5.2	0.03	0.13

Multivariate linear model [age, NART, severe hypoglycaemia category (naive/exposed), APOE category (ε4+ or ε4-), duration of diabetes].

9.4.4 Apolipoprotein-E Genotype and Severe Hypoglycaemia

There was no statistical evidence of a meaningful interaction between APOE genotype, previous exposure to severe hypoglycaemia and cognitive ability (APOE × Severe Hypoglycaemia interaction: F=0.66, p=0.68, Eta^2=0.05). The cognitive ability of subjects with the APOE ε4 allele, when subdivided into those exposed previously to severe hypoglycaemia (n=15) and those naïve to severe hypoglycaemia (n=6), is summarised in Table 9.2. No significant difference in cognitive ability was demonstrated between the subgroups, although the numbers of subjects in the exploratory analysis are sufficiently small, to preclude the exclusion of a non-significant difference. On the basis of the present pilot data no trends were identified to suggest that a clinically meaningful interaction between APOE genotype and exposure to severe hypoglycaemia would be likely, even if a larger study were undertaken.

9.5 DISCUSSION

The present study identified a significant cognitive disadvantage in association with the presence of the APOE ε4 allele in adults with Type 1 diabetes. The differences in performance observed did not encompass all aspects of cognitive ability, were gender-specific and appeared to be evident at a younger age (39 years) than has been previously reported in people who do not have diabetes. The cognitive deficits observed in the present study were limited to performance on tests of current intellectual performance (WAIS-R performance IQ subtests) and frontal lobe and executive functions (Borkowski Verbal Fluency). Significant differences in ability were only observed in women with Type 1 diabetes. Women with one or more ε4 allele exhibited significantly poorer performance on the Object Assembly, Block Design and Digit Symbol tests of the WAIS-R and poorer fontal lobe and executive function, as determined by performance in the Borkowski Verbal Fluency task. Performance on the particular WAIS-R subtests

disadvantaged by possession of the ε4-allele provide measures of visual constructional ability (OA: lesions affecting the occipital lobes, particularly on the non-dominant side), visuo-spatial ability (BD: most strongly affected by non-dominant hemisphere lesions, typically those involving the posterior aspects of the parietal lobe), psychomotor speed, visual-motor speed and short-term visual memory (DSST: non-specific cortical localisation). The brain areas sub-serving these specific modalities are anatomically located in the non-dominant occipital lobe and non-dominant posterior parietal lobe. The Digit Symbol test provides a measure of psychomotor speed, visual-motor speed and short-term visual memory and is sensitive to subtle differences in ability but poorly localised in the cerebral hemispheres (Lezak, 2004). Poorer performance in these subtests has been associated with lesions of the non-dominant hemisphere, with particular localisation to the non-dominant occipital lobe (object assembly) and the posterior region of the non-dominant parietal lobe (block design subtest) (Lezak, 2004).

Regional cerebral glucose utilisation (CMR_{glu}) is known to be influenced by APOE genotype in apparently cognitively normal healthy middle-aged adults many years before the clinical presentation of dementia of the Alzheimer's type (Small et al., 2000). Reductions in the CMR_{glu} have been identified using 18-Fluoro-Deoxyglucose PET in the parietal lobes (Reiman et al., 2001;Reiman et al., 2004;Reiman et al., 2005;Small et al., 2000;Reiman et al., 2005), posterior cingulate gyrus (medial temporal lobe), temporal lobes and pre-frontal cortex (Reiman et al., 2001;Reiman et al., 2004;Reiman et al., 2005) of apparently healthy middle-aged adults with one or more APOE ε4 allele. Reiman et al also identified a relationship between APOE ε4 gene dose and reduced CMR_{glu}, such that ε4 heterozygotes exhibited reduced metabolism in the brain regions described above, but greater reductions were observed in ε4 homozygotes. These reports of altered regional brain glucose metabolism are consistent with the cognitive findings of the present study. Only performance on those specific cognitive ability tests which are anatomically

associated with the cortical areas identified by the above PET studies appeared to be detrimentally affected in APOE ε4 allele carriers.

Female susceptibility to APOE ε4-associated cognitive disadvantage is not a novel observation and has been described across a number of cognitive domains. Mortensen et al prospectively measured WAIS-R performance in healthy participants from the age of 50 to 80 years and observed poorer WAIS-R performance IQ scores, and a more rapid rare of cognitive decline, in 80 year old women with the ε4 allele (Mortensen and Hogh, 2001). These women performed more poorly on the Object Assembly and Block Design performance subtests, and tended to score less well on the Digit Symbol test, an identical pattern to the cognitive disadvantage observed in female participants with Type 1 diabetes in the present study, albeit at a considerably greater chronological age. The mechanisms underlying these clinical observations are not well defined but animal models may offer some clues to causality. In an APOE knockout mouse model, where human APOE isoforms substituted host isoforms, only female ε4 mice exhibited memory and spatial-ability deficits whereas male ε4 mice showed no deficits (Raber et al., 1998). Thereafter, the same research group identified that Androgens may be important in the pathogenesis of APOE ε4 related cognitive disadvantage: treating male ε4 mice with an androgen-receptor antagonist produced memory and spatial impairment, whereas treating female ε4 mice with Androgens reversed ε4-associated cognitive deficits (Raber et al., 2000).

Age influences the effect of APOE ε4 on cognitive ability: neuropsychological performance in healthy children (Turic et al., 2001) and healthy young adults (Yu et al., 2000) is not affected by the ε4 allele but middle-aged, otherwise healthy adults (mean age 46 years) have been observed to have impaired learning and memory ability (Flory et al., 2000). The younger age (median age 39 years) at which ε4-associated cognitive disadvantage was observed in adults with type 1

diabetes in the present study, implies premature susceptibility compared with non-diabetic individuals.

Laboratory and clinical evidence supports the concept of ε4-associated premature cognitive ageing in diabetes. Elderly people with type 2 diabetes and ε4 exhibit greater cognitive decrement (Kalmijn et al., 1996) and an accelerated cognitive decline (Haan et al., 1999) compared to age-matched ε4 healthy controls; APOE from people with diabetes is irreversibly glycated and exhibits less in-vitro bioactivity (Shuvaev et al., 1999) when compared to non-diabetic controls. APOE bioactivity appears to have clinical relevance as outcomes from a variety of cerebral pathologies are poorer in individuals possessing the least biologically active E4-isoform (Smith, 2000). As the present study did not include a healthy comparator group, the notion that APOE ε4 is associated with premature cognitive ageing in type 1 diabetes is speculative at present, whereas this has been demonstrated in type 2 diabetes (Haan et al., 1999;Kalmijn et al., 1996).

The present study did not support the hypothesis that polymorphism of the APOE gene may contribute to the susceptibility to hypoglycaemia-induced cognitive decrement in people with type 1 diabetes. The number of subjects in each ε4 sub-group (n=15 vs. n=6) does not give sufficient power to allow definitive conclusions and further, appropriately powered, investigation is required to evaluate this hypothesis. Irrespective of the influence of APOE alleles, cognitive performance was unaffected by severe hypoglycaemia *per se*, consistent with the conclusions of the Diabetes Control and Complications Trial (1996) and the Stockholm Diabetes Intervention Study (Reichard et al., 1991), in which many participants had type 1 diabetes of relatively short duration, but conflicting with retrospective cross-sectional studies (Langan et al., 1991;Lincoln et al., 1996;Sachon et al., 1992;Wredling RAM et al., 1992), which examined older subjects with

diabetes of long duration.

In conclusion, the data in this modestly-powered study suggest that APOE ε4 confers a cognitive disadvantage in young women who have type 1 diabetes. Further investigation is required to verify these findings and to determine whether the APOE ε4 allele is associated with premature or accelerated cognitive ageing in people who have type 1 diabetes, the diabetes-specific factors that may be mediating any such disadvantage, and the possible interaction with gender.

CHAPTER 10

CONCLUSIONS AND FUTURE DIRECTIONS

10. CONCLUSIONS AND FUTURE DIRECTIONS

The series of clinical studies presented in this thesis identified associations between the clinical complications of Type 1 diabetes and the age of disease onset and related these circumscribed differences in cognitive ability and brain structure, each apparently specific to the particular type of diabetes complication. The studies discussed below did not include a "healthy" non-diabetic control group and were designed to explore the effects of "diabetes-specific" factors on the cognitive and brain structural outcomes of interest. As such, the effects of Type 1 diabetes per se remain relatively under explored, most notably with respect to the subtleties of brain structure.

10.1 Severe Hypoglycaemia and the Brain in Type 1 Diabetes

Repeated exposure to severe hypoglycaemia did not appear to be associated with measurable differences in cognitive ability or brain structure in the cohort of subjects examined within this study, within the significant limitations imposed by the collection of retrospective data summarising preceeding severe hypoglycaemia exposure. Previous cross-sectional studies have suggested a modest cognitive decrement may accrue following repeated exposure to severe hypoglycaemia whereas large scale prospective studies have not confirmed this association. The present study was not without limitations: not all cognitive domains were examined and the neuroimaging methodologies utilised assessed global macroscopic structural changes but only assessed detailed volumetric measurements in specific regions of interest. Therefore, it cannot be definitively concluded from the present data that severe hypoglycaemia exposure was not detrimental to all aspects of cognitive ability or brain structure given these caveats. Despite these caveats, the present findings with respect to severe hypoglycaemia exposure are reassuring and do not infer that any substantial differences in cognitive performance or brain structure are likely in younger people with Type 1 diabetes. Future research could utilise different forms of MRI

neuroimaging to search for very subtle structural brain associations, particularly Voxel Based Morphometry (VBM) which has been recently shown to be a sensitive technique for identifying subtle regional differences in gray matter density (Musen G., 2006), although the practical meaning of such subtle change remains questionable.

10.2 Diabetic Retinopathy and the Brain in Type 1 Diabetes

The present study identified an association between the presence of mild degrees of Diabetic Retinopathy and poorer cognitive abilities, primarily affecting fluid intelligence and information processing speed but also disadvantageous to early visual information processing abilities and the ability to sustain attention and concentration. This observation was consistent with previously published work from Ryan and colleagues (Ryan C.M., 1992) who identified a similar pattern of impairment in neuropsychological performance in association with the presence of peripheral neuropathy. These findings strongly suggest that the presence of microangiopathy is disadvantageous to aspects of human cognitive ability. The structural brain associations of Diabetic Retinopathy were modest, restricted to the presence of SPWML in a regional brain area, and perhaps could represent Type 1 error. The hypothesis that the presence of Diabetic Retinopathy is a surrogate marker for intra-cranial microangiopathy elsewhere in the cerebral circulation is therefore not proven. Future studies could be directed at evaluating the neuroimaging associations of greater microangiopathic burdens, as Proliferative Retinopathy, Nephropathy and Neuropathy. However, neuropathological studies in Type 1 diabetes are lacking and detailed histopathological studies of the brain would garner more valuable information.

10.3 'Early Onset Diabetes' and the Brain in Type 1 Diabetes

The present observations lend support to the hypothesis that organic factors, relating to diabetes *per se* or the development of acute metabolic decompensations in early life, are likely to be largely responsible for the "early onset" of diabetes effect on cognitive abilities and intelligence reported by multiple authors over the last 45 years. The differences in cognitive ability identified in association with "early onset" diabetes were specific, affecting fluid intelligence and information processing speed and are consistent with the reports of others. The differences described in brain structure, with an increased prevalence of subjectively-rated central brain atrophy and greater lateral ventricle cavity volumes, in those with an early childhood onset of Type 1 diabetes is novel and supports an organic process. These differences could not be attributed to any identifiable diabetes-related factor and the present design could not determine whether the associations observed were related to early childhood exposure to severe hypoglycaemia, diabetic keto-acidosis or any other disease-specific factor. The present series of studies comprising this thesis did not attempt to contrast the dieffering effects of a childhood onset of Type 1 diabetes, relative to an onset after the attainment of full intellectual maturity at the age of eighteen years. Such an analysis was not performed, despite two potentially suitable cohorts being available for analysis, due to the interdependent confounding effects of diabetes duration and age. For obvious reasons, it is not possible to match a "childhood onset" and "adult onset" group for both age and disease duration, leading to substantial difficulties in the interpretation of any subsequent outcomes, which may render such an analysis near uninterpretable. Future case-control studies will be required to prospectively chart the neurodevelopmental effects of Type 1 diabetes and identify the key components which mediate the development of central brain atrophy. Prospective assessment of the present cohort may also be of value to determine whether the degree of central brain atrophy alters with time and whether associated differences in cognitive ability emerge.

10.4 Neuroimaging Associations of Type 1 Diabetes

Abnormalities readily apparent on routine MRI of the brain are present in the majority of young adults who have Type 1 diabetes. Those abnormalities most commonly observed were a mild degree of central brain atrophy, mild degrees of Leukoaraiosis and Small Punctate White Matter Lesions. These abnormalities were statistically associated with diabetes-related disease variables but were not independent determinants of performance on a range of cognitive ability tests. The practical significance of these subtle structural brain abnormalities is not apparent at present and prospective assessment will be required to determine their natural history and future associations with cognitive performance. It is unclear whether the presence of cerebral white matter abnormalities is a risk factor for the future development of macrovascular events, or the development of cognitive decline in Type 1 diabetes, as has been noted in chronologically older non-diabetic populations. Diffusion Tensor Imaging, a newer MRI methodology which allows the delineation of white matter tracts (tractography) may assist in clarifying the extent and white matter tract bundles compromised by white matter lesions and therefore give clues as to the cognitive abilities most likely to be disadvantaged.

10.5 Apolipoprotein-E and Type 1 Diabetes

An association between the presence of the APOE ε4 allele and poorer cognitive function was observed in the present study. This independent association was observed only in women and appeared to be of medium effect size. The hypothesis that cognitive abilities would be poorer in those with APOE ε4 who were exposed to severe hypoglycaemia was not proven and the study was underpowered to achieve his aim. It can be concluded that possession of the ε4 allele of the APOE gene is likely to be disadvantageous to cognitive abilities in women with Type 1 diabetes, but whether this will result in more rapid cognitive decline relative to non-ε4 carriers or the

development of early dementia in earlier life is presently not known. A prospective study would be required to determine the validity of the present observations and additionally test the hypotheses that the presence of microangiopathy or intercurrent episodes of severe hypoglycaemia may mediate any cognitive disadvantage. The addition of neuroimaging and a neuropsychological test battery weighted to medial temporal lobe functions would add value to such a study.

REFERENCES

References for Chapter 1

Alex,M, E K Baron, S Goldenberg, H T Blumenthal. An autopsy study of cerebrovascular accident in diabetes mellitus. Circulation 25:663-673, 1962.

Baker AB̃. American Journal of Psychiatry 96:109-127. 1939.

Bale,RN. Brain damage in diabetes mellitus. Br.J.Psychiatry 122:337-341, 1973

DeJong, RN. CNS manifestations of diabetes mellitus. Postgrad.Med. 61:101-107, 1977.

Fineberg S.K., Altschul A. The encephalopathy of hyperinsulinism. Ann Intern Med 36:536-550, 1952.

Fischer AE, Dolger H. Behavior and psychologic problems of young diabetic patients. Archives of Internal Medicinę 78:711-732. 1946.

Franceschi MR, Cecchetto F, Minicucci S, Smizne G, Baio N, Canal N. Cognitive processes in insulin-dependent diabetes: Diabetes Care 7:228-231, 1984.

Grunnet M. Cerebrovascular disease: diabetes and cerebral atherosclerosis. Neurology 13:486-491, 1963.

Johnson PC, K Brendel, E Meezan. Thickened cerebral cortical capillary basement membranes in diabetics: Arch.Pathol.Lab Med 106:214-217, 1982.

Lawrence RD, Mayer A, Nevin S. The pathological changes in the brain in fatal hypoglycaemia̋. Quarterly Journal of Medicine 11:181-201, 1942.

Marchal (deCalvi). Recherces sur les accidents diabetiques, 1864.

Meuter F, W Thomas, D Gruneklee, FA Gries, R Lohmann. Psychometric evaluation of performance in diabetes mellitus: Horm.Metab Res.Suppl 9:9-17, 1980.

Murphy FD, Purtell J. Insulin reaction and the cerebral damage that may occur in diabetes. Journal of Digestive Diseases 1:103-107, 1943.

Plum F. The neurologic complications of diabetes mellitus. Williams RH. Diabetes, p.602-622, 1960. New York, Hoeber.

Reske-Nielsen E, K Lundbaek. Diabetic encephalopathy: diffuse and focal lesions of the brain in long-term diabetes. Acta Neurol.Scand.Suppl 39:273-290, 1963.

Seegen J. Der Diabetes Mellitus. 1893. Berlin , A. Hirschwald.

Tesfaye S, N Chaturvedi, SE Eaton, JD Ward, C Manes, C Ionescu-Tirgoviste, DR Witte, JH Fuller. Vascular risk factors and diabetic neuropathy. N.Engl.J.Med. 352:341-350, 2005.

Woodyat RT. The clinical use of insulin̋. Journal of Metabolic Research 2:793-801. 1922.

References for Chapter 2

The DCCT Investigators. Epidemiology of severe hypoglycemia in the diabetes control and complications trial. Am.J.Med. 90:450-459, 1991.

The DCCT Investigators. Effects of intensive diabetes therapy on neuropsychological function in adults in the Diabetes Control and Complications Trial. Ann.Intern.Med. 124:379-388, 1996.

The DCCT Investigators. Hypoglycemia in the Diabetes Control and Complications Trial. Diabetes 46:271-286, 1997.

Amiel SA, HR Archibald, G Chusney, AJ Williams, EA Gale. Ketone infusion lowers hormonal responses to hypoglycaemia: evidence for acute cerebral utilization of a non-glucose fuel. Clin.Sci.(Lond) 81:189-194, 1991.

Aoki T, T Sato, K Hasegawa, R Ishizaki, M Saiki. Reversible hyperintensity lesion on diffusion-weighted MRI in hypoglycemic coma. Neurology 63:392-393, 2004.

Arky RA, E Veverbrants, E A Abramson. Irreversible hypoglycemia. A complication of alcohol and insulin. JAMA 206:575-578, 1968.

Auer R, H Kalimo, Y Olsson, T Wieloch. The dentate gyrus in hypoglycemia: pathology implicating excitotoxin- mediated neuronal necrosis. Acta Neuropathol.(Berl) 67:279-288, 1985.

Auer RN, J Hugh, E Cosgrove, B Curry. Neuropathologic findings in three cases of profound hypoglycaemia. Clin.Neuropathol. 8:63-68, 1989.

Auer RN, H Kalimo, Y Olsson, B K Siesjo. The temporal evolution of hypoglycemic brain damage. I. Acta Neuropathol.(Berl), 67:13-24, 1985a.

Auer RN, H Kalimo, Y Olsson, B K Siesjo. The temporal evolution of hypoglycemic brain damage II. Acta Neuropathol.(Berl) 67:25-36, 1985c.

Auer RN, B K Siesjo. Hypoglycaemia: brain neurochemistry and neuropathology: Baillieres Clin.Endocrinol.Metab. 7:611-625, 1993.

Austin EJ, I J Deary. Effects of repeated hypoglycemia on cognitive function: a psychometrically validated reanalysis of the Diabetes Control and Complications Trial data. Diabetes Care 22:1273-1277, 1999.

Baker AB. American Journal of Psychiatry 96:109-127, 1939.

Bakshi R, M F Morcos, T F Gabryel, P Dandona. Is fluid-attenuated inversion recovery MRI more sensitive than conventional MRI for hypoglycemic brain injury? Neurology 55:1064-1065, 2000.

Bale RN. Brain damage in diabetes mellitus. Br.J.Psychiatry 122:337-341, 1973.

Bischof MG, V Mlynarik, A Brehm, E Bernroider, M Krssak, E Bauer, C Madl, M Bayerle-Eder, W Waldhausl, M Roden. Brain energy metabolism during hypoglycaemia in healthy and type 1 diabetic subjects. Diabetologia 47:648-651, 2004.

Boeve BF, D G Bell, J H Noseworthy. Bilateral temporal lobe MRI changes in uncomplicated hypoglycemic coma. Can.J.Neurol.Sci. 22:56-58, 1995.

Brands AM, G J Biessels, E H de Haan, L J Kappelle, R P Kessels. The effects of type 1 diabetes on cognitive performance: a meta-analysis. Diabetes Care 28:726-735, 2005.

Chalmers J, M T Risk, D M Kean, R Grant, B Ashworth, I W Campbell. Severe amnesia after hypoglycemia. Clinical, psychometric, and magnetic resonance imaging correlations. Diabetes Care 14:922-925, 1991.

Chan R, S Erbay, S Oljeski, D Thaler, R Bhadelia. Case report: hypoglycemia and diffusion-weighted imaging. J.Comput.Assist.Tomogr. 27:420-423, 2003.

Deary IJ, J R Crawford, D A Hepburn, S J Langan, L M Blackmore, B M Frier. Severe hypoglycemia and intelligence in adult patients with insulin- treated diabetes. Diabetes 42:341-344, 1993.

Donnelly LA, A D Morris, B M Frier, J D Ellis, P T Donnan, R Durrant, M M Band, G Reekie, G P Leese. Frequency and predictors of hypoglycaemia in Type 1 and insulin-treated Type 2 diabetes: a population-based study. Diabet.Med. 22:749-755, 2005.

Evans ML, D Hopkins, I A MacDonald, S A Amiel. Alanine infusion during hypoglycaemia partly supports cognitive performance in healthy human subjects. Diabet.Med. 21:440-446, 2004.

Evans ML, K Matyka, J Lomas, A Pernet, I C Cranston, I Macdonald, S A Amiel. Reduced counterregulation during hypoglycemia with raised circulating nonglucose lipid substrates: evidence for regional differences in metabolic capacity in the human brain? J.Clin.Endocrinol.Metab, 83:2952-2959, 1998.

Fazekas JF, R W Alman, A E Parrish. Irreversible post-hypoglycemic coma. Am.J.Med.Sci. 222:640-643, 1951.

Finelli PF. Diffusion-weighted MR in hypoglycemic coma. Neurology 57:933, 2001.

Gold AE, S M Marshall. Cortical blindness and cerebral infarction associated with severe hypoglycaemia. Diabetes Care 19:1001-1003, 1996.

Gruetter R. Glycogen: The forgotten cerebral energy store. J.Neurosci.Res. 74:179-183, 2003.

Guzman M, C Blazquez. Is there an astrocyte-neuron ketone body shuttle? Trends Endocrinol.Metab. 12:169-173, 2001.

Holemans X, M Dupuis, N Misson, J F Vanderijst. Reversible amnesia in a Type 1 diabetic patient and bilateral hippocampal lesions on magnetic resonance imaging (MRI). Diabet.Med. 18:761-763, 2001.

Jacobson AM, G Musen, C M Ryan, N Silvers, P Cleary, B Waberski, A Burwood, K Weinger, M Bayless, W Dahms, J Harth. Long-term effect of diabetes and its treatment on cognitive function. N.Engl.J.Med. 356:1842-1852, 2007.

Kalimo H, Y Olsson. Effects of severe hypoglycemia on the human brain. Neuropathological case reports. Acta Neurol.Scand. 62:345-356, 1980.

Koppel BS, M Daras. Transient Hypodensity on CT Scan during Hypoglycemia. Eur Neurol 33:80-82, 1993.

Kramer L, P Fasching, C Madl, B Schneider, P Damjancic, W Waldhausl, K Irsigler, G Grimm. Previous episodes of hypoglycemic coma are not associated with permanent cognitive brain dysfunction in IDDM patients on intensive insulin treatment. Diabetes 47:1909-1914, 1998.

Langan SJ, I J Deary, D A Hepburn, B M Frier. Cumulative cognitive impairment following recurrent severe hypoglycaemia in adult patients with insulin-treated diabetes mellitus. Diabetologia 34:337-344, 1991.

Lawrence RD, Mayer A, Nevin S. Quarterly Journal of Medicine 11:181-201, 1942.

Lincoln NB, R M Faleiro, C Kelly, B A Kirk, W J Jeffcoate. Effect of long-term glycemic control on cognitive function. Diabetes Care 19:656-658, 1996.

Lunetta M, A R Damanti, G Fabbri, M Lombardo, M Di Mauro, L Mughini. Evidence by magnetic resonance imaging of cerebral alterations of atrophy type in young insulin-dependent diabetic patients. J.Endocrinol.Invest 17:241-245, 1994.

MacLeod KM, D A Hepburn, B M Frier. Frequency and morbidity of severe hypoglycaemia in insulin-treated diabetic patients. Diabet.Med. 10:238-245, 1993.

Maekawa S, M Aibiki, K Kikuchi, S Kikuchi, K Umakoshi. Time related changes in reversible MRI findings after prolonged hypoglycaemia. Clin.Neurol.Neurosurg. 108:511-513, 2006.

Maran A, I Cranston, J Lomas, I Macdonald, S A Amiel. Protection by lactate of cerebral function during hypoglycaemia. Lancet 343:16-20, 1994.

Maran A, C Crepaldi, S Trupiani, T Lucca, E Jori, I A MacDonald, A Tiengo, A Avogaro, S Del Prato. Brain function rescue effect of lactate following hypoglycaemia is not an adaptation process in both normal and type I diabetic subjects. Diabetologia 43:733-741, 2000.

Mason GF, K F Petersen, V Lebon, D L Rothman, G I Shulman. Increased brain monocarboxylic acid transport and utilization in type 1 diabetes. Diabetes 55:929-934, 2006.

McCall AL. Cerebral glucose metabolism in diabetes mellitus. Eur.J.Pharmacol. 490:147-158, 2004.

McEwen BS, L P Reagan. Glucose transporter expression in the central nervous system: relationship to synaptic function. Eur.J.Pharmacol. 490:13-24, 2004.

Meer A, B Dubois, D Dormont, A Grimaldi. [Value of magnetic resonance imaging after severe hypoglycemic coma]. Presse Med. 17:1368, 1988.

Nelson H, J R Willison. National Adult Reading Test (NART) Test Manual. 2nd ed. 1991. NFER-Nelson, Windsor, U.K.

Olney JW. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Science 164:719-721, 1969.

Olney JW. Excitatory transmitter neurotoxicity. Neurobiol.Aging 15:259-260, 1994.

Owen OE, A P Morgan, H G Kemp, J M Sullivan, M G Herrera, G F Cahill, Jr. Brain metabolism during fasting. J.Clin.Invest 46:1589-1595, 1967.

Pardridge WM. Advances in cell biology of blood-brain barrier transport. Semin.Cell Biol. 2:419-426, 1991.

Patrick AW, I W Campbell. Fatal hypoglycaemia in insulin-treated diabetes mellitus: clinical features and neuropathological changes. Diabet.Med. 7:349-354, 1990.

Perros P, I J Deary, R J Sellar, J J Best, B M Frier. Brain abnormalities demonstrated by magnetic resonance imaging in adult IDDM patients with and without a history of recurrent severe hypoglycaemia. Diabetes Care 20:1013-1018, 1997.

Perros P, R J Sellar, B M Frier. Chronic pontine dysfunction following insulin-induced hypoglycemia in an IDDM patient. Diabetes Care 17:725-727, 1994.

Rajbhandari SM, T Powell, G A Davies-Jones, J D Ward, 1998, Central pontine myelinolysis and ataxia: an unusual manifestation of hypoglycaemia. Diabet.Med. 15:259-261, 1998.

Reichard P, A Britz, U Rosenqvist. Intensified conventional insulin treatment and neuropsychological impairment. BMJ 303:1439-1442, 1991.

Richardson ML, R E Kinard, M B Gray. CT of generalized gray matter infarction due to hypoglycaemia. AJNR Am.J.Neuroradiol. 2:366-367, 1981.

Ryan CM, T M Williams, D N Finegold, T J Orchard. Cognitive dysfunction in adults with type 1 (insulin-dependent) diabetes mellitus of long duration: effects of recurrent hypoglycaemia and other chronic complications. Diabetologia 36:329-334, 1993.

Sachon C, A Grimaldi, J P Digy, B Pillon, B Dubois, F Thervet. Cognitive function, insulin-dependent diabetes and hypoglycaemia. J.Intern.Med. 231:471-475, 1992.

Sakel M. The methodical use of hypoglycemia in the treatment of psychoses (1937). Am.J.Psychiatry 151:240-247, 1994.

Skenazy JA, E D Bigler. Neuropsychological findings in diabetes mellitus. J.Clin.Psychol. 40:246-258, 1984.

Smith D, A Pernet, W A Hallett, E Bingham, P K Marsden, S A Amiel. Lactate: a preferred fuel for human brain metabolism in vivo. J.Cereb.Blood Flow Metab. 23:658-664, 2003.

Snoek FJ, d van, V, R J Heine, E H de Haan. No differences in attentional functioning between type 1 diabetic patients with and without a history of severe hypoglycaemia. Diabetes Care 21:1568-1569, 1998.

Sokoloff L. Measurement of local cerebral glucose utilization and its relation to local functional activity in the brain: Adv.Exp.Med.Biol. 291:21-42, 1991.

Suh SW, J P Bergher, C M Anderson, J L Treadway, K Fosgerau, R A Swanson. Astrocyte Glycogen Sustains Neuronal Activity during Hypoglycemia: Studies with the Glycogen Phosphorylase Inhibitor CP-316,819 ([R-R*,S*]-5-Chloro-N-[2-hydroxy-3-(methoxymethylamino)-3-oxo-1-(phenylmet hyl)propyl]-1H-indole-2-carboxamide): J.Pharmacol.Exp.Ther. 321:45-50, 2007.

Tattersall RB. In Frier BM and Fisher BM. pp55-88, 1999. Churchill Livingstone, London, UK.

Vannucci RC, S J Vannucci. Hypoglycemic brain injury: Semin.Neonatol. 6:147-155, 2001.

Veneman T, A Mitrakou, M Mokan, P Cryer, J Gerich. Effect of hyperketonemia and hyperlacticacidemia on symptoms, cognitive dysfunction, and counterregulatory hormone responses during hypoglycemia in normal humans: Diabetes 43:1311-1317, 1994.

Warren,RE, B M Frier. Hypoglycaemia and cognitive function: Diabetes Obes.Metab. 7:493-503, 2005.

Wredling R, S Levander, U Adamson, P E Lins. Permanent neuropsychological impairment after recurrent episodes of severe hypoglycaemia in man: Diabetologia 33:152-157, 1990.

Yoneda Y, S Yamamoto. Cerebral cortical laminar necrosis on diffusion-weighted MRI in hypoglycaemic encephalopathy: Diabet.Med. 22:1098-1100, 2005.

References for Chapter 3

The DCCT Research Group. Epidemiology of severe hypoglycemia in the diabetes control and complications trial. Am.J.Med. 90:450-459, 1991

The DCCT Research Group. Effects of intensive diabetes therapy on neuropsychological function in adults in the Diabetes Control and Complications Trial: Ann.Intern.Med. 124:379-388, 1996.

Ack,M, I Miller, W B Weil. Intelligence of children with diabetes mellitus. Pediatrics 28:764-770, 1961.

Alvarez,M, J Rovet. Attentional functioning in children and adolescents with IDDM. Diabetes Care 20:803-810, 1997.

Austin,EJ, I J Deary. Effects of repeated hypoglycemia on cognitive function: a psychometrically validated reanalysis of the Diabetes Control and Complications Trial data: Diabetes Care 22:1273-1277, 1999.

Bjorgaas,M, R Gimse, T Vik, T Sand. Cognitive function in type 1 diabetic children with and without episodes of severe hypoglycaemia: Acta Paediatr. 86:148-153, 1997.

Cameron,FJ, M J Kean, R M Wellard, G A Werther, J J Neil, T E Inder. Insights into the acute cerebral metabolic changes associated with childhood diabetes: Diabet.Med. 22:648-653, 2005.

Christiaens,FJ, L D Mewasingh, C Christophe, S Goldman, B Dan. Unilateral cortical necrosis following status epilepticus with hypoglycemia: Brain Dev. 25:107-112, 2003.

Daneman,D, M Frank, K Perlman, J Tamm, R Ehrlich. Severe hypoglycemia in children with insulin-dependent diabetes mellitus: frequency and predisposing factors: J.Pediatr. 115:681-685, 1989.

Davis,EA, T W Jones. Hypoglycemia in children with diabetes: incidence, counterregulation and cognitive dysfunction: J.Pediatr.Endocrinol.Metab. 11(Suppl 1)177-182, 1998.

Durston,S, H E Hulshoff Pol, B J Casey, J N Giedd, J K Buitelaar, H van Engeland. Anatomical MRI of the developing human brain: what have we learned?: J.Am.Acad.Child Adolesc.Psychiatry 40:1012-1020, 2001.

Edge,JA, M E Ford-Adams, D B Dunger. Causes of death in children with insulin dependent diabetes 1990-96: Arch.Dis.Child. 81:318-323, 1999.

Edge,JA, M M Hawkins, D L Winter, D B Dunger. The risk and outcome of cerebral oedema developing during diabetic ketoacidosis: Arch.Dis.Child. 85:16-22, 2001.

Edge,JA, R W Jakes, Y Roy, M Hawkins, D Winter, M E Ford-Adams, N P Murphy, A Bergomi, B Widmer, D B Dunger. The UK case-control study of cerebral oedema complicating diabetic ketoacidosis in children: Diabetologia 49:2002-2009, 2006b.

Edge,JA, Y Roy, A Bergomi, N P Murphy, M E Ford-Adams, K K Ong, D B Dunger. Conscious level in children with diabetic ketoacidosis is related to severity of acidosis and not to blood glucose concentration: Pediatr.Diabetes 7:11-15, 2006a.

Fallstrom,K, On the personality structure in diabetic schoolchildren aged 7-15 years: Acta Paediatr.Scand.Suppl. 1-71, 1974.

Figueroa,RE, W H Hoffman, Z Momin, A Pancholy, G G Passmore, J Allison. Study of subclinical cerebral edema in diabetic ketoacidosis by magnetic resonance imaging T2 relaxometry and apparent diffusion coefficient maps: Endocr.Res. 31:345-355, 2005.

Glaser,NS, S L Wootton-Gorges, M H Buonocore, J P Marcin, A Rewers, J Strain, J DiCarlo, E K Neely, P Barnes, N Kuppermann. Frequency of sub-clinical cerebral edema in children with diabetic ketoacidosis: Pediatr.Diabetes 7:75-80, 2006.

Glaser,NS, S L Wootton-Gorges, J P Marcin, M H Buonocore, J DiCarlo, E K Neely, P Barnes, J Bottomly, N Kuppermann. Mechanism of cerebral edema in children with diabetic ketoacidosis: J.Pediatr. 145:164-171, 2004.

Golden,MP, B P Russell, G M Ingersoll, D L Gray, K M Hummer. Management of diabetes mellitus in children younger than 5 years of age: Am.J.Dis.Child. 139:448-452, 1985.

Greene,SA, I G Jefferson, J D Baum. Cerebral oedema complicating diabetic ketoacidosis: Dev.Med.Child Neurol. 32:633-638, 1990.

Hagen,JW, C R Barclay, B J Anderson, D J Feeman, S S Segal, G Bacon, G W Goldstein. Intellective functioning and strategy use in children with insulin-dependent diabetes mellitus: Child Dev. 61:1714-1727, 1990.

Hermoye,L, C Saint-Martin, G Cosnard, S K Lee, J Kim, M C Nassogne, R Menten, P Clapuyt, P K Donohue, K Hua, S Wakana, H Jiang, P C van Zijl, S Mori. Pediatric diffusion tensor imaging: normal database and observation of the white matter maturation in early childhood: Neuroimage. 29:493-504, 2006.

Hershey,T, N Bhargava, M Sadler, N H White, S Craft. Conventional versus intensive diabetes therapy in children with type 1 diabetes: effects on memory and motor speed: Diabetes Care 22:1318-1324, 1999.

Hershey,T, S Craft, N Bhargava, N H White. Memory and insulin dependent diabetes mellitus (IDDM): effects of childhood onset and severe hypoglycemia: J.Int.Neuropsychol.Soc. 3:509-520, 1997.

Hershey,T, R Lillie, M Sadler, N H White. A prospective study of severe hypoglycemia and long-term spatial memory in children with type 1 diabetes: Pediatr.Diabetes 5:63-71, 2004.

Hoffman,WH, M F Casanova, J A Bauza, G G Passmore, E A Sekul. Computer analysis of magnetic resonance imaging of the brain in children and adolescents after treatment of diabetic ketoacidosis: J.Diabetes Complications 13:176-181, 1999.

Hoffman,WH, C M Steinhart, T el Gammal, S Steele, A R Cuadrado, P K Morse. Cranial CT in children and adolescents with diabetic ketoacidosis: AJNR Am.J.Neuroradiol. 9:733-739, 1988.

Holmes,CS, L C Richman. Cognitive profiles of children with insulin-dependent diabetes: J.Dev.Behav.Pediatr. 6:323-326, 1985.

Jacobson,AM, G Musen, C M Ryan, N Silvers, P Cleary, B Waberski, A Burwood, K Weinger, M Bayless, W Dahms, J Harth. Long-term effect of diabetes and its treatment on cognitive function: N.Engl.J.Med. 356:1842-1852, 2007.

Kaufman,FR, K Epport, R Engilman, M Halvorson. Neurocognitive functioning in children diagnosed with diabetes before age 10 years: J.Diabetes Complications 13:31-38, 1999.

Keane,S, A Gallagher, S Ackroyd, M A McShane, J A Edge. Cerebral venous thrombosis during diabetic ketoacidosis: Arch.Dis.Child. 86:204-205, 2002.

Krane,EJ, M A Rockoff, J K Wallman, J I Wolfsdorf. Subclinical brain swelling in children during treatment of diabetic ketoacidosis: N.Engl.J.Med. 312:1147-1151, 1985.

Lebrun,C, P Thomas, M Chatel, J L Sadoul. Acute peduncular hemiplegia following diabetic ketoacidosis in a 12-year-old boy: Diabetes Care 17:624-625, 1994.

Lenroot,RK, J N Giedd. Brain development in children and adolescents: insights from anatomical magnetic resonance imaging: Neurosci.Biobehav.Rev. 30:718-729, 2006.

McAloon,J, D Carson, P Crean. Cerebral oedema complicating diabetic ketoacidosis: Acta Paediatr.Scand. 79:115-117, 1990.

McCarthy,AM, S Lindgren, M A Mengeling, E Tsalikian, J Engvall. Factors associated with academic achievement in children with type 1 diabetes: Diabetes Care 26:112-117, 2003.

McCarthy,AM, S Lindgren, M A Mengeling, E Tsalikian, J C Engvall. Effects of diabetes on learning in children: Pediatrics 109:E9, 2002.

Northam,EA, P J Anderson, R Jacobs, M Hughes, G L Warne, G A Werther. Neuropsychological profiles of children with type 1 diabetes 6 years after disease onset. Diabetes Care 24:1541-1546, 2001.

Northam,EA, P J Anderson, G A Werther, G L Warne, R G Adler, D Andrewes. Neuropsychological complications of IDDM in children 2 years after disease onset. Diabetes Care 21:379-384, 1998.

Parker,H, P G Swift, J L Botha, N T Raymond. Early onset diabetes: parents' views: Diabet.Med. 11:593-596, 1994.

Reichard,P, A Britz, U Rosenqvist. Intensified conventional insulin treatment and neuropsychological impairment: BMJ 303:1439-1442, 1991.

Roe,TF, T O Crawford, K R Huff, G Costin, F R Kaufman, M D Nelson, Jr. Brain infarction in children with diabetic ketoacidosis: J.Diabetes Complications 10:100-108, 1996.

Rogers,B, I Sills, M Cohen, F G Seidel. Diabetic ketoacidosis. Neurologic collapse during treatment followed by severe developmental morbidity: Clin.Pediatr.(Phila) 29:451-456, 1990.

Rovet,J, R Ehrlich, M Hoppe. Behaviour problems in children with diabetes as a function of sex and age of onset of disease: J.Child Psychol.Psychiatry 28:477-491, 1987b.

Rovet,JF, R M Ehrlich. The effect of hypoglycemic seizures on cognitive function in children with diabetes: a 7-year prospective study: J.Pediatr. 134:503-506, 1999.

Rovet,JF, R M Ehrlich, D Czuchta, M Akler. Psychoeducational characteristics of children and adolescents with insulin-dependent diabetes mellitus: J.Learn.Disabil. 26:7-22, 1993.

Rovet,JF, R M Ehrlich, M Hoppe. Intellectual deficits associated with early onset of insulin-dependent diabetes mellitus in children: Diabetes Care 10:510-515, 1987a.

Ryan,C, C Longstreet, L Morrow. The effects of diabetes mellitus on the school attendance and school achievement of adolescents: Child Care Health Dev. 11:229-240, 1985b.

Ryan,C, A Vega, A Drash. Cognitive deficits in adolescents who developed diabetes early in life: Pediatrics 75:921-927, 1985a.

Schoenle,EJ, D Schoenle, L Molinari, R H Largo. Impaired intellectual development in children with Type I diabetes: association with HbA1 c, age at diagnosis and sex: Diabetologia 45:108-114, 2002.

Shrier,DA, D K Shibata, H Z Wang, Y Numaguchi, J M Powers. Central brain herniation secondary to juvenile diabetic ketoacidosis: AJNR Am.J.Neuroradiol. 20:1885-1888, 1999.

Strudwick,SK, C Carne, J Gardiner, J K Foster, E A Davis, T W Jones. Cognitive functioning in children with early onset type 1 diabetes and severe hypoglycemia: J.Pediatr. 147:680-685, 2005.

Ternand,C, V L Go, J E Gerich, M W Haymond. Endocrine pancreatic response of children with onset of insulin-requiring diabetes before age 3 and after age 5: J.Pediatr. 101:36-39, 1982.

The DCCT Research Group. Hypoglycemia in the Diabetes Control and Complications Trial. Diabetes 46, 271-286, 1997.

Tranel,D, P J Eslinger. Effects of early onset brain injury on the development of cognition and behavior: introduction to the special issue: Dev.Neuropsychol. 18:273-280, 2000.

Vannucci,RC, S J Vannucci. Hypoglycemic brain injury: Semin.Neonatol. 6:147-155, 2001.

Wolters,CA, S L Yu, J W Hagen, R Kail. Short-term memory and strategy use in children with insulin-dependent diabetes mellitus: J.Consult Clin.Psychol. 64:1397-1405, 1996.

Wysocki,T, M A Harris, N Mauras, L Fox, A Taylor, S C Jackson, N H White. Absence of adverse effects of severe hypoglycemia on cognitive function in school-aged children with diabetes over 18 months: Diabetes Care 26:1100-1105, 2003.

References for Chapter 4

Early Treatment Diabetic Retinopathy Study Research Group, 1991, Grading diabetic retinopathy from stereoscopic color fundus photographs--an extension of the modified Airlie House classification. ETDRS report number 10: Ophthalmology 98: 786-806.

The Diabetes Control and Complications Stduy Group, 1995, Effect of intensive diabetes management on macrovascular events and risk factors in the Diabetes Control and Complications Trial: Am.J.Cardiol., v. 75, p. 894-903.

The Diabetes Control and Complications Stduy Group, 1998, The absence of a glycemic threshold for the development of long-term complications: the perspective of the Diabetes Control and Complications Trial: Diabetes, v. 45, p. 1289-1298.

The Diabetes Control and Complications Stduy Group. 1998, Early worsening of diabetic retinopathy in the Diabetes Control and Complications Trial: Arch.Ophthalmol., v. 116, p. 874-886.

The Diabetes Control and Complications Stduy Group, 2000b, Effect of pregnancy on microvascular complications in the diabetes control and complications trial. The Diabetes Control and Complications Trial Research Group: Diabetes Care, v. 23, p. 1084-1091.

The Diabetes Control and Complications Stduy Group, 2000a, Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group: N.Engl.J.Med., v. 342, p. 381-389.

The Diabetes Control and Complications Stduy Group, 2002, Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus: JAMA, v. 287, p. 2563-2569.

Alex,M, E K Baron, S Goldenberg, H T Blumenthal, 1962, An autopsy study of cerebrovascular accident in diabetes mellitus: Circulation, v. 25, p. 663-673.

Bellassoued,M, C Mhiri, C Triki, M Abid, 2001, [Hemichorea caused by striatal infarct in a young type 1 diabetic patient]: Rev.Neurol.(Paris), v. 157, p. 1287-1289.

Chase,HP, S K Garg, G Marshall, C L Berg, S Harris, W E Jackson, R E Hamman, 1991, Cigarette smoking increases the risk of albuminuria among subjects with type I diabetes: JAMA, v. 265, p. 614-617.

Chaturvedi,N, A K Sjoelie, M Porta, S J Aldington, J H Fuller, M Songini, E M Kohner, 2001, Markers of insulin resistance are strong risk factors for retinopathy incidence in type 1 diabetes: Diabetes Care, v. 24, p. 284-289.

Chaturvedi,N, A K Sjolie, J M Stephenson, H Abrahamian, M Keipes, A Castellarin, Z Rogulja-Pepeonik, J H Fuller, 1998, Effect of lisinopril on progression of retinopathy in normotensive people with type 1 diabetes. The EUCLID Study Group. EURODIAB Controlled Trial of Lisinopril in Insulin-Dependent Diabetes Mellitus: Lancet, v. 351, p. 28-31.

Chung,SS, S K Chung, 2005, Aldose reductase in diabetic microvascular complications: Curr.Drug Targets., v. 6, p. 475-486.

Corder,EH, A M Saunders, W J Strittmatter, D E Schmechel, P C Gaskell, G W Small, A D Roses, J L Haines, M A Pericak-Vance, 1993, Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families: Science, v. 261, p. 921-923.

Crawford,FC, R D Vanderploeg, M J Freeman, S Singh, M Waisman, L Michaels, L Abdullah, D Warden, R Lipsky, A Salazar, M J Mullan, 2002, APOE genotype influences acquisition and recall following traumatic brain injury: Neurology, v. 58, p. 1115-1118.

Davis,TM, D G Bruce, W A Davis, 2005, Predictors of first stroke in Type 1 diabetes: The Fremantle Diabetes Study: Diabet.Med., v. 22, p. 551-553.

De Leeuw,FE, J C De Groot, E Achten, M Oudkerk, L M Ramos, R Heijboer, A Hofman, J Jolles, J van Gijn, M M Breteler, 2001, Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study: J.Neurol.Neurosurg.Psychiatry, v. 70, p. 9-14.

De Leeuw,FE, F Richard, J C De Groot, C M van Duijn, A Hofman, J van Gijn, M M Breteler, 2004, Interaction between hypertension, apoE, and cerebral white matter lesions: Stroke, v. 35, p. 1057-1060.

Dejgaard,A, A Gade, H Larsson, V Balle, A Parving, H H Parving, 1991b, Evidence for diabetic encephalopathy: Diabet.Med., v. 8, p. 162-167.

Dejgaard,A, A Gade, H Larsson, V Balle, A Parving, H H Parving, 1991a, Evidence for diabetic encephalopathy: Diabet.Med., v. 8, p. 162-167.

DeJong,RN, 1977, CNS manifestations of diabetes mellitus: Postgrad.Med., v. 61, p. 101-107.

Dodson,PM, G Y Lip, S M Eames, J M Gibson, D G Beevers, 1996, Hypertensive retinopathy: a review of existing classification systems and a suggestion for a simplified grading system: J.Hum.Hypertens., v. 10, p. 93-98.

Dufouil,C, A Kersaint-Gilly, V Besancon, C Levy, E Auffray, L Brunnereau, A Alperovitch, C Tzourio, 2001, Longitudinal study of blood pressure and white matter hyperintensities: the EVA MRI Cohort: Neurology, v. 56, p. 921-926.

Englund,E, 2002, Neuropathology of white matter lesions in vascular cognitive impairment: Cerebrovasc.Dis., v. 13 Suppl 2, p. 11-15.

Fazekas,F, F Barkhof, L O Wahlund, L Pantoni, T Erkinjuntti, P Scheltens, R Schmidt, 2002, CT and MRI rating of white matter lesions: Cerebrovasc.Dis., v. 13 Suppl 2, p. 31-36.

Fazekas,F, R Kleinert, H Offenbacher, F Payer, R Schmidt, G Kleinert, H Radner, H Lechner, 1991, The morphologic correlate of incidental punctate white matter hyperintensities on MR images: AJNR Am.J.Neuroradiol., v. 12, p. 915-921.

Flory,JD, S B Manuck, R E Ferrell, C M Ryan, M F Muldoon, 2000, Memory performance and the apolipoprotein E polymorphism in a community sample of middle-aged adults: Am.J.Med.Genet., v. 96, p. 707-711.

Fuller,JH, L K Stevens, S L Wang, 2001, Risk factors for cardiovascular mortality and morbidity: the WHO Mutinational Study of Vascular Disease in Diabetes: Diabetologia, v. 44 Suppl 2, p. S54-S64.

Genuth,S, W Sun, P Cleary, D R Sell, W Dahms, J Malone, W Sivitz, V M Monnier, 2005, Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes: Diabetes, v. 54, p. 3103-3111.

Giorgino,F, L Laviola, P P Cavallo, B Solnica, J Fuller, N Chaturvedi, 2004, Factors associated with progression to macroalbuminuria in microalbuminuric Type 1 diabetic patients: the EURODIAB Prospective Complications Study: Diabetologia, v. 47, p. 1020-1028.

Grunnet,M. Cerebrovascular disease: diabetes and cerebral atherosclerosis. Neurology 13, 486-491. 1963.

Ikram,MK, F J De Jong, E J Van Dijk, N D Prins, A Hofman, M M Breteler, P T de Jong, 2006, Retinal vessel diameters and cerebral small vessel disease: the Rotterdam Scan Study: Brain, v. 129, p. 182-188.

Inoue,T, H Fushimi, Y Yamada, F Udaka, M Kameyama, 1996, Asymptomatic multiple lacunae in diabetics and non-diabetics detected by brain magnetic resonance imaging: Diabetes Res.Clin.Pract., v. 31, p. 81-86.

Jacobson,AM, G Musen, C M Ryan, N Silvers, P Cleary, B Waberski, A Burwood, K Weinger, M Bayless, W Dahms, J Harth, 2007, Long-term effect of diabetes and its treatment on cognitive function: N.Engl.J.Med., v. 356, p. 1842-1852.

Jordan,BD, N R Relkin, L D Ravdin, A R Jacobs, A Bennett, S Gandy, 1997, Apolipoprotein E epsilon4 associated with chronic traumatic brain injury in boxing: JAMA, v. 278, p. 136-140.

Karamanos,B, M Porta, M Songini, Z Metelko, Z Kerenyi, G Tamas, R Rottiers, L K Stevens, J H Fuller, 2000, Different risk factors of microangiopathy in patients with type I diabetes mellitus of short versus long duration. The EURODIAB IDDM Complications Study: Diabetologia, v. 43, p. 348-355.

Klein,BE, R Klein, P E McBride, K J Cruickshanks, M Palta, M D Knudtson, S E Moss, J O Reinke, 2004, Cardiovascular disease, mortality, and retinal microvascular characteristics in type 1 diabetes: Wisconsin epidemiologic study of diabetic retinopathy: Arch.Intern.Med., v. 164, p. 1917-1924.

Klein,R, B E Klein, S E Moss, T Y Wong, L Hubbard, K J Cruickshanks, M Palta, 2003, Retinal vascular abnormalities in persons with type 1 diabetes: the Wisconsin Epidemiologic Study of Diabetic Retinopathy: XVIII: Ophthalmology, v. 110, p. 2118-2125.

Knopman,DS, T H Mosley, D J Catellier, A R Sharrett, 2005, Cardiovascular risk factors and cerebral atrophy in a middle-aged cohort: Neurology, v. 65, p. 876-881.

Krolewski,AS, A Czyzyk, D Janeczko, J Kopczynski, 1977, Mortality from cardiovascular diseases among diabetics: Diabetologia, v. 13, p. 345-350.

Kruit,MC, M A van Buchem, P A Hofman, J T Bakkers, G M Terwindt, M D Ferrari, L J Launer, 2004, Migraine as a risk factor for subclinical brain lesions: JAMA, v. 291, p. 427-434.

Kutner,KC, D M Erlanger, J Tsai, B Jordan, N R Relkin, 2000, Lower cognitive performance of older football players possessing apolipoprotein E epsilon4: Neurosurgery, v. 47, p. 651-657.

Laing,SP, A J Swerdlow, L M Carpenter, S D Slater, A C Burden, J L Botha, A D Morris, N R Waugh, W Gatling, E A Gale, C C Patterson, Z Qiao, H Keen, 2003, Mortality from cerebrovascular disease in a cohort of 23 000 patients with insulin-treated diabetes: Stroke, v. 34, p. 418-421.

Launer,LJ, 2004, Epidemiology of white matter lesions: Top.Magn Reson.Imaging, v. 15, p. 365-367.

Lawson,JS, D L Erdahl, T N Monga, C E Bird, M W Donald, D H Surridge, F J Letemendia, 1984, Neuropsychological function in diabetic patients with neuropathy: Br.J.Psychiatry, v. 145, p. 263-268.

Lehr,HA, 2000, Microcirculatory dysfunction induced by cigarette smoking: Microcirculation., v. 7, p. 367-384.

Leiter,LA, 2005, The prevention of diabetic microvascular complications of diabetes: is there a role for lipid lowering?: Diabetes Res.Clin.Pract., v. 68 Suppl 2, p. S3-14.

Liao,D, L Cooper, J Cai, J Toole, N Bryan, G Burke, E Shahar, J Nieto, T Mosley, G Heiss, 1997, The prevalence and severity of white matter lesions, their relationship with age, ethnicity, gender, and cardiovascular disease risk factors: the ARIC Study: Neuroepidemiology, v. 16, p. 149-162.

Liao,D, L Cooper, J Cai, J F Toole, N R Bryan, R G Hutchinson, H A Tyroler, 1996, Presence and severity of cerebral white matter lesions and hypertension, its treatment, and its control. The ARIC Study. Atherosclerosis Risk in Communities Study: Stroke, v. 27, p. 2262-2270.

Longstreth,W, Jr., E K Larsen, R Klein, T Y Wong, A R Sharrett, D Lefkowitz, T A Manolio, 2007, Associations between findings on cranial magnetic resonance imaging and retinal photography in the elderly: the Cardiovascular Health Study: Am.J.Epidemiol., v. 165, p. 78-84.

Longstreth,WT, Jr., A M Arnold, T A Manolio, G L Burke, N Bryan, C A Jungreis, D O'Leary, P L Enright, L Fried, 2000, Clinical correlates of ventricular and sulcal size on cranial magnetic resonance imaging of 3,301 elderly people. The Cardiovascular Health Study. Collaborative Research Group: Neuroepidemiology, v. 19, p. 30-42.

Louko,AM, J Vilkki, T Niskakangas, 2006, ApoE genotype and cognition after subarachnoid haemorrhage: a longitudinal study: Acta Neurol.Scand., v. 114, p. 315-319.

Lunetta,M, A R Damanti, G Fabbri, M Lombardo, M Di Mauro, L Mughini, 1994, Evidence by magnetic resonance imaging of cerebral alterations of atrophy type in young insulin-dependent diabetic patients: J.Endocrinol.Invest, v. 17, p. 241-245.

Manolio,TA, G L Burke, D H O'Leary, G Evans, N Beauchamp, L Knepper, B Ward, 1999, Relationships of cerebral MRI findings to ultrasonographic carotid atherosclerosis in older adults : the Cardiovascular Health Study. CHS Collaborative Research Group: Arterioscler.Thromb.Vasc.Biol., v. 19, p. 356-365.

Mitchell,P, J J Wang, T Y Wong, W Smith, R Klein, S R Leeder, 2005, Retinal microvascular signs and risk of stroke and stroke mortality: Neurology, v. 65, p. 1005-1009.

Mungas,D, W J Jagust, B R Reed, J H Kramer, M W Weiner, N Schuff, D Norman, W J Mack, L Willis, H C Chui, 2001, MRI predictors of cognition in subcortical ischemic vascular disease and Alzheimer's disease: Neurology, v. 57, p. 2229-2235.

Nathan,DM, P A Cleary, J Y Backlund, S M Genuth, J M Lachin, T J Orchard, P Raskin, B Zinman, 2005, Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes: N.Engl.J.Med., v. 353, p. 2643-2653.

Nicholl,ID, A W Stitt, J E Moore, A J Ritchie, D B Archer, R Bucala, 1998, Increased levels of advanced glycation endproducts in the lenses and blood vessels of cigarette smokers: Mol.Med., v. 4, p. 594-601.

Orchard,TJ, J S Dorman, R E Maser, D J Becker, A L Drash, D Ellis, R E LaPorte, L H Kuller, 1990, Prevalence of complications in IDDM by sex and duration. Pittsburgh Epidemiology of Diabetes Complications Study II: Diabetes, v. 39, p. 1116-1124.

Orchard,TJ, K Y Forrest, D Ellis, D J Becker, 1997, Cumulative glycemic exposure and microvascular complications in insulin-dependent diabetes mellitus. The glycemic threshold revisited: Arch.Intern.Med., v. 157, p. 1851-1856.

Pantoni,L, J H Garcia, 1995, The significance of cerebral white matter abnormalities 100 years after Binswanger's report. A review: Stroke, v. 26, p. 1293-1301.

Pendleton,N, A Payton, E H van den Boogerd, F Holland, P Diggle, P M Rabbitt, M A Horan, J Worthington, W E Ollier, 2002, Apolipoprotein E genotype does not predict decline in intelligence in healthy older adults: Neurosci.Lett., v. 324, p. 74-76.

Pinholt,M, J L Frederiksen, M Christiansen, 2006, The association between apolipoprotein E and multiple sclerosis: Eur.J.Neurol., v. 13, p. 573-580.

Plomin,R, G E McClearn, D L Smith, S Vignetti, M J Chorney, K Chorney, C P Venditti, S Kasarda, L A Thompson, D K Detterman, ., 1994, DNA markers associated with high versus low IQ: the IQ Quantitative Trait Loci (QTL) Project: Behav.Genet., v. 24, p. 107-118.

Reichard,P, 1992, Risk factors for progression of microvascular complications in the Stockholm Diabetes Intervention Study (SDIS): Diabetes Res.Clin.Pract., v. 16, p. 151-156.

Reiman,EM, R J Caselli, K Chen, G E Alexander, D Bandy, J Frost, 2001, Declining brain activity in cognitively normal apolipoprotein E varepsilon 4 heterozygotes: A foundation for using positron emission tomography to efficiently test treatments to prevent Alzheimer's disease: Proc.Natl.Acad.Sci.U.S.A, v. 98, p. 3334-3339.

Reiman,EM, K Chen, G E Alexander, R J Caselli, D Bandy, D Osborne, A M Saunders, J Hardy, 2004, Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia: Proc.Natl.Acad.Sci.U.S.A, v. 101, p. 284-289.

Reiman,EM, K Chen, G E Alexander, R J Caselli, D Bandy, D Osborne, A M Saunders, J Hardy, 2005, Correlations between apolipoprotein E epsilon4 gene dose and brain-imaging measurements of regional hypometabolism: Proc.Natl.Acad.Sci.U.S.A, v. 102, p. 8299-8302.

Rennick PM, Wilder RM, Sargent J, Ashley BJ. Retinopathy as an indicator of cognitive-perceptual-motor impairment in diabetic adults. Proc 76th Ann Conv Am Psychol Assoc , 473-474. 1968.

Reske-Nielsen,E, K Lundbaek. Diabetic encephalopathy: diffuse and focal lesions of the brain in long-term diabetes. Acta Neurol.Scand. 39[Supp. 4], 273-290. 1963b.

Reske-Nielsen,E, K Lundbaek. Diabetic encephalopathy: diffuse and focal lesions of the brain in long-term diabetes. Acta Neurol.Scand. 39[Supp. 4], 273-290. 1963a.

Ryan,CM, T M Williams, D N Finegold, T J Orchard, 1993, Cognitive dysfunction in adults with type 1 (insulin-dependent) diabetes mellitus of long duration: effects of recurrent hypoglycaemia and other chronic complications: Diabetologia, v. 36, p. 329-334.

Ryan,CM, T M Williams, T J Orchard, D N Finegold, 1992, Psychomotor slowing is associated with distal symmetrical polyneuropathy in adults with diabetes mellitus: Diabetes, v. 41, p. 107-113.

Savitz,J, M Solms, R Ramesar, 2006, The molecular genetics of cognition: dopamine, COMT and BDNF: Genes Brain Behav., v. 5, p. 311-328.

Schalkwijk,CG, N Chaturvedi, H Twaafhoven, V W van Hinsbergh, C D Stehouwer, 2002, Amadori-albumin correlates with microvascular complications and precedes nephropathy in type 1 diabetic patients: Eur.J.Clin.Invest, v. 32, p. 500-506.

Schmidt,R, C Enzinger, S Ropele, H Schmidt, F Fazekas, 2003, Progression of cerebral white matter lesions: 6-year results of the Austrian Stroke Prevention Study: Lancet, v. 361, p. 2046-2048.

Schmidt,R, F Fazekas, G Kleinert, H Offenbacher, K Gindl, F Payer, W Freidl, K Niederkorn, H Lechner, 1992, Magnetic resonance imaging signal hyperintensities in the deep and subcortical white matter. A comparative study between stroke patients and normal volunteers: Arch.Neurol., v. 49, p. 825-827.

Schmidt,R, L J Launer, L G Nilsson, A Pajak, S Sans, K Berger, M M Breteler, M de Ridder, C Dufouil, R Fuhrer, S Giampaoli, A Hofman, 2004, Magnetic resonance imaging of the brain in diabetes: the Cardiovascular Determinants of Dementia (CASCADE) Study: Diabetes, v. 53, p. 687-692.

Seshadri,S, P A Wolf, A Beiser, M F Elias, R Au, C S Kase, R B D'Agostino, C DeCarli, 2004, Stroke risk profile, brain volume, and cognitive function: the Framingham Offspring Study: Neurology, v. 63, p. 1591-1599.

Silbert,LC, J F Quinn, M M Moore, E Corbridge, M J Ball, G Murdoch, G Sexton, J A Kaye, 2003, Changes in premorbid brain volume predict Alzheimer's disease pathology: Neurology, v. 61, p. 487-492.

Skenazy,JA, E D Bigler, 1984, Neuropsychological findings in diabetes mellitus: J.Clin.Psychol., v. 40, p. 246-258.

Small,GW, L M Ercoli, D H Silverman, S C Huang, S Komo, S Y Bookheimer, H Lavretsky, K Miller, P Siddarth, N L Rasgon, J C Mazziotta, S Saxena, H M Wu, M S Mega, J L Cummings, A M Saunders, M A Pericak-Vance, A D Roses, J R Barrio, M E Phelps, 2000, Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer's disease: Proc.Natl.Acad.Sci.U.S.A, v. 97, p. 6037-6042.

Smith,JD, 2000, Apolipoprotein E4: an allele associated with many diseases: Ann.Med., v. 32, p. 118-127.

Staehelin,HB, P Perrig-Chiello, C Mitrache, A R Miserez, W J Perrig, 1999, Apolipoprotein E genotypes and cognitive functions in healthy elderly persons: Acta Neurol.Scand., v. 100, p. 53-60.

Stettler,C, S Allemann, P Juni, C A Cull, R R Holman, M Egger, S Krahenbuhl, P Diem, 2006, Glycemic control and macrovascular disease in types 1 and 2 diabetes mellitus: Meta-analysis of randomized trials: Am.Heart J., v. 152, p. 27-38.

Sudlow,C, N A Martinez Gonzalez, J Kim, C Clark, 2006, Does apolipoprotein E genotype influence the risk of ischemic stroke, intracerebral hemorrhage, or subarachnoid hemorrhage? Systematic review and meta-analyses of 31 studies among 5961 cases and 17,965 controls: Stroke, v. 37, p. 364-370.

Teasdale,GM, J A Nicoll, G Murray, M Fiddes, 1997, Association of apolipoprotein E polymorphism with outcome after head injury: Lancet, v. 350, p. 1069-1071.

Thomas,MC, M Rosengard-Barlund, V Mills, M Ronnback, S Thomas, C Forsblom, M E Cooper, M R Taskinen, G Viberti, P H Groop, 2006, Serum lipids and the progression of nephropathy in type 1 diabetes: Diabetes Care, v. 29, p. 317-322.

Tooke,JE, A C Shore, R A Cohen, C Kluft, 1996, Diabetic angiopathy: tracking down the culprits: J.Diabetes Complications, v. 10, p. 173-181.

Turic,D, P J Fisher, R Plomin, M J Owen, 2001, No association between apolipoprotein E polymorphisms and general cognitive ability in children: Neurosci.Lett., v. 299, p. 97-100.

Wardlaw,JM, K J Ferguson, C Graham, 2004, White matter hyperintensities and rating scales-observer reliability varies with lesion load: J.Neurol., v. 251, p. 584-590.

Warpeha,KM, U Chakravarthy, 2003, Molecular genetics of microvascular disease in diabetic retinopathy: Eye, v. 17, p. 305-311.

Williams,ME, 2006, New potential agents in treating diabetic kidney disease: the fourth act: Drugs, v. 66, p. 2287-2298.

Wong,T, P Mitchell, 2007, The eye in hypertension: Lancet, v. 369, p. 425-435.

Wong,TY, R Klein, A R Sharrett, D J Couper, B E Klein, D P Liao, L D Hubbard, T H Mosley, 2002, Cerebral white matter lesions, retinopathy, and incident clinical stroke: JAMA, v. 288, p. 67-74.

Wong,TY, T H Mosley, Jr., R Klein, B E Klein, A R Sharrett, D J Couper, L D Hubbard, 2003, Retinal microvascular changes and MRI signs of cerebral atrophy in healthy, middle-aged people: Neurology, v. 61, p. 806-811.

Wright,AF, 2005, Neurogenetics II: complex disorders: J.Neurol.Neurosurg.Psychiatry, v. 76, p. 623-631.

Yousem,DM, W S Tasman, R I Grossman, 1991, Proliferative retinopathy: absence of white matter lesions at MR imaging: Radiology, v. 179, p. 229-230.

Yu,YW, C H Lin, S P Chen, C J Hong, S J Tsai, 2000, Intelligence and event-related potentials for young female human volunteer apolipoprotein E epsilon4 and non-epsilon4 carriers: Neurosci.Lett., v. 294, p. 179-181.

References for Chapter 5

Northam,EA, P J Anderson, R Jacobs, M Hughes, G L Warne, G A Werther. Neuropsychological profiles of children with type 1 diabetes 6 years after
disease onset. Diabetes Care 24[9], 1541-1546. 2001.

Ryan,CM, D J Becker, 1999, Hypoglycemia in children with type 1 diabetes mellitus. Risk factors, cognitive function, and management: Endocrinol.Metab Clin.North Am., v. 28, p. 883-900.

References for Chapter 6

Early Treatment Diabetic Retinopathy Study Research Group ,1991, Grading diabetic retinopathy from stereoscopic color fundus photographs--an extension of the modified Airlie House classification. ETDRS report number 10: Ophthalmology, v. 98, p. 786-806.

Alcott,D, R Swann, A Grafham, 1999, The effect of training on rater reliability on the scoring of the NART: Br.J.Clin.Psychol., v. 38 (Pt 4), p. 431-434.

Aldington,SJ, E M Kohner, S Meuer, R Klein, A K Sjolie, 1995, Methodology for retinal photography and assessment of diabetic retinopathy: the EURODIAB IDDM complications study: Diabetologia, v. 38, p. 437-444.

Borkowski,JG, A L Benton. Word fluency and brain damage. Neuropsychologia 5, 135-140. 1967.

Breteler,MM, N M van Amerongen, J C Van Swieten, J J Claus, D E Grobbee, J van Gijn, A Hofman, F van Harskamp, 1994b, Cognitive correlates of ventricular enlargement and cerebral white matter lesions on magnetic resonance imaging. The Rotterdam Study: Stroke, v. 25, p. 1109-1115.

Breteler,MM, J C Van Swieten, M L Bots, D E Grobbee, J J Claus, J H van den Hout, F van Harskamp, H L Tanghe, P T de Jong, J van Gijn, ., 1994a, Cerebral white matter lesions, vascular risk factors, and cognitive function in a population-based study: the Rotterdam Study: Neurology, v. 44, p. 1246-1252.

Cockburn,J, J Keene, T Hope, P Smith, 2000, Progressive decline in NART score with increasing dementia severity: J.Clin.Exp.Neuropsychol., v. 22, p. 508-517.

Crawford,JR, I J Deary, J Starr, L J Whalley, 2001, The NART as an index of prior intellectual functioning: a retrospective validity study covering a 66-year interval: Psychol.Med., v. 31, p. 451-458.

Deary I.J., S J Langan, D A Hepburn, B M Frier. Which abilities does the PASAT test? Pers.Indiv Diff 12, 983-987. 1991.

Deary,IJ, J R Crawford, D A Hepburn, S J Langan, L M Blackmore, B M Frier, 1993, Severe hypoglycemia and intelligence in adult patients with insulin- treated diabetes: Diabetes, v. 42, p. 341-344.

Fazekas,F, J B Chawluk, A Alavi, H I Hurtig, R A Zimmerman, 1987, MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging: AJR Am.J.Roentgenol., v. 149, p. 351-356.

Jensen,AR. Individual differences in the Hick paradigm. Vernon, P. A. Speed of Information Processing and Intelligence . 1987. NJ, Norwood, NJ, Ablex.

Langan,SJ, I J Deary, D A Hepburn, B M Frier, 1991, Cumulative cognitive impairment following recurrent severe hypoglycaemia in adult patients with insulin-treated diabetes mellitus: Diabetologia, v. 34, p. 337-344.

Lawrie,SM, H Whalley, J N Kestelman, S S Abukmeil, M Byrne, A Hodges, J E Rimmington, J J Best, D G Owens, E C Johnstone. Magnetic resonance imaging of brain in people at high risk of developing schizophrenia. Lancet 353[9146], 30-33. 1999.

Lezak,MD. Neuropsychological Assessment. [Fourth Edition], 1-1016. 2004. Oxford, Oxford University Press.

Longstreth,WT, Jr., T A Manolio, A Arnold, G L Burke, N Bryan, C A Jungreis, P L Enright, D O'Leary, L Fried, 1996, Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study: Stroke, v. 27, p. 1274-1282.

Mantyla,R, T Erkinjuntti, O Salonen, H J Aronen, T Peltonen, T Pohjasvaara, C G Standertskjold-Nordenstam, 1997, Variable agreement between visual rating scales for white matter hyperintensities on MRI. Comparison of 13 rating scales in a poststroke cohort: Stroke, v. 28, p. 1614-1623.

McCrimmon,RJ, Deary IJ, B J H Huntly, K J M MacLeod, B M Frier. Visual information processing during controlled hypoglycaemia in humans. Brain 119, 1277-1287. 1996.

Mirsen TR, Lee DH, Wong CJ, Diaz JF, Fox AJ, Hachinski VC, Merskey H. Clinical correlates of white-matter changes on magnetic resonance imaging scans
of the brain. Arch.Neurol. 48[10], 1015-1021. 1991.

Nelson,H, J R Willison. National Adult Reading Test (NART) Test Manual. 2nd ed. 1991. NFER-Nelson, Windsor, U.K.

O'Carroll,RE, 1987, The inter-rater reliability of the National Adult Reading Test (NART): a pilot study: Br.J.Clin.Psychol., v. 26 (Pt 3), p. 229-230.

Pantoni,L, J H Garcia, 1995, The significance of cerebral white matter abnormalities 100 years after Binswanger's report. A review: Stroke, v. 26, p. 1293-1301.

Perros,P, I J Deary, R J Sellar, J J Best, B M Frier, 1997, Brain abnormalities demonstrated by magnetic resonance imaging in adult IDDM patients with and without a history of recurrent severe hypoglycemia: Diabetes Care, v. 20, p. 1013-1018.

Perros,P, B M Frier, 1997, The long-term sequelae of severe hypoglycemia on the brain in insulin-dependent diabetes mellitus: Horm.Metab Res., v. 29, p. 197-202.

Shimada,K, A Kawamoto, K Matsubayashi, T Ozawa, 1990, Silent cerebrovascular disease in the elderly. Correlation with ambulatory pressure: Hypertension, v. 16, p. 692-699.

The DCCT Research Group. Hypoglycemia in the Diabetes Control and Complications Trial. Diabetes 46, 271-286. 1997.

Van Swieten,JC, A Hijdra, P J Koudstaal, J van Gijn, 1990, Grading white matter lesions on CT and MRI: a simple scale: J.Neurol.Neurosurg.Psychiatry, v. 53, p. 1080-1083.

Wahlund,LO, I Agartz, O Almqvist, H Basun, L Forssell, J Saaf, L Wetterberg, 1990, The brain in healthy aged individuals: MR imaging: Radiology, v. 174, p. 675-679.

Wechsler,D. Manual for the Wechsler adult intelligence scale-revised. 1981. Psychological Corporation, New York.

Wenham,PR, W H Price, G Blandell, 1991, Apolipoprotein E genotyping by one-stage PCR: Lancet, v. 337, p. 1158-1159.

Zigmond,AS, R P Snaith. The Hospital Anxiety and Depression Scale. Acta Psychiatr Scand 67, 361-370. 1983.

References for Chapter 7

Early Treatment Diabetic Retinopathy Study Research Group, 1991, Grading diabetic retinopathy from stereoscopic color fundus photographs--an extension of the modified Airlie House classification: ETDRS report number 10. Ophthalmology, v. 98, p. 786-806.

The Diabetes Control and Complications Stduy Group,1995, Effect of intensive diabetes management on macrovascular events and risk factors in the Diabetes Control and Complications Trial: Am.J.Cardiol., v. 75, p. 894-903.

The Diabetes Control and Complications Stduy Group, 1996, Effects of intensive diabetes therapy on neuropsychological function in adults in the Diabetes Control and Complications Trial: Ann.Intern.Med., v. 124, p. 379-388.

The Diabetes Control and Complications Stduy Group, 1997, Hypoglycemia in the Diabetes Control and Complications Trial. The Diabetes Control and Complications Trial Research Group: Diabetes, v. 46, p. 271-286.

Ack,M, I Miller, W B Weil. Intelligence of children with diabetes mellitus. Pediatrics 28, 764-770. 1961.

Alvarez,M, J Rovet. Attentional functioning in children and adolescents with IDDM. Diabetes Care 2:803-810. 1997.

Auer,RN, B K Siesjo, 1993, Hypoglycaemia: brain neurochemistry and neuropathology: Baillieres Clin.Endocrinol.Metab, v. 7, p. 611-625.

Austin,EJ, I J Deary, 1999, Effects of repeated hypoglycemia on cognitive function: a psychometrically validated reanalysis of the Diabetes Control and Complications Trial data: Diabetes Care, v. 22, p. 1273-1277.

Bakshi,R, M F Morcos, T F Gabryel, P Dandona, 2000, Is fluid-attenuated inversion recovery MRI more sensitive than conventional MRI for hypoglycemic brain injury?: Neurology, v. 55, p. 1064-1065.

Bjorgaas,M, R Gimse, T Vik, T Sand, 1997, Cognitive function in type 1 diabetic children with and without episodes of severe hypoglycaemia: Acta Paediatr., v. 86, p. 148-153.

Boeve,BF, D G Bell, J H Noseworthy, 1995, Bilateral temporal lobe MRI changes in uncomplicated hypoglycemic coma: Can.J.Neurol.Sci., v. 22, p. 56-58.

Borkowski,JG, A L Benton. Word fluency and brain damage. Neuropsychologia 5, 135-140. 1967.

Breteler,MM, N M van Amerongen, J C Van Swieten, J J Claus, D E Grobbee, J van Gijn, A Hofman, F van Harskamp, 1994b, Cognitive correlates of ventricular enlargement and cerebral white matter lesions on magnetic resonance imaging. The Rotterdam Study: Stroke, v. 25, p. 1109-1115.

Breteler,MM, J C Van Swieten, M L Bots, D E Grobbee, J J Claus, J H van den Hout, F van Harskamp, H L Tanghe, P T de Jong, J van Gijn, ., 1994a, Cerebral white matter lesions, vascular risk factors, and cognitive function in a population-based study: the Rotterdam Study: Neurology, v. 44, p. 1246-1252.

Chabriat,H, C Sachon, M Levasseur, A Grimaldi, S Pappata, D Rougemont, M C Masure, A De Recondo, Y Samson, 1994, Brain metabolism after recurrent insulin induced hypoglycaemic episodes: a PET study: J.Neurol.Neurosurg.Psychiatry, v. 57, p. 1360-1365.

Chalmers,J, M T Risk, D M Kean, R Grant, B Ashworth, I W Campbell, 1991, Severe amnesia after hypoglycemia. Clinical, psychometric, and magnetic resonance imaging correlations: Diabetes Care, v. 14, p. 922-925.

Chan,R, S Erbay, S Oljeski, D Thaler, R Bhadelia, 2003, Case report: hypoglycemia and diffusion-weighted imaging: J.Comput.Assist.Tomogr., v. 27, p. 420-423.

Deary I.J., S J Langan, D A Hepburn, B M Frier. Which abilities does the PASAT test? Pers.Indiv Diff 12, 983-987. 1991.

Deary,IJ, J R Crawford, D A Hepburn, S J Langan, L M Blackmore, B M Frier, 1993, Severe hypoglycemia and intelligence in adult patients with insulin- treated diabetes: Diabetes, v. 42, p. 341-344.

Dejgaard,A, A Gade, H Larsson, V Balle, A Parving, H H Parving, 1991, Evidence for diabetic encephalopathy: Diabet.Med., v. 8, p. 162-167.

Fazekas,F, J B Chawluk, A Alavi, H I Hurtig, R A Zimmerman, 1987, MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging: AJR Am.J.Roentgenol., v. 149, p. 351-356.

Finelli,PF, 2001, Diffusion-weighted MR in hypoglycemic coma: Neurology, v. 57, p. 933.

Gold,AE, S M Marshall, 1996, Cortical blindness and cerebral infarction associated with severe hypoglycemia: Diabetes Care, v. 19, p. 1001-1003.

Grunnet,M. Cerebrovascular disease: diabetes and cerebral atherosclerosis. Neurology 13, 486-491. 1963.

Hershey,T, R Lillie, M Sadler, N H White, 2003, Severe hypoglycemia and long-term spatial memory in children with type 1 diabetes mellitus: a retrospective study: J.Int.Neuropsychol.Soc., v. 9, p. 740-750.

Hershey,T, D C Perantie, S L Warren, E C Zimmerman, M Sadler, N H White, 2005, Frequency and timing of severe hypoglycemia affects spatial memory in children with type 1 diabetes: Diabetes Care, v. 28, p. 2372-2377.

Holemans,X, M Dupuis, N Misson, J F Vanderijst, 2001, Reversible amnesia in a Type 1 diabetic patient and bilateral hippocampal lesions on magnetic resonance imaging (MRI): Diabet.Med., v. 18, p. 761-763.

Holmes,CS, L C Richman, 1985, Cognitive profiles of children with insulin-dependent diabetes: J.Dev.Behav.Pediatr., v. 6, p. 323-326.

Jensen,AR. Individual differences in the Hick paradigm. Vernon, P. A. Speed of Information Processing and Intelligence . 1987. NJ, Norwood, NJ, Ablex.

Klein,BE, R Klein, P E McBride, K J Cruickshanks, M Palta, M D Knudtson, S E Moss, J O Reinke, 2004, Cardiovascular disease, mortality, and retinal microvascular characteristics in type 1 diabetes: Wisconsin epidemiologic study of diabetic retinopathy: Arch.Intern.Med., v. 164, p. 1917-1924.

Koppel,BS, M Daras. Transient Hypodensity on CT Scan during Hypoglycemia. Eur Neurol 33, 80-82. 1993.

Laing,SP, A J Swerdlow, L M Carpenter, S D Slater, A C Burden, J L Botha, A D Morris, N R Waugh, W Gatling, E A Gale, C C Patterson, Z Qiao, H Keen, 2003, Mortality from cerebrovascular disease in a cohort of 23 000 patients with insulin-treated diabetes: Stroke, v. 34, p. 418-421.

Langan,SJ, I J Deary, D A Hepburn, B M Frier, 1991, Cumulative cognitive impairment following recurrent severe hypoglycaemia in adult patients with insulin-treated diabetes mellitus: Diabetologia, v. 34, p. 337-344.

Lawrie,SM, H Whalley, J N Kestelman, S S Abukmeil, M Byrne, A Hodges, J E Rimmington, J J Best, D G Owens, E C Johnstone. Magnetic resonance imaging of brain in people at high risk of developing schizophrenia. Lancet 353[9146], 30-33. 1999.

Lincoln,NB, R M Faleiro, C Kelly, B A Kirk, W J Jeffcoate, 1996, Effect of long-term glycemic control on cognitive function: Diabetes Care, v. 19, p. 656-658.

Longstreth,WT, Jr., T A Manolio, A Arnold, G L Burke, N Bryan, C A Jungreis, P L Enright, D O'Leary, L Fried, 1996, Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study: Stroke, v. 27, p. 1274-1282.

Lunetta,M, A R Damanti, G Fabbri, M Lombardo, M Di Mauro, L Mughini, 1994, Evidence by magnetic resonance imaging of cerebral alterations of atrophy type in young insulin-dependent diabetic patients: J.Endocrinol.Invest, v. 17, p. 241-245.

Maekawa,S, M Aibiki, K Kikuchi, S Kikuchi, K Umakoshi, 2006, Time related changes in reversible MRI findings after prolonged hypoglycemia: Clin.Neurol.Neurosurg., v. 108, p. 511-513.

Mantyla,R, T Erkinjuntti, O Salonen, H J Aronen, T Peltonen, T Pohjasvaara, C G Standertskjold-Nordenstam, 1997, Variable agreement between visual rating scales for white matter hyperintensities on MRI. Comparison of 13 rating scales in a poststroke cohort: Stroke, v. 28, p. 1614-1623.

McCall,AL, 1992, The impact of diabetes on the CNS: Diabetes, v. 41, p. 557-570.

McCrimmon,RJ, Deary IJ, B J H Huntly, K J M MacLeod, B M Frier. Visual information processing during controlled hypoglycaemia in humans. Brain 119, 1277-1287. 1996.

Mirsen TR, Lee DH, Wong CJ, Diaz JF, Fox AJ, Hachinski VC, Merskey H. Clinical correlates of white-matter changes on magnetic resonance imaging scans
of the brain. Arch.Neurol. 48[10], 1015-1021. 1991.

Nathan,DM, P A Cleary, J Y Backlund, S M Genuth, J M Lachin, T J Orchard, P Raskin, B Zinman, 2005, Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes: N.Engl.J.Med., v. 353, p. 2643-2653.

Nelson,H, J R Willison. National Adult Reading Test (NART) Test Manual. 2nd ed. 1991. NFER-Nelson, Windsor, U.K.

Northam,EA, P J Anderson, R Jacobs, M Hughes, G L Warne, G A Werther. Neuropsychological profiles of children with type 1 diabetes 6 years after disease onset. Diabetes Care 24[9], 1541-1546. 2001.

Northam,EA, P J Anderson, G A Werther, G L Warne, R G Adler, D Andrewes. Neuropsychological complications of IDDM in children 2 years after disease onset. Diabetes Care 21, 379-384. 1998.

Northam,EA, P J Anderson, G A Werther, G L Warne, D Andrewes. Predictors of change in the neuropsychological profiles of children with type 1 diabetes 2 years after disease onset. Diabetes Care 22[9], 1438-1444. 1999.

Pantoni,L, J H Garcia, 1995, The significance of cerebral white matter abnormalities 100 years after Binswanger's report. A review: Stroke, v. 26, p. 1293-1301.

Perros,P, I J Deary, R J Sellar, J J Best, B M Frier, 1997, Brain abnormalities demonstrated by magnetic resonance imaging in adult IDDM patients with and without a history of recurrent severe hypoglycemia: Diabetes Care, v. 20, p. 1013-1018.

Perros,P, R J Sellar, B M Frier, 1994, Chronic pontine dysfunction following insulin-induced hypoglycemia in an IDDM patient: Diabetes Care, v. 17, p. 725-727.

Rajbhandari,SM, T Powell, G A Davies-Jones, J D Ward, 1998, Central pontine myelinolysis and ataxia: an unusual manifestation of hypoglycaemia: Diabet.Med., v. 15, p. 259-261.

Reichard,P, A Britz, U Rosenqvist, 1991, Intensified conventional insulin treatment and neuropsychological impairment: BMJ, v. 303, p. 1439-1442.

Reske-Nielsen,E, K Lundbaek. Diabetic encephalopathy: diffuse and focal lesions of the brain in long-term diabetes. Acta Neurol.Scand. 39[Supp. 4], 273-290. 1963.

Richardson,ML, R E Kinard, M B Gray, 1981, CT of generalized gray matter infarction due to hypoglycemia: AJNR Am.J.Neuroradiol., v. 2, p. 366-367.

Rovet,JF, R M Ehrlich, 1999, The effect of hypoglycemic seizures on cognitive function in children with diabetes: a 7-year prospective study: J.Pediatr., v. 134, p. 503-506.

Rovet,JF, R M Ehrlich, D Czuchta, M Akler, 1993, Psychoeducational characteristics of children and adolescents with insulin-dependent diabetes mellitus: J.Learn.Disabil., v. 26, p. 7-22.

Rovet,JF, R M Ehrlich, M Hoppe, 1988, Specific intellectual deficits in children with early onset diabetes mellitus: Child Dev., v. 59, p. 226-234.

Ryan,C, C Longstreet, L Morrow, 1985b, The effects of diabetes mellitus on the school attendance and school achievement of adolescents: Child Care Health Dev., v. 11, p. 229-240.

Ryan,C, A Vega, A Drash, 1985a, Cognitive deficits in adolescents who developed diabetes early in life: Pediatrics, v. 75, p. 921-927.

Ryan,CM, D J Becker, 1999, Hypoglycemia in children with type 1 diabetes mellitus. Risk factors, cognitive function, and management: Endocrinol.Metab Clin.North Am., v. 28, p. 883-900.

Sachon,C, A Grimaldi, J P Digy, B Pillon, B Dubois, F Thervet, 1992, Cognitive function, insulin-dependent diabetes and hypoglycaemia: J.Intern.Med., v. 231, p. 471-475.

Schoenle,EJ, D Schoenle, L Molinari, R H Largo, 2002, Impaired intellectual development in children with Type I diabetes: association with HbA1 c, age at diagnosis and sex: Diabetologia, v. 45, p. 108-114.

Service,FJ, 1995, Hypoglycemia: Med.Clin.North Am., v. 79, p. 1-8.

Shimada,K, A Kawamoto, K Matsubayashi, T Ozawa, 1990, Silent cerebrovascular disease in the elderly. Correlation with ambulatory pressure: Hypertension, v. 16, p. 692-699.

Stettler,C, S Allemann, P Juni, C A Cull, R R Holman, M Egger, S Krahenbuhl, P Diem, 2006, Glycemic control and macrovascular disease in types 1 and 2 diabetes mellitus: Meta-analysis of randomized trials: Am.Heart J., v. 152, p. 27-38.

Strachan,MW, I J Deary, F M Ewing, B M Frier, 2000, Recovery of cognitive function and mood after severe hypoglycemia in adults with insulin-treated diabetes: Diabetes Care, v. 23, p. 305-312.

Van Swieten,JC, A Hijdra, P J Koudstaal, J van Gijn, 1990, Grading white matter lesions on CT and MRI: a simple scale: J.Neurol.Neurosurg.Psychiatry, v. 53, p. 1080-1083.

Wechsler,D. Manual for the Wechsler adult intelligence scale-revised. 1981. Psychological Corporation, New York.

Wredling,R, S Levander, U Adamson, P E Lins, 1990, Permanent neuropsychological impairment after recurrent episodes of severe hypoglycaemia in man: Diabetologia, v. 33, p. 152-157.

Wysocki,T, M A Harris, N Mauras, L Fox, A Taylor, S C Jackson, N H White, 2003, Absence of adverse effects of severe hypoglycemia on cognitive function in school-aged children with diabetes over 18 months: Diabetes Care, v. 26, p. 1100-1105.

Yoneda,Y, S Yamamoto, 2005, Cerebral cortical laminar necrosis on diffusion-weighted MRI in hypoglycaemic encephalopathy: Diabet.Med., v. 22, p. 1098-1100.

Yousem,DM, W S Tasman, R I Grossman, 1991, Proliferative retinopathy: absence of white matter lesions at MR imaging: Radiology, v. 179, p. 229-230.

Zigmond,AS, R P Snaith. The Hospital Anxiety and Depression Scale. Acta Psychiatr Scand 67, 361-370. 1983.

References for Chapter 8

The Diabetes Control and Complications Stduy Group, 1991, Grading diabetic retinopathy from stereoscopic color fundus photographs--an extension of the modified Airlie House classification. ETDRS report number 10. Early Treatment Diabetic Retinopathy Study Research Group: Ophthalmology, v. 98, p. 786-806.

The Diabetes Control and Complications Stduy Group, 1997, Hypoglycemia in the Diabetes Control and Complications Trial. The Diabetes Control and Complications Trial Research Group: Diabetes, v. 46, p. 271-286.

Auer,RN, B K Siesjo, 1993, Hypoglycaemia: brain neurochemistry and neuropathology: Baillieres Clin.Endocrinol.Metab, v. 7, p. 611-625.

Bakshi,R, M F Morcos, T F Gabryel, P Dandona, 2000, Is fluid-attenuated inversion recovery MRI more sensitive than conventional MRI for hypoglycemic brain injury?: Neurology, v. 55, p. 1064-1065.

Boeve,BF, D G Bell, J H Noseworthy, 1995, Bilateral temporal lobe MRI changes in uncomplicated hypoglycemic coma: Can.J.Neurol.Sci., v. 22, p. 56-58.

Boone,KB, B L Miller, I M Lesser, C M Mehringer, E Hill-Gutierrez, M A Goldberg, N G Berman, 1992, Neuropsychological correlates of white-matter lesions in healthy elderly subjects. A threshold effect: Arch.Neurol., v. 49, p. 549-554.

Borkowski,JG, A L Benton. Word fluency and brain damage. Neuropsychologia 5, 135-140. 1967.

Breteler,MM, N M van Amerongen, J C Van Swieten, J J Claus, D E Grobbee, J van Gijn, A Hofman, F van Harskamp, 1994b, Cognitive correlates of ventricular enlargement and cerebral white matter lesions on magnetic resonance imaging. The Rotterdam Study: Stroke, v. 25, p. 1109-1115.

Breteler,MM, J C Van Swieten, M L Bots, D E Grobbee, J J Claus, J H van den Hout, F van Harskamp, H L Tanghe, P T de Jong, J van Gijn, ., 1994a, Cerebral white matter lesions, vascular risk factors, and cognitive function in a population-based study: the Rotterdam Study: Neurology, v. 44, p. 1246-1252.

Chalmers,J, M T Risk, D M Kean, R Grant, B Ashworth, I W Campbell, 1991, Severe amnesia after hypoglycemia. Clinical, psychometric, and magnetic resonance imaging correlations: Diabetes Care, v. 14, p. 922-925.

Chan,R, S Erbay, S Oljeski, D Thaler, R Bhadelia, 2003, Case report: hypoglycemia and diffusion-weighted imaging: J.Comput.Assist.Tomogr., v. 27, p. 420-423.

Christiaens,FJ, L D Mewasingh, C Christophe, S Goldman, B Dan, 2003, Unilateral cortical necrosis following status epilepticus with hypoglycemia: Brain Dev., v. 25, p. 107-112.

Craft,S, G S Watson, 2004, Insulin and neurodegenerative disease: shared and specific mechanisms: Lancet Neurol., v. 3, p. 169-178.

De Groot,JC, F E De Leeuw, M Oudkerk, J van Gijn, A Hofman, J Jolles, M M Breteler, 2000, Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study: Ann.Neurol., v. 47, p. 145-151.

Deary I.J., S J Langan, D A Hepburn, B M Frier. Which abilities does the PASAT test? Pers.Indiv Diff 12, 983-987. 1991.

Deary,IJ, J R Crawford, D A Hepburn, S J Langan, L M Blackmore, B M Frier, 1993, Severe hypoglycemia and intelligence in adult patients with insulin- treated diabetes: Diabetes, v. 42, p. 341-344.

DeCarli,C, D G Murphy, M Tranh, C L Grady, J V Haxby, J A Gillette, J A Salerno, A Gonzales-Aviles, B Horwitz, S I Rapoport, ., 1995, The effect of white matter hyperintensity volume on brain structure, cognitive performance, and cerebral metabolism of glucose in 51 healthy adults: Neurology, v. 45, p. 2077-2084.

Dejgaard,A, A Gade, H Larsson, V Balle, A Parving, H H Parving, 1991, Evidence for diabetic encephalopathy: Diabet.Med., v. 8, p. 162-167.

Desmond,DW, 2002, Cognition and white matter lesions: Cerebrovasc.Dis., v. 13 Suppl 2, p. 53-57.

Durston,S, H E Hulshoff Pol, B J Casey, J N Giedd, J K Buitelaar, H van Engeland, 2001, Anatomical MRI of the developing human brain: what have we learned?: J.Am.Acad.Child Adolesc.Psychiatry, v. 40, p. 1012-1020.

Edge,JA, M E Ford-Adams, D B Dunger, 1999, Causes of death in children with insulin dependent diabetes 1990-96: Arch.Dis.Child, v. 81, p. 318-323.

Englund,E, 2002, Neuropathology of white matter lesions in vascular cognitive impairment: Cerebrovasc.Dis., v. 13 Suppl 2, p. 11-15.

Fallstrom,K, 1974, On the personality structure in diabetic schoolchildren aged 7-15 years: Acta Paediatr.Scand.Suppl, p. 1-71.

Fazekas,F, F Barkhof, L O Wahlund, L Pantoni, T Erkinjuntti, P Scheltens, R Schmidt, 2002, CT and MRI rating of white matter lesions: Cerebrovasc.Dis., v. 13 Suppl 2, p. 31-36.

Fazekas,F, J B Chawluk, A Alavi, H I Hurtig, R A Zimmerman, 1987, MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging: AJR Am.J.Roentgenol., v. 149, p. 351-356.

Fazekas,F, R Kleinert, H Offenbacher, F Payer, R Schmidt, G Kleinert, H Radner, H Lechner, 1991, The morphologic correlate of incidental punctate white matter hyperintensities on MR images: AJNR Am.J.Neuroradiol., v. 12, p. 915-921.

Finelli,PF, 2001, Diffusion-weighted MR in hypoglycemic coma: Neurology, v. 57, p. 933.

Fuller,JH, L K Stevens, S L Wang, 2001, Risk factors for cardiovascular mortality and morbidity: the WHO Mutinational Study of Vascular Disease in Diabetes: Diabetologia, v. 44 Suppl 2, p. S54-S64.

Gold,AE, S M Marshall, 1996, Cortical blindness and cerebral infarction associated with severe hypoglycemia: Diabetes Care, v. 19, p. 1001-1003.

Greene,SA, I G Jefferson, J D Baum, 1990, Cerebral oedema complicating diabetic ketoacidosis: Dev.Med.Child Neurol., v. 32, p. 633-638.

Greenwood,PM, T Sunderland, J L Friz, R Parasuraman, 2000, Genetics and visual attention: selective deficits in healthy adult carriers of the epsilon 4 allele of the apolipoprotein E gene: Proc.Natl.Acad.Sci.U.S.A, v. 97, p. 11661-11666.

Hoffman,WH, M F Casanova, J A Bauza, G G Passmore, E A Sekul, 1999, Computer analysis of magnetic resonance imaging of the brain in children and adolescents after treatment of diabetic ketoacidosis: J.Diabetes Complications, v. 13, p. 176-181.

Hoffman,WH, C M Steinhart, T el Gammal, S Steele, A R Cuadrado, P K Morse, 1988, Cranial CT in children and adolescents with diabetic ketoacidosis: AJNR Am.J.Neuroradiol., v. 9, p. 733-739.

Holemans,X, M Dupuis, N Misson, J F Vanderijst, 2001, Reversible amnesia in a Type 1 diabetic patient and bilateral hippocampal lesions on magnetic resonance imaging (MRI): Diabet.Med., v. 18, p. 761-763.

Holl,RW, G E Lang, M Grabert, E Heinze, G K Lang, K M Debatin, 1998, Diabetic retinopathy in pediatric patients with type-1 diabetes: effect of diabetes duration, prepubertal and pubertal onset of diabetes, and metabolic control: J.Pediatr., v. 132, p. 790-794.

Ikram,MA, H A Vrooman, M W Vernooij, L F van der, A Hofman, L A van der, W J Niessen, M M Breteler, 2007, Brain tissue volumes in the general elderly population The Rotterdam Scan Study: Neurobiol.Aging.

Iwai,A, T Sakamoto, Y Kinoshita, J Yokota, T Yoshioka, T Sugimoto, 1987, Computed tomographic imaging of the brain in after hypoglycemia coma: Neuroradiology, v. 29, p. 398-400.

Jacobson,AM, G Musen, C M Ryan, N Silvers, P Cleary, B Waberski, A Burwood, K Weinger, M Bayless, W Dahms, J Harth, 2007, Long-term effect of diabetes and its treatment on cognitive function: N.Engl.J.Med., v. 356, p. 1842-1852.

Jensen,AR. Individual differences in the Hick paradigm. Vernon, P. A. Speed of Information Processing and Intelligence . 1987. NJ, Norwood, NJ, Ablex.

Junque,C, J Pujol, P Vendrell, O Bruna, M Jodar, J C Ribas, J Vinas, A Capdevila, J L Marti-Vilalta, 1990, Leuko-araiosis on magnetic resonance imaging and speed of mental processing: Arch.Neurol., v. 47, p. 151-156.

Keane,S, A Gallagher, S Ackroyd, M A McShane, J A Edge, 2002, Cerebral venous thrombosis during diabetic ketoacidosis: Arch.Dis.Child, v. 86, p. 204-205.

Klein,BE, R Klein, P E McBride, K J Cruickshanks, M Palta, M D Knudtson, S E Moss, J O Reinke, 2004, Cardiovascular disease, mortality, and retinal microvascular characteristics in type 1 diabetes: Wisconsin epidemiologic study of diabetic retinopathy: Arch.Intern.Med., v. 164, p. 1917-1924.

Koppel,BS, M Daras. Transient Hypodensity on CT Scan during Hypoglycemia. Eur Neurol 33, 80-82. 1993.

Kostraba,JN, J S Dorman, T J Orchard, D J Becker, Y Ohki, D Ellis, B H Doft, L A Lobes, R E LaPorte, A L Drash, 1989, Contribution of diabetes duration before puberty to development of microvascular complications in IDDM subjects: Diabetes Care, v. 12, p. 686-693.

Krane,EJ, M A Rockoff, J K Wallman, J I Wolfsdorf, 1985, Subclinical brain swelling in children during treatment of diabetic ketoacidosis: N.Engl.J.Med., v. 312, p. 1147-1151.

Kruit,MC, M A van Buchem, P A Hofman, J T Bakkers, G M Terwindt, M D Ferrari, L J Launer, 2004, Migraine as a risk factor for subclinical brain lesions: JAMA, v. 291, p. 427-434.

Kullberg,CE, M Abrahamsson, H J Arnqvist, K Finnstrom, J Ludvigsson, 2002, Prevalence of retinopathy differs with age at onset of diabetes in a population of patients with Type 1 diabetes: Diabet.Med., v. 19, p. 924-931.

Langan,SJ, I J Deary, D A Hepburn, B M Frier, 1991, Cumulative cognitive impairment following recurrent severe hypoglycaemia in adult patients with insulin-treated diabetes mellitus: Diabetologia, v. 34, p. 337-344.

Lenroot,RK, J N Giedd, 2006, Brain development in children and adolescents: insights from anatomical magnetic resonance imaging: Neurosci.Biobehav.Rev., v. 30, p. 718-729.

Longstreth,WT, Jr., A M Arnold, T A Manolio, G L Burke, N Bryan, C A Jungreis, D O'Leary, P L Enright, L Fried, 2000, Clinical correlates of ventricular and sulcal size on cranial magnetic resonance imaging of 3,301 elderly people. The Cardiovascular Health Study. Collaborative Research Group: Neuroepidemiology, v. 19, p. 30-42.

Longstreth,WT, Jr., T A Manolio, A Arnold, G L Burke, N Bryan, C A Jungreis, P L Enright, D O'Leary, L Fried, 1996, Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study: Stroke, v. 27, p. 1274-1282.

Lunetta,M, A R Damanti, G Fabbri, M Lombardo, M Di Mauro, L Mughini, 1994, Evidence by magnetic resonance imaging of cerebral alterations of atrophy type in young insulin-dependent diabetic patients: J.Endocrinol.Invest, v. 17, p. 241-245.

Maekawa,S, M Aibiki, K Kikuchi, S Kikuchi, K Umakoshi, 2006, Time related changes in reversible MRI findings after prolonged hypoglycemia: Clin.Neurol.Neurosurg., v. 108, p. 511-513.

Mantyla,R, T Erkinjuntti, O Salonen, H J Aronen, T Peltonen, T Pohjasvaara, C G Standertskjold-Nordenstam, 1997, Variable agreement between visual rating scales for white matter hyperintensities on MRI. Comparison of 13 rating scales in a poststroke cohort: Stroke, v. 28, p. 1614-1623.

McAloon,J, D Carson, P Crean, 1990, Cerebral oedema complicating diabetic ketoacidosis: Acta Paediatr.Scand., v. 79, p. 115-117.

McCrimmon,RJ, Deary IJ, B J H Huntly, K J M MacLeod, B M Frier. Visual information processing during controlled hypoglycaemia in humans. Brain 119, 1277-1287. 1996.

McEwen,BS, L P Reagan, 2004, Glucose transporter expression in the central nervous system: relationship to synaptic function: Eur.J.Pharmacol., v. 490, p. 13-24.

Meer,A, B Dubois, D Dormont, A Grimaldi, 1988, [Value of magnetic resonance imaging after severe hypoglycemic coma]: Presse Med., v. 17, p. 1368.

Mirsen TR, Lee DH, Wong CJ, Diaz JF, Fox AJ, Hachinski VC, Merskey H. Clinical correlates of white-matter changes on magnetic resonance imaging scans
of the brain. Arch.Neurol. 48[10], 1015-1021. 1991.

Musen,G, I K Lyoo, C R Sparks, K Weinger, J Hwang, C M Ryan, D C Jimerson, J Hennen, P F Renshaw, A M Jacobson, 2006, Effects of type 1 diabetes on gray matter density as measured by voxel-based morphometry: Diabetes, v. 55, p. 326-333.

Nathan,DM, P A Cleary, J Y Backlund, S M Genuth, J M Lachin, T J Orchard, P Raskin, B Zinman, 2005, Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes: N.Engl.J.Med., v. 353, p. 2643-2653.

Nelson,H, J R Willison. National Adult Reading Test (NART) Test Manual. 2nd ed. 1991. NFER-Nelson, Windsor, U.K.

Northam,EA, P J Anderson, R Jacobs, M Hughes, G L Warne, G A Werther. Neuropsychological profiles of children with type 1 diabetes 6 years after
disease onset. Diabetes Care 24[9], 1541-1546. 2001.

Olsen,BS, A K Sjolie, P Hougaard, J Johannesen, K Marinelli, B B Jacobsen, H B Mortensen, 2004, The significance of the prepubertal diabetes duration for the development of retinopathy and nephropathy in patients with type 1 diabetes: J.Diabetes Complications, v. 18, p. 160-164.

Pantoni,L, J H Garcia, 1995, The significance of cerebral white matter abnormalities 100 years after Binswanger's report. A review: Stroke, v. 26, p. 1293-1301.

Perros,P, I J Deary, R J Sellar, J J Best, B M Frier, 1997, Brain abnormalities demonstrated by magnetic resonance imaging in adult IDDM patients with and without a history of recurrent severe hypoglycemia: Diabetes Care, v. 20, p. 1013-1018.

Perros,P, R J Sellar, B M Frier, 1994, Chronic pontine dysfunction following insulin-induced hypoglycemia in an IDDM patient: Diabetes Care, v. 17, p. 725-727.

Rajbhandari,SM, T Powell, G A Davies-Jones, J D Ward, 1998, Central pontine myelinolysis and ataxia: an unusual manifestation of hypoglycaemia: Diabet.Med., v. 15, p. 259-261.

Reske-Nielsen,E, K Lundbaek. Diabetic encephalopathy: diffuse and focal lesions of the brain in long-term diabetes. Acta Neurol.Scand. 39[Supp. 4], 273-290. 1963.

Richardson,ML, R E Kinard, M B Gray, 1981, CT of generalized gray matter infarction due to hypoglycemia: AJNR Am.J.Neuroradiol., v. 2, p. 366-367.

Roe,TF, T O Crawford, K R Huff, G Costin, F R Kaufman, M D Nelson, Jr., 1996, Brain infarction in children with diabetic ketoacidosis: J.Diabetes Complications, v. 10, p. 100-108.

Rogers,B, I Sills, M Cohen, F G Seidel, 1990, Diabetic ketoacidosis. Neurologic collapse during treatment followed by severe developmental morbidity: Clin.Pediatr.(Phila), v. 29, p. 451-456.

Rovet,J, R Ehrlich, M Hoppe, 1987, Behaviour problems in children with diabetes as a function of sex and age of onset of disease: J.Child Psychol.Psychiatry, v. 28, p. 477-491.

Rovet,JF, R M Ehrlich, 1999, The effect of hypoglycemic seizures on cognitive function in children with diabetes: a 7-year prospective study: J.Pediatr., v. 134, p. 503-506.

Ryan,C, A Vega, A Drash, 1985, Cognitive deficits in adolescents who developed diabetes early in life: Pediatrics, v. 75, p. 921-927.

Schmidt,R, C Enzinger, S Ropele, H Schmidt, F Fazekas, 2003, Progression of cerebral white matter lesions: 6-year results of the Austrian Stroke Prevention Study: Lancet, v. 361, p. 2046-2048.

Schoenle,EJ, D Schoenle, L Molinari, R H Largo, 2002, Impaired intellectual development in children with Type I diabetes: association with HbA1 c, age at diagnosis and sex: Diabetologia, v. 45, p. 108-114.

Shimada,K, A Kawamoto, K Matsubayashi, T Ozawa, 1990, Silent cerebrovascular disease in the elderly. Correlation with ambulatory pressure: Hypertension, v. 16, p. 692-699.

Shrier,DA, D K Shibata, H Z Wang, Y Numaguchi, J M Powers, 1999, Central brain herniation secondary to juvenile diabetic ketoacidosis: AJNR Am.J.Neuroradiol., v. 20, p. 1885-1888.

Stettler,C, S Allemann, P Juni, C A Cull, R R Holman, M Egger, S Krahenbuhl, P Diem, 2006, Glycemic control and macrovascular disease in types 1 and 2 diabetes mellitus: Meta-analysis of randomized trials: Am.Heart J., v. 152, p. 27-38.

Van Swieten,JC, A Hijdra, P J Koudstaal, J van Gijn, 1990, Grading white matter lesions on CT and MRI: a simple scale: J.Neurol.Neurosurg.Psychiatry, v. 53, p. 1080-1083.

Vannucci,RC, S J Vannucci, 2001, Hypoglycemic brain injury: Semin.Neonatol., v. 6, p. 147-155.

Wahlund,LO, I Agartz, O Almqvist, H Basun, L Forssell, J Saaf, L Wetterberg, 1990, The brain in healthy aged individuals: MR imaging: Radiology, v. 174, p. 675-679.

Wardlaw,JM, K J Ferguson, C Graham, 2004, White matter hyperintensities and rating scales-observer reliability varies with lesion load: J.Neurol., v. 251, p. 584-590.

Wechsler,D. Manual for the Wechsler adult intelligence scale-revised. 1981. Psychological Corporation, New York.

Ylikoski,R, A Ylikoski, T Erkinjuntti, R Sulkava, R Raininko, R Tilvis, 1993, White matter changes in healthy elderly persons correlate with attention and speed of mental processing: Arch.Neurol., v. 50, p. 818-824.

Yoneda,Y, S Yamamoto, 2005, Cerebral cortical laminar necrosis on diffusion-weighted MRI in hypoglycaemic encephalopathy: Diabet.Med., v. 22, p. 1098-1100.

Yousem,DM, W S Tasman, R I Grossman, 1991, Proliferative retinopathy: absence of white matter lesions at MR imaging: Radiology, v. 179, p. 229-230.

References for Chapter 9

The Diabetes Control and Complications Stduy Group, 1991, Epidemiology of severe hypoglycemia in the diabetes control and complications trial. The DCCT Research Group: Am.J.Med., v. 90, p. 450-459.

The Diabetes Control and Complications Stduy Group, 1996, Effects of intensive diabetes therapy on neuropsychological function in adults in the Diabetes Control and Complications Trial: Ann.Intern.Med., v. 124, p. 379-388.

Arky,RA, E Veverbrants, E A Abramson, 1968, Irreversible hypoglycemia. A complication of alcohol and insulin: JAMA, v. 206, p. 575-578.

Austin,EJ, I J Deary, 1999, Effects of repeated hypoglycemia on cognitive function: a psychometrically validated reanalysis of the Diabetes Control and Complications Trial data: Diabetes Care, v. 22, p. 1273-1277.

Borkowski,JG, A L Benton. Word fluency and brain damage. Neuropsychologia 5, 135-140. 1967.

Corder,EH, A M Saunders, W J Strittmatter, D E Schmechel, P C Gaskell, G W Small, A D Roses, J L Haines, M A Pericak-Vance, 1993, Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families: Science, v. 261, p. 921-923.

Crawford,FC, R D Vanderploeg, M J Freeman, S Singh, M Waisman, L Michaels, L Abdullah, D Warden, R Lipsky, A Salazar, M J Mullan, 2002, APOE genotype influences acquisition and recall following traumatic brain injury: Neurology, v. 58, p. 1115-1118.

Deary I.J., S J Langan, D A Hepburn, B M Frier. Which abilities does the PASAT test? Pers.Indiv Diff 12, 983-987. 1991.

Deary,IJ, J R Crawford, D A Hepburn, S J Langan, L M Blackmore, B M Frier, 1993, Severe hypoglycemia and intelligence in adult patients with insulin- treated diabetes: Diabetes, v. 42, p. 341-344.

Ferguson,SC, A Blane, P Perros, R J McCrimmon, J J Best, J Wardlaw, I J Deary, B M Frier, 2003, Cognitive ability and brain structure in type 1 diabetes: relation to microangiopathy and preceding severe hypoglycemia: Diabetes, v. 52, p. 149-156.

Flory,JD, S B Manuck, R E Ferrell, C M Ryan, M F Muldoon, 2000, Memory performance and the apolipoprotein E polymorphism in a community sample of middle-aged adults: Am.J.Med.Genet., v. 96, p. 707-711.

Haan,MN, L Shemanski, W J Jagust, T A Manolio, L Kuller, 1999, The role of APOE epsilon4 in modulating effects of other risk factors for cognitive decline in elderly persons: JAMA, v. 282, p. 40-46.

Jacobson,AM, G Musen, C M Ryan, N Silvers, P Cleary, B Waberski, A Burwood, K Weinger, M Bayless, W Dahms, J Harth, 2007, Long-term effect of diabetes and its treatment on cognitive function: N.Engl.J.Med., v. 356, p. 1842-1852.

Jensen,AR. Individual differences in the Hick paradigm. Vernon, P. A. Speed of Information Processing and Intelligence . 1987. NJ, Norwood, NJ, Ablex.

Jordan,BD, 2000, Chronic traumatic brain injury associated with boxing: Semin.Neurol., v. 20, p. 179-185.

Jordan,BD, N R Relkin, L D Ravdin, A R Jacobs, A Bennett, S Gandy, 1997, Apolipoprotein E epsilon4 associated with chronic traumatic brain injury in boxing: JAMA, v. 278, p. 136-140.

Kalmijn,S, E J Feskens, L J Launer, D Kromhout, 1996, Cerebrovascular disease, the apolipoprotein e4 allele, and cognitive decline in a community-based study of elderly men: Stroke, v. 27, p. 2230-2235.

Kutner,KC, D M Erlanger, J Tsai, B Jordan, N R Relkin, 2000, Lower cognitive performance of older football players possessing apolipoprotein E epsilon4: Neurosurgery, v. 47, p. 651-657.

Langan,SJ, I J Deary, D A Hepburn, B M Frier, 1991, Cumulative cognitive impairment following recurrent severe hypoglycaemia in adult patients with insulin-treated diabetes mellitus: Diabetologia, v. 34, p. 337-344.

Lezak,MD. Neuropsychological Assessment. [Fourth Edition], 1-1016. 2004. Oxford, Oxford University Press.

Lincoln,NB, R M Faleiro, C Kelly, B A Kirk, W J Jeffcoate, 1996, Effect of long-term glycemic control on cognitive function: Diabetes Care, v. 19, p. 656-658.

Louko,AM, J Vilkki, T Niskakangas, 2006, ApoE genotype and cognition after subarachnoid haemorrhage: a longitudinal study: Acta Neurol.Scand., v. 114, p. 315-319.

Maclullich AMJ, Seckl JR, Starr JM, Deary IJ. The Biology of Intelligence: From Association to Mechanism. Intelligence 26[2], 63-73. 1998.

McCrimmon,RJ, Deary IJ, B J H Huntly, K J M MacLeod, B M Frier. Visual information processing during controlled hypoglycaemia in humans. Brain 119, 1277-1287. 1996.

Mortensen,EL, P Hogh, 2001, A gender difference in the association between APOE genotype and age-related cognitive decline: Neurology, v. 57, p. 89-95.

Nelson,H, J R Willison. National Adult Reading Test (NART) Test Manual. 2nd ed. 1991. NFER-Nelson, Windsor, U.K.

Pendleton,N, A Payton, E H van den Boogerd, F Holland, P Diggle, P M Rabbitt, M A Horan, J Worthington, W E Ollier, 2002, Apolipoprotein E genotype does not predict decline in intelligence in healthy older adults: Neurosci.Lett., v. 324, p. 74-76.

Pinholt,M, J L Frederiksen, M Christiansen, 2006, The association between apolipoprotein E and multiple sclerosis: Eur.J.Neurol., v. 13, p. 573-580.

Plomin,R, G E McClearn, D L Smith, S Vignetti, M J Chorney, K Chorney, C P Venditti, S Kasarda, L A Thompson, D K Detterman, ., 1994, DNA markers associated with high versus low IQ: the IQ Quantitative Trait Loci (QTL) Project: Behav.Genet., v. 24, p. 107-118.

Raber,J, D Wong, M Buttini, M Orth, S Bellosta, R E Pitas, R W Mahley, L Mucke, 1998, Isoform-specific effects of human apolipoprotein E on brain function revealed in ApoE knockout mice: increased susceptibility of females: Proc.Natl.Acad.Sci.U.S.A, v. 95, p. 10914-10919.

Raber,J, D Wong, G Q Yu, M Buttini, R W Mahley, R E Pitas, L Mucke, 2000, Apolipoprotein E and cognitive performance: Nature, v. 404, p. 352-354.

Reichard,P, A Britz, U Rosenqvist, 1991, Intensified conventional insulin treatment and neuropsychological impairment: BMJ, v. 303, p. 1439-1442.

Reichard,P, M Pihl, U Rosenqvist, J Sule, 1996, Complications in IDDM are caused by elevated blood glucose level: the Stockholm Diabetes Intervention Study (SDIS) at 10-year follow up: Diabetologia, v. 39, p. 1483-1488.

Reiman,EM, R J Caselli, K Chen, G E Alexander, D Bandy, J Frost, 2001, Declining brain activity in cognitively normal apolipoprotein E varepsilon 4 heterozygotes: A foundation for using positron emission tomography to efficiently test treatments to prevent Alzheimer's disease: Proc.Natl.Acad.Sci.U.S.A, v. 98, p. 3334-3339.

Reiman,EM, K Chen, G E Alexander, R J Caselli, D Bandy, D Osborne, A M Saunders, J Hardy, 2004, Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia: Proc.Natl.Acad.Sci.U.S.A, v. 101, p. 284-289.

Reiman,EM, K Chen, G E Alexander, R J Caselli, D Bandy, D Osborne, A M Saunders, J Hardy, 2005, Correlations between apolipoprotein E epsilon4 gene dose and brain-imaging measurements of regional hypometabolism: Proc.Natl.Acad.Sci.U.S.A, v. 102, p. 8299-8302.

Sachon,C, A Grimaldi, J P Digy, B Pillon, B Dubois, F Thervet, 1992, Cognitive function, insulin-dependent diabetes and hypoglycaemia: J.Intern.Med., v. 231, p. 471-475.

Shuvaev,VV, J Fujii, Y Kawasaki, H Itoh, R Hamaoka, A Barbier, O Ziegler, G Siest, N Taniguchi, 1999, Glycation of apolipoprotein E impairs its binding to heparin: identification of the major glycation site: Biochim.Biophys.Acta, v. 1454, p. 296-308.

Small,GW, L M Ercoli, D H Silverman, S C Huang, S Komo, S Y Bookheimer, H Lavretsky, K Miller, P Siddarth, N L Rasgon, J C Mazziotta, S Saxena, H M Wu, M S Mega, J L Cummings, A M Saunders, M A Pericak-Vance, A D Roses, J R Barrio, M E Phelps, 2000, Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer's disease: Proc.Natl.Acad.Sci.U.S.A, v. 97, p. 6037-6042.

Smith,JD, 2000, Apolipoprotein E4: an allele associated with many diseases: Ann.Med., v. 32, p. 118-127.

Staehelin,HB, P Perrig-Chiello, C Mitrache, A R Miserez, W J Perrig, 1999, Apolipoprotein E genotypes and cognitive functions in healthy elderly persons: Acta Neurol.Scand., v. 100, p. 53-60.

Sudlow,C, N A Martinez Gonzalez, J Kim, C Clark, 2006, Does apolipoprotein E genotype influence the risk of ischemic stroke, intracerebral hemorrhage, or subarachnoid hemorrhage? Systematic review and meta-analyses of 31 studies among 5961 cases and 17,965 controls: Stroke, v. 37, p. 364-370.

Teasdale,GM, J A Nicoll, G Murray, M Fiddes, 1997, Association of apolipoprotein E polymorphism with outcome after head injury: Lancet, v. 350, p. 1069-1071.

The DCCT Research Group. Hypoglycemia in the Diabetes Control and Complications Trial. Diabetes 46, 271-286. 1997.

Turic,D, P J Fisher, R Plomin, M J Owen, 2001, No association between apolipoprotein E polymorphisms and general cognitive ability in children: Neurosci.Lett., v. 299, p. 97-100.

Wechsler,D. Manual for the Wechsler adult intelligence scale-revised. 1981. Psychological Corporation, New York.

Wenham,PR, W H Price, G Blandell, 1991, Apolipoprotein E genotyping by one-stage PCR: Lancet, v. 337, p. 1158-1159.

Wredling RAM, Theorell PGT, Roll HM, Lins PERS, Adamson UKC. Psychosocial state of patients with IDDM prone to recurrent episodes of severe hypoglycemia. Diabetes Care 15, 518-521. 1992.

Wredling,R, S Levander, U Adamson, P E Lins, 1990, Permanent neuropsychological impairment after recurrent episodes of severe hypoglycaemia in man: Diabetologia, v. 33, p. 152-157.

Wright,AF, 2005, Neurogenetics II: complex disorders: J.Neurol.Neurosurg.Psychiatry, v. 76, p. 623-631.

Yu,YW, C H Lin, S P Chen, C J Hong, S J Tsai, 2000, Intelligence and event-related potentials for young female human volunteer apolipoprotein E epsilon4 and non-epsilon4 carriers: Neurosci.Lett., v. 294, p. 179-181.

Zigmond,AS, R P Snaith. The Hospital Anxiety and Depression Scale. Acta Psychiatr Scand 67, 361-370. 1983.

APPENDIX 1 ABSTRACTS AND COMMUNICATIONS

Oral Presentations

1. `**Ferguson S.C.**, McCrimmon R.J., Perros P., Best J.J.K., Deary I.J., Frier B.M.

'Quantitative magnetic resonance imaging of the brain in insulin-dependent diabetic patients: effect of recurrent severe hypoglycaemia and advanced microvascular disease on brain structure and cognitive function'.

Anglo-Danish-Dutch Diabetes Group Meeting, Vaals, May 1999.

2. **S.C. Ferguson**, R.J. McCrimmon, P. Perros, J.J.K. Best, I.J. Deary, B.M. Frier.

'Severe hypoglycaemia, cognition and MRI structural abnormalities in young patients with type 1 diabetes'.

European Association for the Study of Diabetes Annual Conference, Brussels, (September 1999).

3. **Ferguson S.C.**, McCrimmon R.J., Perros P., Blane A., Best J.J.K.,

Wardlaw J., Deary I.J., Frier B.M.

'Complications of type 1 diabetes: impact on brain structure and function'.

Junior Diabetes Group Meeting, Dunkeld, October 2000 – Prize presentation.

4. **Ferguson S.C.,** McCrimmon R.J., Blane A., Perros P., Best J.J.K., Wardlaw J., Frier B.M., Deary I.J.

'The onset *age of type 1 diabetes influences intellectual ability and brain structure'.*

in adulthood'.

Junior Diabetes Group Meeting, Dunkeld, October 2002 – Prize presentation.

5. ***Ferguson S.C.,*** *McCrimmon R.J., Blane A., Perros P., Best J.J.K., Wardlaw J., Frier B.M., Deary I.J.*

'Is the brain a target organ for damage in type 1 diabetes?'

Caledonian Prize Lecture, Caledonian Society for Endocrinology meeting (Dec 2002).

Abstract: Scottish Medical Journal

6. ***Ferguson S.C.,*** *Blane A., Wardlaw J., Perros P., McCrimmon R.J., Deary I.J., Frier B.M.*

'The onset age of type 1 diabetes influences intellectual ability and brain structure in adulthood.'

Association of Physicians of Great Britain and Ireland Annual Meeting (April 2003).

Abstract: Quarterly Journal of Medicine, *2003.*

Poster presentations

1. **Ferguson SC**, McCrimmon RJ, Blane A, Perros P, Best JJK, Deary IJ, Frier BM.
'Microvascular complications of type 1 diabetes may be more detrimental to the human brain than severe hypoglycaemia'.
American Diabetes Association, San Antonio (June 2000).

2. **Ferguson SC**, Evans JC, Ellard S, Hattersley AT, Deary IJ, Frier BM.
Apolipoprotein-e allotype influences cognitive function in type 1 diabetes but not susceptibility to hypoglycemic brain injury
American Diabetes Association, Philadelphia (June 2001).

3. **S.C. Ferguson**, R.J. McCrimmon, A. Blane, J.J.K. Best, J. Wardlaw, P. Perros, I.J. Deary, B.M. Frier.
Cognitive and structural brain abnormalities in young patients with type 1 diabetes: effects of severe hypoglycaemia and microvascular disease.
Association of Physicians Meeting (Edinburgh 2001).

4. 4. **Ferguson S.C.,** McCrimmon R.J., Blane A., Perros P., Best J.J.K., Wardlaw J., Frier B.M., Deary I.J.

'The onset age of type 1 diabetes influences intellectual ability and brain structure

in adulthood'.

Diabetes UK/Scottish Executive Meeting (Nov 2002).

APPENDIX 2 PUBLICATIONS

Publications

1: Ferguson SC, Blane A, Wardlaw J, Frier BM, Perros P, McCrimmon RJ, Deary IJ.
Influence of an early-onset age of type 1 diabetes on cerebral structure and cognitive function.
Diabetes Care. 2005 Jun;28(6):1431-7.

2: Ferguson SC, Blane A, Perros P, McCrimmon RJ, Best JJ, Wardlaw J, Deary IJ,
Frier BM.
Cognitive ability and brain structure in type 1 diabetes: relation to microangiopathy and preceding severe hypoglycemia.
Diabetes. 2003 Jan;52(1):149-56.

3: Ferguson SC, Deary IJ, Perros P, Evans JC, Ellard S, Hattersley AT, Frier BM.
Apolipoprotein-e influences aspects of intellectual ability in type 1 diabetes.
Diabetes. 2003 Jan;52(1):145-8. Erratum in: Diabetes. 2003 Jul;52(7):1877.