INSULIN-INDUCED HYPOGLYCAEMIA IN HUMANS

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To Jackie, Jamie, Paul and Dad for providing much needed love, support and encouragement

Omni Nunc Arte Magistra

Motto of Robert Gordon's College, Aberdeen
DECLARATION

a) This thesis was composed by Dr Mark William John Strachan

b) Studies 1, 2 and 4 were performed, analysed and written primarily by myself. In Study 1, Dr Fiona Ewing and Dr Stewart Ferguson assisted in the execution of glucose clamp procedures. In Study 3, Ms Hagosa Abraha and Dr Roy Sherwood, from King's College, London, performed the assays of the serum markers of neuronal damage. Dr Alistair Lammie, from the Department of Pathology, University of Edinburgh, reported the neuropathological findings in 'Case 1' and 'Case 2'. Dr Petros Perros, from the Freeman Hospital, Newcastle, provided the clinical details of 'Case 3' and obtained the blood samples for analyses of the serum markers. All other aspects of Study 3 were performed by myself.

c) I hold the degree of MB ChB (Honours) Edinburgh

d) This thesis has not been submitted for any other degree, diploma or professional qualification.

Mark WJ Strachan

Date: 10\textsuperscript{th} November 2000
Hypoglycaemia is the commonest complication of insulin-treated diabetes. In the initial Chapters of this thesis, the physiological and clinical aspects of hypoglycaemia are described, and the literature examining the effects of both acute and recurrent hypoglycaemia on cognitive function in humans is reviewed. The remaining Chapters describe original experimental studies in which the neuro-cognitive sequelae and the prevention of insulin-induced hypoglycaemia were examined.

Acute hypoglycaemia was induced in 16 non-diabetic subjects using a modified hyperinsulinaemic glucose clamp technique and caused predictable decrements in cognitive performance. However, acute hypoglycaemia had no effect on the function of the peripheral nervous system, as assessed by nerve conduction studies performed on the dominant median and common peroneal nerves. This lack of effect of acute hypoglycaemia on peripheral nerve function suggests that peripheral neurones do not have the same obligate requirement for glucose as a metabolic fuel as neurones of the central nervous system.

The temporal changes in mood states and cognitive functions following a single, spontaneous episode of severe hypoglycaemia in 20 people with insulin-treated diabetes were examined. Recovery from any acute cognitive decrement following severe hypoglycaemia was complete by 1.5 days, although decreased levels of 'happiness' and 'energy' appeared to take longer to recover. Compared to 'control' subjects who had not experienced severe hypoglycaemia for over one year, the 'hypo' subjects had persistent cognitive decrements and altered mood states which may have been a consequence of previous exposure to recurrent episodes of severe hypoglycaemia.

The identification of serum markers that could predict the degree of neuronal damage and prognosis of patients after severe hypoglycaemia would have considerable clinical value. Neurone-Specific Enolase (NSE) and Protein S-100 (S-100) are markers of acute neuronal damage in various neurological disorders. Serum concentrations of these markers did not rise in 16 diabetic subjects who experienced an episode of severe hypoglycaemia and who made a complete neurological recovery. However, serum concentrations of the markers did rise in two of three patients who died following an episode of severe hypoglycaemia. These preliminary results suggest that measurement of serum concentrations of NSE and S-100 may have a future role in evaluating clinical outcome following an episode of severe hypoglycaemia which is associated with neurological damage.

Insulin lispro is a rapid-acting analogue of human insulin which is associated with a diminished frequency of nocturnal hypoglycaemia. With certain meal types, however, the use of lispro could theoretically be associated with an increased risk of post-prandial hypoglycaemia. The glucodynamics of pre- and post-prandially administered lispro were examined following test meals in which the proportion of the carbohydrate and fat, and liquid and solid constituents was varied. The optimum timing of injection of lispro was, in part, dependent on the constituents of the test meals. Optimum glucodynamics for a high carbohydrate/low fat meal were obtained with pre-prandial administration of lispro. However, large decremental glucose excursions were seen when lispro was injected before meals with a high fat/low carbohydrate content, resulting in an increased risk of post-prandial hypoglycaemia. A more favourable glucodynamic pattern was observed when lispro was injected after high fat/low carbohydrate meals.
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SUMMARY OF STUDY 1

Background and Aims
Acute hypoglycaemia causes impairment of cognitive function in people with and without diabetes. There is evidence that profound and prolonged acute hypoglycaemia can facilitate the development of a peripheral neuropathy. However, few controlled studies have assessed the impact of a more moderate degree and duration of acute hypoglycaemia on the function of the peripheral nervous system. This study assessed the impact of 60 minutes of acute hypoglycaemia on parameters of peripheral neural function.

It has also been suggested that individual differences in intelligence and speed of information processing may be mediated by the speed of nerve conduction within the central nervous system, and that an indirect measure of this can be obtained by measuring peripheral nerve conduction velocities. Therefore, a secondary aim of the investigation was, within a single study, to examine comprehensively the effects of controlled hypoglycaemia on different levels of human information processing. By testing all such levels during hypoglycaemia in the same group of subjects, it was possible to investigate the lower level processes that may contribute to the decrements in higher-level mental performance.

Methods
Sixteen non-diabetic humans underwent two separate hyperinsulinaemic glucose clamp procedures on different study days, in a counterbalanced fashion. On one occasion euglycaemia was maintained (blood glucose 5.0 mmol/l) and on the other occasion hypoglycaemia was induced (blood glucose 2.6 mmol/l). During each condition, subjects performed a combined psychometric, cognitive-experimental and psychophysical test battery. Nerve conduction studies were also performed on the dominant-side median and common peroneal nerves and measurements were made of the motor nerve conduction velocities, the amplitudes of the motor action potentials, and the ratios of the amplitudes after proximal and distal stimulation (a measure of conduction block).
Results
Hypoglycaemia caused impaired performance of general cognitive and information processing tasks at the cognitive-experimental and psychophysical level (p<0.05). However, peripheral motor nerve conduction velocities and the amplitudes of motor action potentials were unaffected (p>0.05). In addition there was no evidence of peripheral neural conduction block. The common peroneal nerve conduction velocities reduced with time in both conditions (p<0.01), but no effect of time was observed with any of the other neurophysiological parameters.

Conclusions
Multiple levels of information processing in the brain were altered while peripheral nerve function remained intact. This implies that peripheral neural function may not be used as a surrogate marker of speed of neuronal conduction within the central nervous system in future studies attempting to determine the underlying neural mechanisms of intelligence. The lack of effect of acute hypoglycaemia on peripheral nerve function also suggests that peripheral neurones do not have the same obligate requirement for glucose as a metabolic fuel as neurones of the central nervous system. The change in common peroneal nerve conduction velocities requires further investigation, but may indicate an effect of hyperinsulinaemia on peripheral neural function.
SUMMARY OF STUDY 2

Background and Aim
Acute hypoglycaemia in non-diabetic and diabetic humans impairs cognitive functions and alters mood states. There is considerable evidence from experimental studies that cognitive decrements induced by moderate degrees and durations of acute hypoglycaemia, and probably alterations in mood state, return to baseline parameters within 45 - 60 minutes of restoration of normoglycaemia. However, no previous studies have examined the time required for cognitive functions and moods to return to normal after an acute episode of severe hypoglycaemia. The aim of the present study was, therefore, to examine the temporal changes in mood states and cognitive functions following a single, spontaneous episode of severe hypoglycaemia occurring in people with insulin-treated diabetes.

Methods
Cognitive functions and moods were studied prospectively in 20 subjects with insulin-treated diabetes who had experienced a spontaneous episode of severe hypoglycaemia ('hypos'), and 20 matched subjects with insulin-treated diabetes who had not experienced severe hypoglycaemia in the preceding year ('controls'). A detailed battery of cognitive function tests and mood scales was administered at 1.5, 9 and 30 days following the severe hypoglycaemia, and at similar intervals for the 'controls'.

Results
The 'hypo' subjects had a history of a greater number of episodes of severe hypoglycaemia (p<0.01). For the majority of cognitive tests, no evidence was observed of a 'hangover' effect of acute hypoglycaemia on cognitive function (p>0.05). A trend was noted for levels of Hedonic Tone (p=0.08) and Energetic Arousal (p=0.05) to improve with time in the 'hypo' subjects, but not in the 'controls'. However, the 'hypo' subjects had chronically elevated levels of depression (p=0.01) and anxiety (p<0.05) and persistently performed more poorly in several cognitive tests such as the Digit Symbol (p<0.01) and the Stroop (p<0.01) tasks. The
differences in cognitive performance between the groups disappeared after statistical adjustment for previous severe hypoglycaemia history.

**Conclusions**

These results suggest that, in the main, recovery from any acute cognitive decrement following severe hypoglycaemia was complete by 1.5 days, although decreased levels of 'happiness' and 'energy' may take longer to recover. The persistent cognitive decrements and altered mood states noted in the 'hypo' subjects may be a consequence of previous exposure to recurrent episodes of severe hypoglycaemia.
SUMMARY OF STUDY 3

Background and Aim
The ability of severe hypoglycaemia to induce both transient and permanent neurological abnormalities, and rarely death, is well recognised. Clinicians often have difficulty in determining the prognosis of unconscious patients admitted following an episode of severe hypoglycaemia. The identification of serum markers that could predict the degree of neuronal damage and prognosis of patients after severe hypoglycaemia would have considerable clinical value. Neurone-Specific Enolase (NSE) and Protein S-100 (S-100) are markers of acute neuronal damage in patients with various neurological disorders. The aim of the present study was to determine if these proteins have a role in predicting clinical outcome following severe hypoglycaemia by measuring changes in their serum concentrations with time in patients who did and did not make a complete neurological recovery following severe hypoglycaemia.

Methods
Serum concentrations of NSE and S-100 were measured at various time intervals in 16 patients with insulin-treated diabetes following an episode of severe hypoglycaemia, which did not cause permanent neurological impairment (the 'hypo' subjects), and in three diabetic patients who died following an episode of severe hypoglycaemia. The proteins were also measured in 10 insulin-treated diabetic subjects who had not experienced an episode of severe hypoglycaemia for at least one year (the 'control' subjects).

Results
No difference in serum concentrations of NSE and S-100 was observed between the 'hypo' and 'control' subjects at either 36 hours or seven days after the episode of severe hypoglycaemia (p>0.05). However, in two of the three subjects who died, NSE and S-100 concentrations were markedly elevated.
Conclusions

These preliminary results suggest that any neuronal injury occurring during an episode of severe hypoglycaemia, which is not associated with permanent neurological deficit, is insufficient to provoke elevation of these markers. However, measurement of serum concentrations of NSE and S-100 may have a role in evaluating clinical outcome following an episode of severe hypoglycaemia that is associated with neurological damage.
SUMMARY OF STUDY 4

Background and Aim
Insulin lispro is an analogue of human insulin, which has a rapid onset of action and a relatively short duration of action, allowing administration immediately before food. Global studies, in patients with Type 1 diabetes, have suggested that therapy with insulin lispro is associated with a diminished frequency of hypoglycaemia. However, the Scottish diet has a much higher content of fat than is ingested in many other European countries. In the context of a high fat diet, the rapid onset of action of pre-prandially administered insulin lispro could result in early post-prandial hypoglycaemia. In such circumstances, post-prandial injection of lispro may be more appropriate. The aim of the present study was to compare the glucodynamics of pre- and post-prandially administered lispro following test meals in which the proportions of the carbohydrate and fat, and liquid and solid, constituents were varied.

Methods
20 subjects with Type 1 diabetes were allocated into two groups. Group 1 subjects (n=10) received isocaloric high carbohydrate/low fat breakfasts and high fat/low carbohydrate breakfasts which had a marked liquid component. Group 2 subjects (n=10) received isocaloric test meals with equivalent carbohydrate and fat proportions to those in Group 1, but with a more marked solid phase. For each test meal, lispro was injected pre-prandially (10 minutes before) on one occasion and post-prandially (20 minutes after) on another. Blood glucose levels were measured every 15 minutes for a two hour period, and the glucose excursions (difference in blood glucose levels from baseline) calculated.

Results
For all meal types studied, pre-prandially administered lispro produced significantly smaller glucose excursions (p<0.05). In the case of the high carbohydrate/low fat breakfasts, the excursions observed following pre-prandial lispro were incremental, although a small negative excursion occurred in the first 45 minutes after ingestion of
the more solid meal. Pre-prandial lispro produced decremental excursions after consumption of the high fat/low carbohydrate breakfasts and in the case of the more solid meal this was particularly marked. Post-prandially administered lispro resulted in incremental glucose excursions after the high fat meals.

**Conclusions**

The optimum timing of injection of lispro is, in part, dependent on the constituents of the meal to be ingested. If a high carbohydrate/low fat meal is to be consumed, pre-prandial administration of lispro is superior. However, the large decremental glucose excursions seen when lispro was injected before meals with a high fat/low carbohydrate content (particularly when the food was mainly in the solid phase) would result in an increased risk of early post-prandial hypoglycaemia. In such circumstances, post-prandial administration of lispro may be more appropriate.
CHAPTER 1

PATHOPHYSIOLOGICAL AND CLINICAL ASPECTS OF HYPOGLYCAEMIA
PART A: INTRODUCTION

1.1 HISTORICAL BACKGROUND

In 1910, Porges reported the earliest biochemically-confirmed observation of hypoglycaemia in humans. He described the histories of three patients with Addison's disease in whom blood glucose concentrations fell below 0.067% (approximately 3.7 mmol/l)\(^1\). However, it was not until the pioneering work of Frederick Banting, Charles Best, James Collip and John Macleod in Toronto in the early 1920's\(^2\) that hypoglycaemia became firmly recognised as a clinical entity. These investigators were responsible for the isolation, purification and subsequent clinical application of insulin in the treatment of diabetes mellitus. In early experiments, the Toronto investigators noted that rabbits injected with insulin became increasingly hungry as their blood glucose concentrations fell, often to the point that the animals would eat wood shavings or paper\(^3\). The rabbits would eventually go on to develop convulsions and lapse into unconsciousness and, if left untreated, would ultimately die\(^3\). Such 'hypoglycaemic reactions' could be reversed following the injection of a solution of glucose.

Hypoglycaemia was also observed in some of the first human diabetic patients treated with insulin\(^4\) and indeed several patients who were administered these early insulin preparations died from what were later demonstrated to be 'hypoglycaemic reactions'\(^2\). This led the pre-eminent diabetologist of the era, Elliott P. Joslin, to observe that "insulin is not a cure for diabetes, but is a potent preparation, alike for evil and for good"\(^5\).

Over the last 80 years there have been considerable advances in insulin therapy. Isophane and lente insulins, with a much more prolonged duration of action than soluble insulin, were developed in the middle of the last century. There were subsequent improvements in the purity of pork and beef insulin preparations and, in the 1980's, advanced molecular genetic techniques lead to the introduction of insulins
with the human amino acid sequence. These human insulins have largely replaced animal insulins in the first-line management of people with Type 1 diabetes. However, none of these advances have addressed the fundamental inadequacies of exogenous insulin therapy. The concentrations of insulin that are administered peripherally are far in excess of those seen physiologically within the hepatic portal circulation. Moreover, while exogenous insulin regimens attempt to mimic the pattern of insulin release observed in the non-diabetic state, they do so poorly and clearly are not able to respond in a physiological manner to variations in blood glucose concentrations. Consequently, hypoglycaemia is still the commonest complication of insulin-treated diabetes and generates as much anxiety in patients as the threat of advanced diabetic complications such as blindness or renal failure. In an attempt to reduce the frequency of hypoglycaemia, genetically engineered analogues of human insulin have been developed, whose time-action profiles are more representative of physiological insulin secretion. However, the magnitude and consequences of hypoglycaemia remain immense. Few people with insulin-treated diabetes escape intermittent exposure and, as a consequence, hypoglycaemia remains the principal limiting factor in achieving good glycaemic control in patients with diabetes.

1.2 DEFINITIONS OF HYPOGLYCAEMIA

1.2.1 Problems with Biochemical Definitions of Hypoglycaemia
It is not possible to provide a precise biochemical criterion for the diagnosis of hypoglycaemia. A progressive decline in blood glucose triggers a hierarchy of events that occur at individual glycaemic thresholds (Figure 1.1), commencing with counterregulation (arterial blood glucose ~3.8 mmol/l), impairment of different cognitive functions (~3.2–2.6 mmol/l) and the onset of symptoms and neurophysiological dysfunction (~3.2–2.4 mmol/l). In clinical practice, however, it is usual only for venous blood glucose levels to be measured, and in the main these are lower than contemporaneous arterialisued blood glucose concentrations (which tend to be measured in research studies involving hypoglycaemia). Venous plasma glucose
concentrations below 3.0 mmol/l may occur readily following an overnight fast or during the course of a prolonged glucose tolerance test\textsuperscript{10}. Moreover, as will be described later, thresholds for the onset of symptoms and counterregulation in patients with Type 1 diabetes may vary depending on preceding glycaemic control. Thus, patients with preceding poor glycaemic control may experience symptoms of hypoglycaemia at venous plasma glucose concentrations substantially in excess of 3.0 mmol/l\textsuperscript{11}. Patients with preceding strict glycaemic control may not experience the onset of symptoms of hypoglycaemia until venous plasma glucose concentrations have fallen below 2.0 mmol/l\textsuperscript{12}. In terms of clinical practice, Diabetes UK has recommended that individuals with diabetes should strive wherever possible to ensure that blood glucose concentrations are always greater than 4.0 mmol/l\textsuperscript{13}.

1.2.2 Clinical Definitions of Hypoglycaemia

Episodes of hypoglycaemia may be usefully subdivided into asymptomatic (biochemical) hypoglycaemia, mild symptomatic hypoglycaemia and severe hypoglycaemia. Severe hypoglycaemia is defined as an episode of hypoglycaemia in which assistance from a third party is required to effect treatment. The definition does not specify a threshold level below which blood glucose concentration must fall before an episode of hypoglycaemia can be considered severe, nor does it specify any particular modalities of treatment.
The earliest manifestation of a falling blood glucose level is the inhibition of endogenous insulin secretion. Thereafter, as blood glucose falls, counterregulatory hormones are released and symptoms are generated. With increasing depth of hypoglycaemia, cognitive impairment develops and ultimately coma, convulsions and death will supervene.

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PART B: PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL RESPONSES TO HYPOGLYCAEMIA

1.3 PHYSIOLOGICAL RESPONSES TO HYPOGLYCAEMIA

1.3.1 Central Nervous System Energy Metabolism

An insight into normal central nervous system energy metabolism is crucial in understanding the physiological responses to, and the neuropathological effects of, hypoglycaemia.

*Glucose Metabolism*

Despite the fact that it performs no mechanical or osmotic activities and constitutes only 2% of normal body weight, the human brain has an enormous metabolic requirement. On average, the brain utilises 20% of the body's oxygen supplies and receives 15% of cardiac output\(^14\). The processes that consume this large amount of energy include the generation of neuronal action potentials and the maintenance of ionic homeostasis, neurotransmitter re-uptake, axoplasmic flow and protein synthesis\(^15\).

Glucose is the principal metabolic fuel of the brain and its oxidation therefore accounts for effectively all of the oxygen consumed by the brain (Figure 1.2). Oxidation of one molecule of glucose yields an energy equivalent of 42 molecules of adenosine triphosphate (ATP) while the anaerobic metabolism of glucose, by glycolysis alone, generates only 2 molecules of ATP. However, the brain can neither synthesise nor store glucose and is entirely dependent upon the cerebral circulation for maintaining its constant supply of fuel. Glucose is transported across the blood-brain barrier and into the brain by specific glucose transport proteins (GLUT 1)\(^16,17\). These molecules act as 'carriers' rather than 'pumps', in that they transport glucose passively down a concentration gradient, without a cellular energy requirement.
FIGURE 1.2: Schematic Representation of Glycolysis and Oxidative Phosphorylation

GLUCOSE

LACTATE ↔ PYRUVATE

CO₂

ACETYL CoA

OXALOACETATE

CITRATE

MALATE

FUMARATE

SUCCINATE

α-KETOGLUTARATE

SUCCINYL CoA

CO₂
Other glucose transport proteins have been identified in brain tissue (GLUT 3, 4 and 5), and these may be responsible for allowing entry of glucose into neurones and glial cells. Under normal circumstances, the facilitated transport of glucose into the brain does not limit the rate of intracellular brain metabolism. However, during hypoglycaemia, glucose transport becomes rate limiting and neuroglycopenia quickly develops.

Glucose extraction from the cerebral circulation depends not only on the number and activity of GLUT 1 transporters, but also on the surface area of the cerebral circulation and cerebral blood flow. The nature of changes in cerebral blood flow may be important in determining its impact on glucose extraction. For example, if cerebral blood flow increases as a consequence of greater capillary recruitment, glucose extraction may be significantly enhanced because of the increased cerebrovascular surface area available for glucose transport. By contrast, increases in linear cerebral blood flow may have a smaller impact on glucose extraction during hypoglycaemia because of the rate-limiting effect of glucose diffusion across the blood-brain barrier.

**Other Cerebral Energy Sources**

The brain has a limited capacity to utilise other energy sources such as ketone bodies, lactate, amino acids and free fatty acids. Ketone bodies (β-hydroxybutyrate, acetoacetate and acetone) are short-chain fatty acids that provide up to 65% of brain oxidative energy metabolism during periods of fasting or carbohydrate deprivation, and so spare the progressive catabolism of muscle proteins that would otherwise be required to maintain endogenous gluconeogenesis. Lactate may also be used as a metabolic fuel by the brain, through its ability to be converted to pyruvate by the enzyme lactate dehydrogenase. In certain forms of neonatal hypoglycaemia, lactate is known to provide up to 30-40% of cerebral energy requirements.

The brain does not appear to require a prolonged period to adapt to use alternate energy sources, since the intravenous infusion of either ketone bodies or lactate
can ameliorate the symptomatic and hormonal responses to insulin-induced hypoglycaemia and prevent cognitive dysfunction. However, outwith the laboratory, the role of ketone bodies and lactate in protecting the brain from the deleterious effects of insulin-induced hypoglycaemia may be more limited, since plasma concentrations are generally low and their rate of uptake by the brain is modest\textsuperscript{15}.

1.3.2 Counterregulation
Because of the reliance of the central nervous system on glucose as a metabolic fuel, multiple mechanisms have evolved to maintain glucose homeostasis (Figure 1.3). A fall in blood glucose is detected by glucose sensors within the brain, located mainly in the ventromedial nuclei of the hypothalamus\textsuperscript{24}, and the hepatic portal system\textsuperscript{25}. Activation of these glucose sensors instigates a cascade of responses to raise blood glucose\textsuperscript{26}. These responses include:

1. The release of counterregulatory hormones which antagonise insulin action and suppress its endogenous secretion.
2. Stimulation of the autonomic nervous system (principally the sympathetic division) which not only promotes counterregulation, but also induces important haemodynamic and other end-organ effects.
3. The generation of warning symptoms that alert the individual to the development of hypoglycaemia and the need to take corrective action.

The principal counterregulatory hormones are glucagon (secreted by pancreatic alpha cells independently of control by the brain), and adrenaline (epinephrine) secreted secondary to sympathetic neural stimulation. Other counterregulatory hormones (cortisol and growth hormone), released through activation of the hypothalamic-pituitary axis, are less powerful. These hormones stimulate the immediate provision of stored glucose from glycogen within the liver (hepatic glycogenolysis) and encourage the synthesis of glucose (gluconeogenesis) within the liver and kidney (Figure 1.3).
Acute hypoglycaemia causes activation of the sympathetic nervous system and the release of glucagon from the pancreas, as well as anterior and posterior pituitary hormones. These act in concert to raise blood glucose concentrations and to generate warning symptoms of hypoglycaemia. Sympathetic activation results in the stimulation of adrenaline secretion from the adrenal medulla and in the release of noradrenaline from sympathetic nerve endings.

1.3.3 Symptoms of Hypoglycaemia

The symptoms of hypoglycaemia are generated directly by neuroglycopenia, and also via acute autonomic stimulation. Common autonomic symptoms are sweating, trembling, pounding heart, hunger and anxiety, while neuroglycopenic symptoms include inability to concentrate, drowsiness, confusion, difficulty with speech and incoordination. Common non-specific symptoms are nausea, tiredness and headache. The symptom profile differs with age. Behavioural changes are observed in children, while the elderly report more neurological symptoms of hypoglycaemia. The early recognition of the onset of symptoms of hypoglycaemia (‘awareness’) is vital for people with insulin-treated diabetes.

1.3.4 Hypoglycaemia and Cerebral Blood Flow

Autonomic activation and secretion of catecholamines provoke haemodynamic changes that modify regional blood flow and increase the circulation of many organs, including the heart, liver and skeletal muscles (Figure 1.3). Most, but not all, studies in humans have demonstrated that acute hypoglycaemia also causes an increase in cerebral blood flow. Tallroth et al demonstrated, in non-diabetic subjects, that acute hypoglycaemia promoted not only an overall increase in cerebral blood flow, but also a significant alteration in the relative distribution of cerebral blood flow, with the highest increments being found in the frontal and parietal lobes. In patients with Type 1 diabetes, the magnitude of the response was less and the increased regional blood flow was more uniform. MacLeod et al also reported alterations in regional blood flow during acute hypoglycaemia in patients with Type 1 diabetes. There were significant increments in relative cerebral blood flow to both superior frontal cortices and the right thalamus, and significant decrements in cerebral blood flow to the right posterior cingulate cortex and the right putamen.

In theory, the increased cerebral blood flow that occurs during acute hypoglycaemia would be consistent with a physiological attempt to increase substrate delivery to the central nervous system at a time of maximum need. Evidence in support of this premise has come from studies in which cerebral blood flow has been manipulated pharmacologically. Increasing cerebral blood flow with the carbonic anhydrase
inhibitor acetazolamide, reduces the symptomatic and adrenaline responses to hypoglycaemia\textsuperscript{19}, while reducing cerebral blood flow with caffeine, markedly increases the symptomatic and counterregulatory hormonal responses to hypoglycaemia\textsuperscript{35,36}.

However, the rise in cerebral blood flow during hypoglycaemia only occurs at very low blood glucose concentrations (less than 2.0 mmol/l), i.e. well below the threshold for the development for cognitive dysfunction\textsuperscript{32}. This appears to be a maladaptive response to hypoglycaemia, since it might be imagined that the whole purpose of increasing substrate delivery would be to preserve cognitive function. However, increasing cerebral blood flow with acetazolamide does not actually reduce the degree of cognitive impairment that occurs during mild hypoglycaemia\textsuperscript{19}. Thus, the brain appears to be differentially sensitive to increased substrate delivery, with the glucose-sensing areas demonstrating much greater responsiveness to increased cerebral blood flow than the cerebral cortex.

1.4 NEUROBIOCHEMICAL AND NEUROPATHOLOGICAL EFFECTS OF HYPOGLYCAEMIA

1.4.1 Differences Between Hypoglycaemic and Hypoxic Brain Injury

For many years, hypoglycaemia was thought to harm the brain by simply starving neurones of their energy supply. Such a theory would, therefore, imply that identical neurobiochemical and neuropathological changes would occur during periods of either oxygen or glucose deprivation. Recent research has shown that this is not the case. Energy failure of cerebral neurones appears to be less marked during hypoglycaemia than during periods of hypoxia-ischaemia. Brain glucose utilisation decreases almost linearly as the blood glucose concentration falls below normal levels, but cerebral oxygen consumption does not fall as much as would be predicted by the reduction in glucose utilisation\textsuperscript{37}. Neuronal ATP levels are reduced to just over one third of the normal value during severe hypoglycaemia whereas, by contrast, ATP levels fall to less than 5% of normal in ischaemic neurones\textsuperscript{37}. These
findings presumably reflect the ability of cerebral neurones to utilise other endogenous substrates as fuels during periods of profound glucose deprivation and account for the ability of the brain to withstand longer periods of hypoglycaemia than of hypoxia-ischaemia, before permanent damage ensues. Nevertheless, the partial energy failure that occurs during mild to moderate hypoglycaemia presumably accounts for the impaired neuronal function that ensues (see Chapter 2).

The development of hypoglycaemia heralds the onset of a variety of other ion and chemical changes within cerebral neurones. Activity of ionic pumps, such as the sodium/potassium ATPase pump, cannot be maintained because of the energy deficit and consequently ions run down their concentration gradients. Increased protein and lipid catabolism occurs and flux through the glycolytic pathway is reduced, resulting in diminished tissue concentrations of lactate and pyruvate (Figure 1.2). The generation of protons by the Krebs cycle is reduced and when this is coupled with a lack of organic acids, tissue alkalosis ensues. This is in direct contrast to the situation during hypoxia-ischaemia where anaerobic metabolism of glucose results in increased tissue concentrations of lactate and where the resultant tissue acidosis is thought to exert a direct 'acidotoxic' effect on neurones.

### 1.4.2 Excitotoxic Brain Injury

Aspartate and glutamate are excitatory neurotransmitters that bind to N-methyl-D-aspartate (NMDA) receptors on the dendrites of neurones. In high concentrations, these neurotransmitters can cause neuronal death that is characterised by destruction of dendrites, axonal sparing and, ultimately, necrosis of the nerve cell body (perikaryon). Such a pattern of 'excitotoxic' cell death is seen in neurones following hypoglycaemia and can be prevented by pre-treatment with NMDA receptor antagonists. The key factor in the development of increased levels of aspartate and glutamate is the decrease in glycolytic flux which reduces concentrations of acetyl co-enzyme A and results in increased concentrations of oxaloacetate (acetyl co-enzyme A normally condenses with oxaloacetate as the first reaction in the Krebs cycle; Figure 1.2). Oxaloacetate can combine with glutamate to produce aspartate and α-ketoglutarate, according to the following equation:
According to Le Chetalier's principle, the excess oxaloacetate drives the chemical reaction to the right resulting in proportionately greater levels of aspartate than glutamate\(^39\). This is again in direct contrast to the pattern seen in cerebral ischaemia where glutamate predominates\(^41\). The mechanisms behind 'excitotoxic' neuronal death remain to be clearly elucidated but probably involve excitotoxic-mediated calcium influx into neurones with subsequent activation of lipolysis, the generation of free radicals and cell membrane breakdown\(^41\).

### 1.4.3 Neuropathological Features of Hypoglycaemic Brain Injury

The striking feature of hypoglycaemic brain damage is 'selective neuronal necrosis' i.e. neurones are affected while other cell types, such as blood vessels and glial cells are not\(^39\). Hypoxia-ischaemia may cause either selective neuronal necrosis or 'pan-necrosis', in which all cell types are affected\(^39\). Pathogenetically, it is generally accepted that excitatory amino acids lead to selective neuronal necrosis, while tissue acidosis results in pan-necrosis\(^39\). In the rat, neuronal necrosis does not start to occur until cessation of electrical activity in the brain, as measured by electroencephalography (EEG), has been present for up to 10 minutes\(^42\). The extent of neuronal necrosis increases as the duration of EEG 'isoelectricity' increases and while, initially, only selective neuroanatomical cell types are affected, ultimately all will be involved if hypoglycaemia is of sufficient severity and duration\(^42\).

The neuropathology of hypoglycaemic brain damage is similar to that of hypoxia-ischaemia but, in the early stages, the pattern of neuronal involvement may allow a distinction between the two neurotoxic mechanisms to be made. In both animal and human brains, neurones in the superficial cerebral cortex tend to be involved more than deeper neurones in hypoglycaemia\(^37,43-45\). Necrosis of neurones within the
dentate gyrus of the hippocampus, with relative sparing of cerebellar Purkinje cells, is also considered to be characteristic of hypoglycaemia. The mechanisms behind the selective pattern of neuronal damage remain to be firmly elucidated, but may reflect differential efficiencies of neuronal glucose transporters and the accumulation of excitotoxins within the CSF. The importance of this latter phenomenon is supported by time course observations of hypoglycaemic neuronal damage in rats. These have demonstrated that areas exposed to the subarachnoid spaces (i.e. cerebral cortex and hippocampus) are affected early, with regions exposed to other brain surfaces, and finally deeper structures, becoming involved at later stages.
PART C: CLINICAL ASPECTS OF HYPOGLYCAEMIA

1.5 FREQUENCY OF HYPOGLYCAEMIA

Over 100, mostly rare, causes of hypoglycaemia have been identified in humans\textsuperscript{10}. In Western clinical practice, the overwhelmingly most common form encountered is hypoglycaemia associated with the treatment of diabetes mellitus with insulin, and it is consideration of this form of hypoglycaemia that will form the basis of this thesis. Hypoglycaemia in diabetic patients is also less frequently related to the use of sulphonylurea drugs, particularly long-acting agents such as glibenclamide\textsuperscript{48,49}.

1.5.1 Frequency of Mild Hypoglycaemia

The true frequency of hypoglycaemia in insulin-treated diabetic patients is difficult to estimate accurately because of differences in definitions of hypoglycaemia. In addition, assessment of hypoglycaemia frequency is hampered because most episodes occur in the community without any input from medical, nursing or paramedical staff, and recall by patients of such episodes is generally poor. This highlights the importance of prospective recording of episodes of hypoglycaemia in clinical research studies.

The average patient with insulin-treated diabetes will probably experience several thousand episodes of mild symptomatic hypoglycaemia over a lifetime with diabetes. In a study of 441 patients with Type 1 diabetes, managed primarily with a conventional insulin regimen of twice daily soluble and isophane insulin, an average of 1.8 episodes of mild symptomatic hypoglycaemia occurred per patient per week\textsuperscript{7}. Asymptomatic hypoglycaemia, detected on routine home blood glucose monitoring, is likely to be even more common, particularly at night and if overall glycaemic control is strict. Thorsteinsson \textit{et al} demonstrated that 10% of blood glucose levels were less than 3.0 mmol/l in patients who had a median blood glucose concentration of 5.0 mmol/l\textsuperscript{50}. In a separate study of nocturnal blood glucose profiles in 31 patients with Type 1 diabetes (mean HbA1c 8.6%), 29% experienced nocturnal
hypoglycaemia (blood glucose less than 3.0 mmol/l) and 67% of these episodes were asymptomatic.

1.5.2 Frequency of Severe Hypoglycaemia
Surveys of frequency of severe hypoglycaemia in unselected northern European populations with insulin-treated diabetes have documented rates of 1.0 to 1.6 episodes per patient per year. In any particular year, around 30% of patients treated with insulin experience one or more episodes of severe hypoglycaemia.

1.6 RISK FACTORS FOR SEVERE HYPOGLYCAEMIA

1.6.1 Conventional Precipitating Factors for Severe Hypoglycaemia
Traditionally, severe hypoglycaemia has been ascribed to relative or absolute insulin excess, with the conventional precipitating factors being grouped into main six categories:

1. Inappropriate insulin injection - e.g. excessive dose, inappropriate time, inappropriate insulin formulation
2. Inadequate exogenous carbohydrate - e.g. missed meal or snack, overnight fast
3. Increased carbohydrate utilisation - e.g. exercise
4. Decreased endogenous glucose production - e.g. excess alcohol consumption
5. Increased insulin sensitivity - e.g. exercise, weight loss
6. Decreased insulin clearance - e.g. renal failure

1.6.2 Severe Hypoglycaemia in the Diabetes Control and Complications Trial
While the above factors undoubtedly contribute to many episodes of severe hypoglycaemia, the results of the Diabetes Control and Complications Trial (DCCT) have suggested that investigators must look beyond these conventional risk factors in an attempt to understand the pathogenesis of severe hypoglycaemia. In the DCCT, 1441 patients with Type 1 diabetes were randomly assigned to either intensive
insulin therapy (based on multiple injection insulin regimens or continuous subcutaneous insulin infusion therapy with an external pump) or conventional insulin therapy (one or two insulin injections daily)\(^6\). The subjects, particularly in the intensive group, were highly motivated and received significant levels of professional support. Subjects were excluded if, in the previous two years, they had experienced more than one episode of severe neurological impairment without warning symptoms of hypoglycaemia, or more than two episodes of seizure or coma, regardless of attributed cause. Over 6.5 years of follow-up, the average glycated haemoglobin (HbA1c) concentration in the intensive group was approximately 7.0% and in the conventional group approximately 8.8%\(^6\).

During the study, the occurrence of an episode of severe hypoglycaemia in an individual patient prompted a review of conventional risk factors and, in instances where probable causes were identified, corrective actions such as re-educating the patient were undertaken\(^5\). Despite this, 3788 episodes of severe hypoglycaemia occurred in the 1441 patients over the course of the study\(^5\).

The main risk factor for severe hypoglycaemia in the DCCT was a history of previous episodes of severe hypoglycaemia\(^5\). Other high risk groups included males, adolescents, C-peptide negative individuals and subjects administering a high total daily insulin dose (>0.6 units/kg)\(^5\). Moreover, subjects in the intensive group were three times more likely to experience severe hypoglycaemia, compared with subjects in the conventional group. At the end of the study, 65% and 35%, respectively, of subjects in the two treatment groups had experienced an episode of severe hypoglycaemia. In both groups, there was a quadratic relationship between HbA1c and risk of severe hypoglycaemia (Figure 1.4), with risk of hypoglycaemia increasing as HbA1c decreased. However, the attained HbA1c did not account for all the difference in risk of severe hypoglycaemia, as subjects in the intensive group still had an excess risk of severe hypoglycaemia after statistical adjustment for HbA1c concentration.
In the DCCT, there was a quadratic relationship between glycated haemoglobin concentration and severe hypoglycaemia, with rate of severe hypoglycaemia rising significantly as HbA1c concentrations fell. The pale line is a regression line estimated as a function of the logarithm of the glycosylated haemoglobin value; the dark lines represent the 95% confidence intervals. The circles indicate the crude rates, within deciles, of the mean HbA1c concentrations during the trial. The arrows mark the mean HbA1c levels attained by subjects in the intensive- and conventionally-treated arms of the study.

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1.6.3 Risk Factors for Severe Hypoglycaemia in Other Studies

The above association between severe hypoglycaemia and HbA1c has been replicated in some, but not all, studies. In a prospective evaluation of the implementation of an intensive insulin therapy programme in 636 patients with Type 1 diabetes, a lower mean HbA1c during the period of the study was associated with severe hypoglycaemia, but there was no linear or quadratic relationship between HbA1c and severe hypoglycaemia. Other risk factors for severe hypoglycaemia in this study were comparable with some of those identified in the DCCT and included previous severe hypoglycaemia, higher daily insulin doses, increased duration of diabetes, younger age at onset of diabetes, lower emotional coping abilities and C-peptide negativity. A recent systematic review has found no evidence to support the premise that treatment with human, as opposed to animal, insulins, is associated with an increased risk of hypoglycaemia.

The role of peripheral autonomic neuropathy in increasing the risk of severe hypoglycaemia has been considered in several studies. In the EURODIAB IDDM Complications Study, the presence of abnormal cardiovascular reflexes was associated with a 1.7-fold increased risk of severe hypoglycaemia. Gold et al also demonstrated that autonomic neuropathy was associated with a small increased risk of severe hypoglycaemia in 60 patients with Type 1 diabetes, but no relationship was demonstrated in the DCCT. The mechanism underlying this association remains to be fully elucidated. Peripheral autonomic neuropathy often co-exists with impaired hypoglycaemia awareness in patients with Type 1 diabetes, presumably because both conditions are associated with diabetes of long duration, but impaired awareness can readily occur in the absence of peripheral autonomic neuropathy.

1.6.4 Acquired Hypoglycaemia Syndromes

Citing the results of the DCCT, Cryer has suggested that the integrity of the glucose counterregulation system may be a pivotal factor in determining whether the relative or absolute hyperinsulinism that frequently occurs in insulin-treated diabetes, ultimately results in the development of hypoglycaemia. The three main acquired syndromes that are associated with an increased risk of severe hypoglycaemia and
impaired glucose counterregulation in patients with Type 1 diabetes are described below.

**Counterregulatory Hormonal Deficiencies**

Hypoglycaemia-induced secretion of glucagon declines in most patients within five years of developing Type 1 diabetes\(^{64,65}\). The cause of the defective glucagon response to hypoglycaemia in Type 1 diabetes is not known, but is presumably mediated by a second messenger as the pancreatic \(\alpha\)-cells react appropriately to other stimuli. Later on in the course of Type 1 diabetes, a defective adrenaline response to hypoglycaemia may also develop\(^{65-67}\). As with the glucagon response, the impaired adrenaline response is hypoglycaemia-specific but, in contrast to glucagon, the adrenaline response exhibits a threshold effect - i.e. an adrenaline response can still be elicited by hypoglycaemia, but only at a lower blood glucose concentration\(^{67}\). If hypoglycaemia develops in patients who have this combined counterregulatory hormonal deficiency, glucose recovery may be seriously compromised. Subjecting such patients to intensified insulin therapy increased the risk of severe hypoglycaemia by 25 times, compared with subjects with an intact adrenaline response\(^{68}\).

**Impaired Awareness of Hypoglycaemia**

In many patients with insulin-treated diabetes, the hypoglycaemia symptom profile alters with time, resulting in impaired perception of the onset of hypoglycaemia. Commonly, autonomic warning symptoms are lost and in their place neuroglycopenic symptoms come to predominate. Around 25% of people treated with insulin develop impaired awareness of hypoglycaemia (IAH) and the prevalence of this problem increases with the duration of insulin treatment\(^{7,61,69}\). Prospective studies have demonstrated that the frequency of severe hypoglycaemia is increased three- to six-fold in patients with IAH compared to those with normal hypoglycaemia awareness\(^{70,71}\).
Altered Glycaemic Thresholds Following Intensive Insulin Therapy

Strict glycaemic control modifies the glycaemic thresholds for counterregulation\textsuperscript{72,73} and the onset of symptoms\textsuperscript{74}. The threshold for autonomic symptoms and the counterregulatory response is therefore set at a much lower blood glucose than usual.

1.6.5 Hypoglycaemia-Associated Central Autonomic Failure

The above acquired hypoglycaemia syndromes tend to segregate together clinically. Patients with glycated haemoglobin concentrations near the non-diabetic range have an increased risk of developing IAH\textsuperscript{75-77}, while the glycaemic thresholds for the onset of symptoms and responses are altered in patients with IAH\textsuperscript{12,63,78,79}. Cryer has suggested that these acquired abnormalities represent a form of central 'hypoglycaemia-associated autonomic failure' in Type 1 diabetes, speculating that recurrent severe hypoglycaemia is the primary cause\textsuperscript{80}. If hypoglycaemia is the precipitant, then it is possible to see how a vicious cycle may become established with the development of the acquired hypoglycaemia syndromes promoting further episodes of severe hypoglycaemia.

Evidence supporting the role of antecedent hypoglycaemia in the pathogenesis of acquired hypoglycaemia syndromes has come from studies demonstrating that the glycaemic thresholds for autonomic and symptom responses to an episode of hypoglycaemia are reduced by exposure to a preceding episode of hypoglycaemia in both healthy individuals\textsuperscript{81-84} and subjects with Type 1 diabetes\textsuperscript{67,85,86}. The duration and depth of antecedent hypoglycaemia determines the responses to a subsequent decline in blood glucose\textsuperscript{87}. In animals, the counterregulatory hormonal response to systemic hypoglycaemia can be reduced if the brain is infused with glucose, i.e. if the cerebral glucose supply is maintained despite peripheral hypoglycaemia\textsuperscript{88,89}. In vitro, the transcription\textsuperscript{90} and translation\textsuperscript{91} of GLUT 1 protein (the blood-brain barrier glucose transport protein) is increased in brain capillary endothelial cells which are deprived of glucose. Moreover, prolonged hypoglycaemia in rats increases the number of GLUT 1 transporters in the blood-brain barrier\textsuperscript{92} and increases the extraction of glucose from the cerebral circulation\textsuperscript{93}. In humans, glucose uptake into the brain is enhanced during acute hypoglycaemia, in non-diabetic subjects subjected
to chronic hypoglycaemia (blood glucose 2.9 mmol/l for 56 hours)\textsuperscript{94}, and in diabetic subjects with strict glycaemic control\textsuperscript{75}. These studies provide a mechanism whereby acquired hypoglycaemia syndromes could develop: repeated severe hypoglycaemia causes upregulation of GLUT 1 transporters in the brain, as a compensatory \textit{adaptive} mechanism to preserve neuronal energy supplies when circulating glucose levels are low. However, the response may be considered \textit{maladaptive} since glucose sensors in the hypothalamus may not detect the systemic hypoglycaemia until much lower blood glucose concentrations are achieved, resulting in elevated glycaemic thresholds for the release of counterregulatory hormones and the development of symptoms.

Other mechanisms whereby antecedent hypoglycaemia may mediate the development of hypoglycaemia-associated autonomic failure have been postulated. Davis \textit{et al} reported that the infusion of cortisol the day before a hypoglycaemic clamp study resulted in comparable blunted counterregulatory hormone responses to those observed following antecedent hypoglycaemia\textsuperscript{95}. Moreover, Tkacs \textit{et al} found that recurrent hypoglycaemia in male Sprague-Dawley rats, lowering blood glucose to levels that impaired counterregulatory responses without the induction of coma, caused apoptotic cell \textit{death} in the arcuate nucleus of the hypothalamus and reduced expression of neuropeptide Y (NPY) and pro-opiomelanocortin (POMC) mRNA\textsuperscript{96}. The loss of arcuate nucleus NPY and POMC neurones could result in defective counterregulatory responses to hypoglycaemia since they project both rostrally and caudally to brain areas involved in the autonomic and pituitary components of counterregulation\textsuperscript{96}.

1.7 MORBIDITY AND MORTALITY OF SEVERE HYPOGLYCAEMIA

Fortunately, the overwhelming majority of patients make a complete recovery following an episode of hypoglycaemia. Aside from cognitive dysfunction and coma, the commonest complication of acute hypoglycaemia is likely to be the precipitation of focal or generalised convulsions. Failure to recognise the occurrence
of hypoglycaemia may lead to an erroneous diagnosis of idiopathic epilepsy being made and the prescription of anticonvulsant drugs which are ineffective in preventing hypoglycaemia-induced convulsions\textsuperscript{97}.

Rarely, severe and protracted hypoglycaemia can cause permanent brain damage, and this often occurs in the context of excessive consumption of alcohol or insulin overdosage (either deliberate or accidental)\textsuperscript{98}. These patients usually present in a coma and cerebral oedema may be present\textsuperscript{99}. Survivors of protracted hypoglycaemic coma may develop cortical and hippocampal atrophy with ventricular enlargement, often associated with a chronic vegetative state\textsuperscript{100}. Transient and permanent focal neurological deficits, such as hemiparesis, 'locked in' syndrome, ataxia, severe amnesia\textsuperscript{101} and cortical blindness\textsuperscript{102}, have been described following severe hypoglycaemia.

Hypoglycaemia is a relatively rare cause of death in insulin-treated patients but the frequency may be underestimated. The 1979 joint survey, by the Medical Services Study Group and the British Diabetic Association, of factors contributing to the deaths of people with diabetes under the age of 50, identified hypoglycaemia as a cause of death in 4\% of the 448 deaths recorded\textsuperscript{103}. More recently, in the British Diabetic Association Cohort Study, hypoglycaemia accounted for 4\% of deaths in men with Type 1 diabetes aged 20 to 49 years and 1\% of deaths in women in the same age group\textsuperscript{104}. Aside from its direct effects on brain metabolism, hypoglycaemia could precipitate death by a variety of mechanisms. In patients with macrovascular disease, the profound haemodynamic and haemorrhheological changes of severe hypoglycaemia\textsuperscript{105} may provoke acute vascular sequelae such as cardiac arrhythmia, myocardial ischaemia and cerebrovascular insufficiency. Cardiac arrhythmia, induced by nocturnal hypoglycaemia, has also been implicated in rare cases of sudden death during sleep in otherwise healthy, young patients with Type 1 diabetes (the 'dead in bed syndrome')\textsuperscript{106,107}. 

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1.8 REDUCING THE FREQUENCY OF SEVERE HYPOGLYCAEMIA

1.8.1 Hypoglycaemia Recognition and Avoidance Strategies
There is no clear consensus on the optimum strategy to manage patients at increased risk of severe hypoglycaemia.

*Home Blood Glucose Monitoring*
No trials have demonstrated that increased use of home blood glucose monitoring reduces the occurrence of severe hypoglycaemia. However, common sense would indicate that patients at risk should be advised to monitor blood glucose concentrations on a regular basis to detect asymptomatic biochemical hypoglycaemia. Indeed, home blood glucose monitoring profiles can predict patients who are at increased risk of severe hypoglycaemia, i.e. those with frequent and extreme low blood glucose readings, and with pronounced variability in day-to-day readings.\(^{108}\)

The majority of episodes of severe hypoglycaemia tend to occur at night,\(^{70,109}\) presumably because sleep reduces the ability to recognise the symptoms of hypoglycaemia. In theory, monitoring of bedtime and fasting blood glucose measurements may help reduce the risk of nocturnal hypoglycaemia. Vervoort et al demonstrated that fasting glucose levels of less than 5.5 mmol/l were predictive of preceding ‘early morning’ hypoglycaemia (between 4.00 and 7.30am) and that ‘early nocturnal’ hypoglycaemia (between 11.00pm and 1.00am) never occurred if blood glucose concentrations before bedtime were greater than 7.5 mmol/l.\(^{51}\)

*Lifestyle and Dietary Measures*
All patients with insulin-treated diabetes should be educated about the need for regular meals and snacks and about the adjustment of carbohydrate intake and insulin dosage before and after exercise. The risks of hypoglycaemia associated with excess alcohol consumption should also be regularly reinforced. The frequency of nocturnal hypoglycaemia may be reduced by the ingestion of a bedtime snack of complex carbohydrate, such as uncooked cornstarch, that is slowly digested and which may
maintain blood glucose concentrations for several hours\textsuperscript{110}. Including protein with the complex carbohydrate at bedtime may be beneficial\textsuperscript{109,111}, although this has been disputed\textsuperscript{112}.

\textit{Increasing Awareness of Hypoglycaemia and Counterregulatory Hormonal Responses}

If glycated haemoglobin concentrations are within or near the non-diabetic range, relaxation of glycaemic control will restore symptomatic and counterregulatory hormonal responses to hypoglycaemia\textsuperscript{113}. IAH (and possibly counterregulatory deficiencies) may be reversed through a total avoidance of hypoglycaemia and, with meticulous diabetic management, this has been achieved in research studies for periods varying from three weeks to one year\textsuperscript{114-116}. Although complete avoidance of hypoglycaemia is clearly beneficial, to attempt this is time-consuming and labour-intensive, and beyond the resources of most specialist centres. As an alternative, Blood Glucose Awareness Training (BGAT) has been developed in the USA for patients with IAH. This involves an intensive period of re-education in which patients are taught to recognise neuroglycopenic cues\textsuperscript{117-119} and initiate appropriate treatment responses. In unaware patients, BGAT reduces the number of episodes of undetected biochemical hypoglycaemia, but does not appear to alter the overall prevalence of hypoglycaemia\textsuperscript{119,120}.

The bedtime administration of the glucagon-releasing amino acid alanine, or the adrenaline-stimulating $\beta_2$-adrenergic agonist terbutaline is more effective than a conventional bedtime snack in reducing the occurrence of nocturnal hypoglycaemia\textsuperscript{121}. Moreover, the frequent ingestion of caffeine augments the symptomatic and counterregulatory hormonal responses to hypoglycaemia in non-diabetic and diabetic subjects\textsuperscript{36}. Concerns about the long-term safety and practicality of these pharmacological agents has limited their clinical role to date, but they do introduce the concept of identifying therapeutic agents that may reduce the risk of severe hypoglycaemia in patients with diabetes.
Altering Insulin Regimens

Reducing total daily insulin dosage is a simple means of avoiding hypoglycaemia, but clearly this may be at the expense of suboptimal glycaemic control. Splitting the evening dose of soluble and intermediate-acting insulin (such that soluble insulin is administered before the evening meal and intermediate insulin before bed) is commonly recommended to patients experiencing frequent nocturnal hypoglycaemia\textsuperscript{109}. Reductions in severe hypoglycaemia have also accrued by replacing multiple daily injection regimens of insulin with continuous subcutaneous insulin infusions (CSII)\textsuperscript{122}, or indeed by substituting bedtime isophane insulin with the overnight use of CSII\textsuperscript{123}. However, in the United Kingdom, at least, cost implications have meant that CSII is not a practical option for most patients.

Other potentially significant resources in the management of patients at risk of severe hypoglycaemia are the new rapid-acting insulin analogues, insulin lispro (Humalog) and insulin aspart (Novorapid). In particular a large body of evidence has emerged about the role of insulin lispro in reducing the frequency of episodes of hypoglycaemia and this will be considered in detail in the following section.

1.8.2 Insulin Lispro

Insulin lispro is a genetically modified form of human insulin in which the sequence of amino acids in the $\beta$-chain has been altered, such that the natural sequence of proline at position B28 and lysine at position B29 has been reversed (Figure 1.5)\textsuperscript{124,125}.

Conventional soluble insulin molecules form dimers in solution, and aggregate further into hexamers in the presence of zinc ions. The relatively delayed onset and prolonged duration of action of soluble insulins is, therefore, explainable by the fact that, following subcutaneous injection, the insulin hexamers have to dissociate before diffusion into the circulation can occur\textsuperscript{126,127}. As a consequence, it is recommended that soluble insulin is injected at least half an hour before food to limit post-prandial glucose excursions\textsuperscript{128-130}. By contrast, the altered amino acid sequence of insulin
FIGURE 1.5: Amino Acid Sequence of Insulin Lispro

Compared with human insulin, the positions of proline at B28 and lysine at B29 of the C-terminal end of the β-chain are reversed.

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lispro leads to a conformational shift in the C-terminal end of the β-chain that sterically hinders the ability of the insulin lispro molecules to form dimers\textsuperscript{126,127}. Therefore, compared to conventional soluble insulins, the onset of action of insulin lispro is quicker and the overall duration of action is shorter (Figure 1.6)\textsuperscript{124,125}. In practical terms this means that insulin lispro can be injected just before meals.

Insulin lispro underwent extensive Phase III clinical trials in over 4000 patients with diabetes before a product licence was granted in April, 1996. Because of the differences in the timing of pre-meal injections of insulin lispro and soluble insulin, the majority of the trials were open-label. The consistent finding of these early trials was that, compared with soluble insulin, insulin lispro did not improve overall glycaemic control (as assessed by glycated haemoglobin concentrations), but did reduce post-prandial glucose excursions by 1.0 - 4.0 mmol/l\textsuperscript{125,131,132}. However, its shorter duration of action meant that fasting and pre-prandial glucose concentrations tended to be higher during treatment with insulin lispro than with soluble insulin\textsuperscript{125,131,132}. More recent studies have suggested that improvements in glycated haemoglobin concentrations can be achieved by optimisation of basal insulin regimens\textsuperscript{133,134}.

The effects of insulin lispro on the frequency of hypoglycaemia have received considerable attention. Therapy with insulin lispro does not impair counterregulatory hormonal and symptomatic responses to hypoglycaemia\textsuperscript{135}. In the largest open-label, crossover study of 1008 patients with Type 1 diabetes, three months treatment with insulin lispro was associated with a 12\% reduction in the frequency of hypoglycaemia\textsuperscript{132}. The greatest proportional reduction occurred in the frequency of nocturnal hypoglycaemia\textsuperscript{132}. This pattern of results has been borne out in several other studies\textsuperscript{131,136} and has a plausible physiological explanation. Nocturnal hypoglycaemia was thought to be associated with the nocturnal hyperinsulinaemia caused by intermediate or long-acting insulins taken before bed or teatime. However, soluble insulin injected before the evening meal, because of its relatively long duration of action (Figure 1.6), may exacerbate early nocturnal
Following subcutaneous injection, levels of insulin lispro peak in the circulation after approximately one hour, and fall rapidly thereafter. By contrast, levels of soluble (regular) insulin rise more slowly, peak between one to three hours and, thereafter, gradually fall to basal levels.

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hyperinsulinaemia and so contribute to the development of nocturnal hypoglycaemia\textsuperscript{137}. The frequency of nocturnal hypoglycaemia may be reduced by insulin lispro because its shorter time-action profile diminishes early nocturnal hyperinsulinaemia.

However, it is important to recognise that a fairly broad definition of hypoglycaemia was utilised by the insulin lispro investigators: 	extit{"patients recorded an episode of hypoglycaemia when they experienced a sign or symptom that they normally associated with hypoglycaemia. In addition a blood glucose measurement during routine blood glucose testing <3.5 mmol/l was counted as hypoglycaemia"}\textsuperscript{132}. This definition would clearly have had the potential to include significant numbers of episodes that either may not have been true hypoglycaemia or may have been of limited clinical relevance. A more robust meta-analysis of the data on episodes of severe hypoglycaemia (defined strictly as episodes of coma requiring treatment with glucagon or intravenous glucose) from eight trials studying 2576 patients with Type 1 diabetes was, therefore, performed\textsuperscript{138}. The total number of episodes of severe hypoglycaemia was small (233) and only 4.4\% of patients receiving soluble insulin experienced such an episode. Therapy with insulin lispro was associated with about a 30\% reduction in the number of patients who developed severe hypoglycaemia, but the absolute reduction was small, with 3.1\% of insulin lispro-treated patients affected\textsuperscript{138}.

Insulin lispro probably does offer lifestyle benefits to people with Type 1 diabetes, in terms of the potential convenience of being able to administer insulin immediately before food. The data on the ability of insulin lispro to reduce the frequency of hypoglycaemia is of reasonable quality, but is not compelling. Further studies are needed in patients who are at particularly high risk of hypoglycaemia, such as those with IAH, to clarify its role in strategies to reduce the incidence of severe hypoglycaemia. In addition, the rapid onset of action of insulin lispro could, theoretically, result in an \textit{increased} risk of early post-prandial hypoglycaemia, particularly if the normal rise in blood glucose concentrations after a meal were delayed (such as after a meal with a high fat content). Therefore, further research
into the effects of insulin lispro on blood glucose concentrations following various
different meal types is required so that patients can be advised on the optimal timing
of administration of insulin lispro.

1.9 SUMMARY

Glucose is the predominant metabolic fuel of the brain, and neuroglycopenia quickly
ensues when blood glucose concentrations fall below normal limits. The prevention
of hypoglycaemia is, therefore, crucial to survival. As a consequence, blood glucose
concentrations are normally maintained within a very narrow range by powerful
neuro-hormonal mechanisms. Several large trials have elucidated the major risk
factors for hypoglycaemia but, even with modern insulin therapies, hypoglycaemia
remains the commonest complication of insulin-treated diabetes and forms the major
barrier to achieving 'normal' blood glucose concentrations.

In recent years, our understanding of the effects of hypoglycaemia on the brain has
increased significantly. In Chapter 2, the literature examining the impact of insulin-
induced hypoglycaemia on cognitive function will be reviewed in detail. In
particular the impact of recurrent episodes of hypoglycaemia on cerebral function
will be considered, as well as the evidence from experimental studies that have
examined the effects of acute hypoglycaemia on cognitive performance.
CHAPTER 2

EFFECTS OF HYPOGLYCAEMIA ON COGNITIVE FUNCTION
2.1 EFFECTS OF ACUTE HYPOGLYCAEMIA ON COGNITIVE FUNCTION

Examination of the reproducible effects of acute hypoglycaemia on cognitive performance became feasible in the 1980’s, when experimental procedures such as the hyperinsulinaemic glucose clamp and the bedside measurement of blood glucose concentration were widely available. Prior to this, observations had been made that blood glucose levels below 3.0 mmol/l, as a consequence of either natural glucose fluctuations\textsuperscript{139} or bolus injection of insulin\textsuperscript{140}, were associated with impaired motor co-ordination, mental speed, concentration and memory. Since then, a large literature on the cognitive effects of acute hypoglycaemia has developed with recent interest focusing on the particular cognitive domains that are affected, factors which moderate individual susceptibility to hypoglycaemia, and the clinical relevance of the cognitive decrements that occur.

2.1.1 Methodological Considerations

The literature examining the effects of acute hypoglycaemia on cognitive function is large and diverse. Before considering the results in detail, it is important to consider the methodological differences that exist between many of the studies and the limitations of some the procedures employed\textsuperscript{9}.

Limitations of Neuropsychological Testing

All studies examining cognitive performance during acute hypoglycaemia utilise neuro-cognitive tests of one form or another. Although much of the variance in human mental abilities is attributable to general intelligence, in clinical practice it is useful to employ tests to examine the various cognitive domains\textsuperscript{141,142}. These domains include attention and concentration, memory (both verbal and non-verbal), abstract thinking, constructional ability and speed of information processing. Full assessment of cognitive function requires, therefore, the administration of a battery of psychometric tests to ensure that sufficient cognitive domains are examined to detect any decrements. However, a bewildering range of psychometric tests is available\textsuperscript{141}. The format of individual tests is very variable, ranging from the relative simplicity of paper and pencil tasks, such as Trail-Making B (from the Halstead-
Reitan battery)\textsuperscript{143} and Digit Symbol (from the Wechsler Adult Intelligence Scale)\textsuperscript{144}, to the electronic complexity of driving simulators\textsuperscript{145}. The lack of 'gold standards' for the validation of psychological tests means that multiple tests of differing nature and complexity are available to examine the parameters of any given cognitive domain. The situation is made more complex since many tests measure more than one aspect of cognitive ability, so that the results from two different tests purporting to examine, for example, 'verbal memory' may not be directly comparable. Thus, comparisons between studies that have used different batteries of cognitive tests must be made with caution, even though they may have been attempting to examine the same nominal cognitive domains\textsuperscript{146}.

The use of large batteries of psychological tests also increases the risk of a type I statistical error, i.e. that a statistically significant result will occur by chance. The difficulties associated with this approach have recently been highlighted by Ryan who has advocated the employment of multivariate statistical procedures in the analysis of study results\textsuperscript{147}. Unfortunately, this approach can only effectively be used if sample sizes are large and the outcome measures are statistically interrelated.

Cognitive tasks are also subject to the effects of practice, i.e. performance improves with repeated administration. Many studies have failed to account for this and have compared cognitive performance during acute hypoglycaemia with that measured during a preceding period of euglycaemia. This may lead to underestimation of the magnitude of cognitive decrements occurring during hypoglycaemia\textsuperscript{9}. Comparing cognitive performance during acute hypoglycaemia with that measured during a separate euglycaemia study day may control the effects of practice. Crucially, the order of the experimental study sessions must be counter-balanced, i.e. half of the subjects must undergo the hypoglycaemia study session first followed by the euglycaemia control session, and the other half must undergo the study sessions in the reverse order. Such a study design may be further refined by including periods of euglycaemia prior to each experimental condition, to take account of day-to-day variations in cognitive performance\textsuperscript{148}. 

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Few studies have reported the order in which cognitive tasks are administered within a given test battery or have provided reassurance that the order has remained consistent. This is clearly important, since fatigue of the subject and the potential for a lag between changing blood glucose concentrations and an effect of cognitive function mean that performance of cognitive tasks may be affected by the duration of the experimental study.

Methods of Inducing Hypoglycaemia

Two main techniques have been employed to induce hypoglycaemia experimentally: the insulin infusion technique and the hyperinsulinaemic glucose clamp. The former involves the intravenous infusion of insulin at variable rates to reach the desired blood glucose concentration, while the latter employs a fixed intravenous infusion rate of insulin with a variable intravenous infusion of dextrose to maintain blood glucose concentrations at the appropriate level. The clamp technique has gained considerable popularity because of the relative ease and speed at which fine adjustments in blood glucose concentrations can be made, allowing measurement of the thresholds of various physiological responses to hypoglycaemia.

However, both techniques have disadvantages. In the insulin infusion technique, the plasma concentrations of insulin vary, while the clamp procedures employ very high concentrations of insulin. Insulin itself has generally been regarded as having no effect on brain tissue, as glucose uptake into neurones was thought to be wholly insulin-independent. However, the insulin-responsive glucose transporter, GLUT 4, has been identified in brain endothelial tissue. Moreover, insulin receptors are found both in the brains of animals and of humans, especially in the hippocampus, hypothalamus and olfactory bulb. Insulin can act as a neuromodulator in the brain, inhibiting synaptic activity, while alterations in plasma insulin concentrations can be related to, or even directly regulate, changes in cognitive function in non-diabetic humans. A degree of reassurance about the validity of utilising pharmacological doses of insulin to assess cognitive responses to hypoglycaemia has been provided by two studies in humans. In these studies, the infusion of differing concentrations of insulin did not affect cognitive responses to
comparable levels of hypoglycaemia\textsuperscript{157,158}, although higher infusion rates did affect neuroendocrine\textsuperscript{157,158} and symptomatic\textsuperscript{157} responses. In a separate study, using more physiological concentrations of insulin, higher plasma insulin concentrations were associated with more pronounced cognitive impairment during hypoglycaemia\textsuperscript{159}.

\textbf{Measurement of Blood Glucose Concentrations}

Knowledge of the nature of blood samples analysed for glucose concentration (plasma or whole blood; arterialised or venous blood) is important in the interpretation of results from hypoglycaemia studies. Plasma glucose concentrations are 10-20\% higher than contemporaneously measured whole blood glucose concentrations\textsuperscript{9}. In certain circumstances, notably post-prandially, there are also significant differences between arterial and venous blood glucose concentrations\textsuperscript{160}.

\textbf{2.1.2 Cognitive Processes Impaired by Acute Hypoglycaemia}

Numerous experimental studies have considered the impact of acute hypoglycaemia on cognitive function and many of these have been subject to detailed review\textsuperscript{9,161}. A summary of some of the studies that have examined the cognitive decrements that occur at specific blood glucose concentrations is provided in Table 2.1. The Table is not meant to provide a comprehensive account of all studies that have examined acute hypoglycaemia and cognitive function, and indeed studies that are discussed in later sections are not included. Instead, it is designed to allow an insight into the glucose thresholds at which decrements occur in the various cognitive domains.

The cognitive tests examined in individual studies have been broadly grouped in Table 2.1 according to the criteria described by Lezak\textsuperscript{141}. The Table clearly illustrates that cognitive function becomes progressively impaired as blood glucose falls and that no cognitive domains are spared. Although individual studies have demonstrated cognitive decrements at glucose concentrations between 3.0-3.4 mmol/l, measures of cognitive function appear to be consistently impaired at blood glucose concentrations of 2.9 mmol/l and below. It has been repeatedly asserted that, during hypoglycaemia, accuracy of performance of a given task tends to be preserved.
TABLE 2.1: Cognitive Tasks Impaired during Acute Hypoglycaemia

<table>
<thead>
<tr>
<th>Study</th>
<th>Subject Number</th>
<th>Status</th>
<th>Blood</th>
<th>Sample Glucose</th>
<th>Method Type</th>
<th>Motor</th>
<th>Attention</th>
<th>Reaction Time (s)</th>
<th>Psychomotor</th>
<th>Inform</th>
<th>Digit</th>
<th>Span</th>
<th>Memory</th>
<th>Verbal Fluency</th>
<th>P300</th>
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<td>ND</td>
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<td>Ipp and Forster, 1987</td>
<td>7</td>
<td>ND</td>
<td>3.6</td>
<td>avb</td>
<td>Infusion</td>
<td>NI</td>
<td>NI</td>
<td>ND(S)</td>
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<td>Clamp</td>
<td>NI</td>
<td>ND</td>
<td>ND(S)</td>
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<td>Clamp</td>
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<td>NI</td>
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<td>Clamp</td>
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</table>
Footnotes to Table 2.1

'ND' - non-diabetic subjects
'I' - performance of the cognitive task was significantly impaired during acute hypoglycaemia
'NI' performance of the cognitive task was not significantly impaired during acute hypoglycaemia
'a' - arterialised blood sample
'V' - venous blood sample
'b' - whole blood sample
'p' - plasma sample
's' - simple reaction time
'c' - choice reaction time
'B' - backward digit span
'F' - forward digit span

*8 subjects underwent a hypoglycaemic clamp and 8 subjects underwent a euglycaemic clamp
at the expense of speed. Moreover, cognitive tasks which are complex and attention-demanding are disrupted more by milder levels of hypoglycaemia than simple motor tasks, such as finger tapping or digit span recall\textsuperscript{9,161}. This latter assertion is not completely borne out by the studies cited in Table 2.1, since few experimental studies have actually examined aspects of motor function at blood glucose concentrations between 2.0-3.0 mmol/l.

2.1.3 Moderators of Cognitive Function during Acute Hypoglycaemia
Several studies have demonstrated a substantial degree of variability in individual responses to the effects of acute hypoglycaemia\textsuperscript{165,166,171,174,179-182}. Some subjects demonstrate profound impairment of cognitive function during mild hypoglycaemia, while others remain unaffected at the lowest concentrations of blood glucose that can safely be induced in the laboratory. There has, therefore, been considerable interest in factors that moderate individual susceptibility to acute hypoglycaemia.

Gonder-Frederick et al\textsuperscript{183} confirmed the wide inter-individual variability in performance of several cognitive tasks (examining motor function, attention and verbal fluency) during hypoglycaemia. Important cognitive decrements occurred at a venous plasma glucose of 2.6 mmol/l in 50% of subjects, but 15% showed little or no disruption of performance. Variations in the glycaemic thresholds for the onset of cognitive impairment, as well as the degree of dysfunction experienced in most tasks, were stable in individual subjects on re-testing three months later. However, paradoxically, individual variations in performance of simple and complex reaction time tasks were not consistent with time and did not correlate with any biological variable measured\textsuperscript{182}. No other studies have examined the repeatability of responses to hypoglycaemia in individuals, and this clearly limits the ability to draw firm conclusions from studies that have examined specific moderators of hypoglycaemia susceptibility. The individual moderators that have been investigated, and which will be considered in turn, include: age, sex, intelligence, the presence or absence of diabetes, glycaemic control, antecedent hypoglycaemia, impaired awareness of
hypoglycaemia (IAH), previous severe hypoglycaemia and duration of hypoglycaemia.

**Age**

Matyka et al compared the effects of acute hypoglycaemia on performance of a four-choice reaction time task in seven non-diabetic men aged 22-26 years and seven non-diabetic men aged 60-70 years\(^\text{184}\). Compared with the younger men, reaction time deteriorated more profoundly in the older men and at a higher glycaemic threshold (arterialised plasma glucose of 3.0 mmol/l in the older men compared with 2.6 mmol/l in the younger men). By contrast, in several other studies, there was no correlation between age and change in cognitive performance during hypoglycaemia\(^\text{180,181,183}\), although the age-range of subjects studied was more limited than in the investigation by Matyka et al\(^\text{184}\).

**Sex**

Significant gender differences in cognitive function during hypoglycaemia were reported in one study\(^\text{181}\). At an arterialised venous glucose of 2.2 mmol/l, women with Type 1 diabetes exhibited less of a performance decrement from baseline than men in measures of attention and mental flexibility. There were no significant gender differences in other cognitive measures, and the differences persisted after adjustment for other potential confounding factors\(^\text{181}\). Somewhat paradoxical results were observed in a further study in which women performed better during mild hypoglycaemia (venous plasma glucose 3.6 mmol/l), but demonstrated comparable cognitive performance to men during more moderate hypoglycaemia (venous plasma glucose 2.6 mmol/l)\(^\text{183}\).

**Intelligence**

It has frequently been asserted that people with a higher IQ level possess more 'brain reserve capacity' in cognitive processing, which may offer a degree of protection from cerebral insults such as hypoglycaemia\(^\text{185}\). This concept was directly investigated by Gold et al\(^\text{186}\), who studied the effects of acute hypoglycaemia on the cognitive performance of 24 non-diabetic individuals who were divided into high and
average IQ groups. As expected, the high IQ group had significantly better performance at baseline in most of the cognitive tasks but, overall during hypoglycaemia, there were few differences in deterioration in performance between the two groups. Following multiple univariate analyses (with the attendant risk of a type 1 statistical error), the high IQ group performed relatively more poorly during hypoglycaemia during two cognitive tasks assessing attention and information processing.

Other studies have also failed to demonstrate a relationship between intelligence (or surrogate markers such as education) and change in cognitive performance during hypoglycaemia\textsuperscript{180-183}.

\textbf{Diagnosis of Diabetes}

Wirsen et al examined 10 men with Type 1 diabetes and 12 non-diabetic men, and found that the cognitive performance of the former group was more affected than the latter by acute hypoglycaemia (arterialised venous glucose 2.0 mmol/l). However, the results of this study must be interpreted with caution as the diabetic subjects started at higher blood glucose concentrations (and therefore had a proportionately greater fall in glucose concentrations). Moreover, although the groups were matched for baseline performance on a battery of cognitive tests, no assessment was reported of pre-morbid cognitive ability. The groups came from different occupational backgrounds (the diabetic men were white collar workers and the non-diabetic men were medical students) and had different durations of education.

There were no differences between diabetic and non-diabetic subjects in deterioration in cognitive performance during acute hypoglycaemia in two other studies\textsuperscript{74,179}. In a third study where diabetic and non-diabetic subjects were studied, there were baseline differences in cognitive performance of the groups which preclude further interpretation of the impact of diabetes per se\textsuperscript{187}.
Other Diabetes-Related Clinical Variables
Several studies have found no relationship between degree of cognitive dysfunction during acute hypoglycaemia and variables such as duration of diabetes\textsuperscript{164,179,181,183} age of onset of diabetes\textsuperscript{182,183} and the magnitude of counterregulatory response to hypoglycaemia\textsuperscript{179-181}. Increased deterioration in cognitive performance during acute hypoglycaemia was associated with a history of coma due to severe hypoglycaemia in one study\textsuperscript{183}.

Strict Glycaemic Control, Recent Antecedent Hypoglycaemia and Impaired Awareness of Hypoglycaemia
The effects of IAH, strict glycaemic control and recent antecedent hypoglycaemia on the cognitive response to hypoglycaemia have been of considerable research interest. However, the inter-relation of these factors makes the field complex and difficult to interpret.

Strict Glycaemic Control
In several studies, there was no correlation between glycated haemoglobin levels and cognitive performance during acute hypoglycaemia\textsuperscript{74,164,179-183}. However, in one early study, lower HbA1c levels were associated with poorer performance on an auditory reaction time task, although subjects with stricter glycaemic control had also experienced significantly more previous episodes of unconsciousness secondary to severe hypoglycaemia\textsuperscript{166}. The interpretation of these studies is limited because a relatively small number of subjects were studied, so increasing the risk of a type 2 statistical error.

In the study by Widom and Simonson\textsuperscript{74}, there was no difference in the median threshold for cognitive dysfunction during hypoglycaemia between diabetic patients with well-controlled (mean total HbA1 8.0%) and poorly controlled (mean total HbA1 11.8%) diabetes. Similar results were observed by Maran et al in an examination of the thresholds for deterioration of four-choice reaction time during acute hypoglycaemia in eight intensively-treated diabetic subjects (mean HbA1c 7.7%) and ten conventionally-treated subjects (mean HbA1c 10.1%)\textsuperscript{188}. By contrast,
Ziegler et al studied seven diabetic subjects with strict glycaemic control (mean HbA1c 6.3%) and 11 with less strict control (mean HbA1c 9.1%) and found that the threshold for prolongation for the latency of the P300 wave (a 'cognitive' brain electrical event-related potential) occurred at a significantly lower glucose concentration in the former group than the latter. Comparable results were obtained in another study of P300 potentials in intensively and conventionally-treated diabetic subjects.

**Antecedent Hypoglycaemia**

It is difficult to obtain an overall perspective of the impact of antecedent hypoglycaemia on cognitive performance during subsequent episodes of hypoglycaemia, because different degrees and durations of antecedent hypoglycaemia have been studied. Moreover, there is no consistent pattern for the duration of the time period between the antecedent hypoglycaemia (or the control euglycaemia condition) and the subsequent hypoglycaemia study. In the following section study results are summarised in order of increasing duration of this time period.

Mellman et al induced hypoglycaemia in nine non-diabetic subjects, 90 minutes after a two hour period of antecedent hypoglycaemia (arterialised plasma glucose 3.2 mmol/l). Two cognitive tasks were utilised - Logical Memory and Digit Symbol Substitution (a psychomotor test); performance of the former task was preserved following antecedent hypoglycaemia, but Digit Symbol Substitution test scores deteriorated equally whether there had been antecedent hypoglycaemia or euglycaemia.

The impact of antecedent nocturnal hypoglycaemia has been considered in two separate studies. Veneman et al demonstrated in 10 non-diabetic volunteers that a single episode of moderate asymptomatic nocturnal hypoglycaemia (plasma glucose 2.4 mmol/l for two hours) diminished the degree of cognitive dysfunction (as assessed by a comprehensive battery of cognitive tasks) during subsequent hypoglycaemia the following day. Fanelli et al also found that antecedent nocturnal hypoglycaemia (arterialised plasma glucose 2.8 mmol/l for 3.5 hours)
reduced overall cognitive dysfunction during subsequent hypoglycaemia in 15 subjects with Type 1 diabetes\textsuperscript{191}. In particular, there was relative preservation in performance of tasks assessing attention and pattern recognition, but not in measures of delayed verbal memory and information processing\textsuperscript{191}.

By contrast, the induction of hypoglycaemia (arterialised plasma glucose 2.6 mmol/l) in 16 non-diabetic subjects for a two hour period in the afternoon prior to a stepped hypoglycaemic clamp the following morning, was associated with no overall change in cognitive performance when compared with results from a stepped hypoglycaemic clamp with antecedent euglycaemia\textsuperscript{192}. However, when performance of individual tasks at specified blood glucose concentrations was considered, there did appear to be reduced deterioration in tasks of attention and pattern recognition when glucose concentrations were reduced from 2.8 mmol/l to 2.5 mmol/l\textsuperscript{192}. Dagogo-Jack et al also found no evidence that antecedent afternoon hypoglycaemia limited the degree of cognitive impairment during hypoglycaemia induced on the following day in 15 subjects with Type 1 diabetes (indeed there was evidence that attention scores were worse following antecedent hypoglycaemia)\textsuperscript{67}. Finally, George et al, demonstrated in eight subjects with Type 1 diabetes that hypoglycaemia (arterialised blood glucose 2.8 mmol/l for two hours) induced two days prior to a subsequent episode of hypoglycaemia had no effect on the threshold for impairment of four-choice reaction time, despite the fact that the noradrenaline response to hypoglycaemia was blunted by antecedent hypoglycaemia\textsuperscript{193}.

Ovalle et al investigated, in six patients with Type 1 diabetes, the impact of the induction of twice weekly periods of hypoglycaemia (plasma glucose 2.8 mmol/l for two hours), for a period of one month, on subsequent cognitive performance during a stepped hypoglycaemic clamp\textsuperscript{194}. Recurrent antecedent hypoglycaemia blunted the subsequent symptomatic and hormonal responses to hypoglycaemia and reduced overall cognitive dysfunction\textsuperscript{194}.
Impaired Awareness of Hypoglycaemia

Scientific assessment of the impact of IAH on cognitive function during acute hypoglycaemia is hampered by the fact that there is not a validated, non-subjective means of defining the phenomenon. Moreover, as has been suggested already, it is also difficult to separate out the confounding impacts of recent antecedent hypoglycaemia and strict glycaemic control, all of which are inter-related. Heller et al studied 15 people with Type 1 diabetes - 11 with IAH and four with normal awareness of hypoglycaemia. Awareness was defined according to symptomatic responses during acute hypoglycaemia. Subjects with IAH had a longer duration of diabetes and lower glycated haemoglobin concentrations than subjects with normal awareness, and demonstrated comparable cognitive performance (as assessed by four-choice reaction time) during acute hypoglycaemia. Subjects were not matched for IQ. By contrast, Gold et al demonstrated statistical trends that Type 1 patients with IAH (defined on the basis of a self-rating scale, in which subjects scored how often they had symptoms during spontaneous episodes of hypoglycaemia) exhibited more profound cognitive dysfunction during acute hypoglycaemia, and that cognitive dysfunction persisted for longer following blood glucose recovery. This result is clearly at odds with many of the foregoing studies, but there is no doubt that the aware and unaware subjects in the study by Gold et al were matched for pre-morbid IQ, duration of diabetes, HbA1c, and exposure to previous episodes of severe hypoglycaemia. There was, however, a 0.5 standard deviation difference in current IQ between the groups (which was not statistically significant), with the unaware subjects having lower mean scores on the Alice Heim 4 task than the aware subjects, and it is conceivable that this may have confounded the study results.

The impact of the restoration of hypoglycaemia awareness, by the avoidance of hypoglycaemia, on the thresholds for cognitive dysfunction during acute hypoglycaemia has also engendered considerable controversy. Cranston et al studied 12 patients with Type 1 diabetes and IAH (six with good glycaemic control and six with poor control) before and after a period of three weeks of complete avoidance of hypoglycaemia (which took a mean of four months to achieve). After avoidance
of hypoglycaemia, hormonal and symptomatic responses during a stepped hypoglycaemic clamp were increased, but the threshold for cognitive dysfunction (as assessed by four-choice reaction time) remained unaltered at an arterialised plasma glucose concentration of 2.8 mmol/l. By contrast, Fanelli et al found, in 16 subjects with Type 1 diabetes of moderate duration and with IAH, that the meticulous prevention of hypoglycaemia for two weeks lowered the threshold for cognitive dysfunction during hypoglycaemia (i.e. occurred at a higher glucose concentration) and decreased the degree of cognitive dysfunction at a given glucose concentration. These changes were maintained following a year of hypoglycaemia avoidance and were also observed in subjects with IAH who had short-term Type 1 diabetes.

**Duration of Hypoglycaemia (Short-Term Cerebral Adaptation)**

The impact of the duration of hypoglycaemia on cognitive function, in effect a measure of the ability of the brain to adapt to hypoglycaemia, has been investigated in several studies. Kerr et al studied changes in reaction times in seven non-diabetic subjects whose arterialised blood glucose concentrations were clamped at 3.5 mmol/l for one hour and then reduced to 3.0 mmol/l for a further hour. Simple reaction times deteriorated initially when the glucose concentration was 3.0 mmol/l, but then improved towards baseline after 60 minutes of sustained hypoglycaemia, suggesting that cerebral adaptation had occurred. However, graphic data from the separate euglycaemic clamp studies did suggest that there was a learning effect for the reaction time task, and no statistical comparison of the results from the hypoglycaemia and euglycaemia clamps was reported. In a further study of six subjects with Type 1 diabetes, arterialised blood glucose levels were clamped at 2.8 mmol/l for 90 minutes. Reaction time scores deteriorated initially, but there was evidence of relative improvement in reaction time as the duration of hypoglycaemia increased. Changes in reaction times were not observed in the euglycaemia arm, although again no direct statistical comparisons were made.

By contrast Gold et al found no evidence of cerebral adaptation following 40-60 minutes of hypoglycaemia (arterialised blood glucose 2.5 mmol/l) in 24 non-diabetic subjects.
This study had several considerable strengths in that an extensive cognitive test battery was utilised with statistical adjustment for potential practice effects by comparing the hypoglycaemia clamp results with those obtained during a separate euglycaemic clamp. The duration of hypoglycaemia was relatively short and it is of course possible that cerebral adaptation could occur after more prolonged durations of hypoglycaemia.

Some evidence in support of this came from a remarkable study by Boyle et al of 12 non-diabetic subjects in whom chronic hypoglycaemia (arterialised blood glucose 2.9 mmol/l) was maintained for 56 hours. Before and after the period of chronic hypoglycaemia, subjects underwent a stepped hypoglycaemic clamp procedure during which cognitive function was repeatedly assessed using a four test battery. Prior to the period of chronic hypoglycaemia, performance in the Stroop and finger tapping tasks deteriorated at an arterialised plasma glucose concentration of 3.05 mmol/l. At the end of the period of chronic hypoglycaemia, performance in these tests did not deteriorate until arterialised plasma glucose had fallen to 2.5 mmol/l. The mechanism behind this putative cerebral adaptation appeared to be a preservation of brain glucose uptake during hypoglycaemia, rather than the utilisation of non-glucose fuels by the brain. However, the results of this study should be viewed with caution since the practice effects on the cognitive tasks were not controlled for and the statistical analysis compared performance at each glucose level within a clamp with its own baseline. Interaction analyses between the clamps were not performed, thereby increasing the risk of a spurious effect in line with the experimental hypothesis.

2.1.4 Clinical Importance of Cognitive Dysfunction during Acute Hypoglycaemia

If the psychometric test performance impairments observed during experimental studies of acute hypoglycaemia are clinically relevant, then these experimental findings have enormous potential implications for patients with diabetes whose performance at work or when driving may become impaired if hypoglycaemia supervenes. However, it is difficult to evaluate the relevance of most experimental
cognitive tasks to everyday life. Because driving is an important daily task involving many cognitive processes, several hypoglycaemia studies have been performed using driving simulators. Hoffman et al found no effect of hypoglycaemia (venous blood glucose 2.7 mmol/l) on driving, although only 10 of 18 subjects with Type 1 diabetes in the study were tested in a driving simulator that was relatively unsophisticated. Utilising a stepped hypoglycaemic clamp, Cox et al demonstrated that driving performance in 25 adults with Type 1 diabetes was significantly disrupted at venous plasma glucose concentration of 2.6 mmol/l, but not 3.6 mmol/l. Hypoglycaemia caused more swerving, spinning, time off the midline of the road and time off the road, as well as more compensatory slow driving. The individual differences in driving performance during hypoglycaemia were stable at re-testing three months later. The driving simulator was, however, relatively basic with non-demanding driving scenarios that lasted only five minutes. In a more recent study, Cox et al evaluated driving performance in 37 adults with Type 1 diabetes during progressive hypoglycaemia, utilising a more sophisticated simulator. The simulator had a wrap-around screen, providing a 160 degree visual field, and a programmed 16 mile course that took approximately 30 minutes to complete. In this study, even relatively minor levels of hypoglycaemia (arterialised blood glucose concentrations 3.4-4.0 mmol/l) were associated with significant driving impairments. Subjects were generally aware of their impaired driving, but the mean glucose level at which subjects took corrective actions (either stopping driving or ingesting carbohydrate) was 2.7 mmol/l. Of the 14 subjects who exhibited severely impaired driving, only eight took corrective actions.

These driving simulator studies have been criticised for not reliably reproducing real driving experiences. Heller and Macdonald suggested that subjects may regard driving simulators as a novelty and the resulting increased arousal could reduce the sensitivity of the task during hypoglycaemia. They also suggested that experimentally-induced hypoglycaemia in a driving simulator would be unlikely to reproduce the effect of mild hypoglycaemia upon a driver after hours of driving along a motorway, where boredom and fatigue are other important factors to influence driving performance. The fact remains, though, that the studies by Cox
and colleagues provide compelling evidence of the deleterious effects of hypoglycaemia on driving ability and highlight the importance of educating people with diabetes about the potential dangers of hypoglycaemia.

2.1.5 Effects of Acute Hypoglycaemia on Mood

The impact of acute hypoglycaemia on mood states has attracted considerably less attention than its effects on cognitive function. Hepburn et al administered the Mackay and Cox Mood Adjective Checklist to 12 non-diabetic men and 15 men with Type 1 diabetes during an insulin infusion technique in which venous blood glucose fell to a mean nadir of approximately 1.5 mmol/l. Levels of tense arousal rose in both groups while energetic arousal decreased significantly. Hypoglycaemia therefore resulted in the development of a state of ‘tense tiredness’. In a further study of two adrenalectomised patients (who had no endogenous adrenaline secretion in response to hypoglycaemia) and 25 healthy non-diabetic subjects, the UWIST Mood Adjective Checklist (MACL) was administered during a similar insulin infusion protocol. The UWIST MACL measures levels of hedonic tone (happiness), tense arousal and energetic arousal. During hypoglycaemia in the healthy subjects, levels of hedonic tone and energetic arousal fell, while levels of tense arousal rose. By contrast, in the two adrenalectomised subjects, there was no change in levels of tense arousal and hedonic tone, following induction of hypoglycaemia, although energetic arousal decreased significantly. These data were interpreted as suggesting that adrenaline secretion during hypoglycaemia may mediate changes in tense arousal and hedonic tone, while alterations in energetic arousal be a direct effect of neuroglycopenia. The major drawback of the above studies was the absence of a euglycaemia control arm, but this was included in a study of 24 non-diabetic subjects by Gold et al who utilised a clamp technique to induce hypoglycaemia (arterialised blood glucose 2.5 mmol/l). Similar results were obtained, with hypoglycaemia resulting in reduced levels of hedonic tone and energetic arousal, and increased levels of tense arousal.

The effects of acute hypoglycaemia on anger have been investigated in at least two studies. Merbis et al assessed mood, using the Profile of Mood States, in 10 subjects
with Type 1 diabetes (nine of whom were male), during a stepped hyperinsulinaemic glucose clamp\textsuperscript{205}. Hypoglycaemia (venous blood glucose 2.0 mmol/l) was associated overall with increased levels of anger, but there was large inter-individual variability. Those subjects who had high pre-test levels of hostility tended to demonstrate higher levels of anger during hypoglycaemia.\textsuperscript{205} McCrimmon \textit{et al} also reported that hypoglycaemia (arterialised blood glucose 2.6 mmol/l), induced using a hyperinsulinaemic glucose clamp technique, caused a significant increase in feelings of anger in 18 non-diabetic subjects and 30 subjects with Type 1 diabetes\textsuperscript{206}. In this study, anger was measured using the State-Trait Anger Expression Inventory and, in contrast to the study by Merbis \textit{et al}\textsuperscript{205}, there was no clear association between an individual's change in reported anger during hypoglycaemia and measures of anger trait and anger expression.

2.1.6 \textit{Summary of Effects of Acute Hypoglycaemia on Cognitive Function}

Although individual studies have demonstrated cognitive decrements at glucose concentrations between 3.0-3.4 mmol/l, all domains of cognitive function appear to be consistently impaired at blood glucose concentrations of 2.9 mmol/l and below. Acute hypoglycaemia increases levels of anger and results in the development of a mood state that can be described as 'tense tiredness'. Studies with driving simulators strongly suggest that the cognitive decrements that occur during experimental hypoglycaemia are clinically relevant. Individual differences in susceptibility to the cognitive effects of acute hypoglycaemia are likely to be mediated by a large number of interacting factors. There is some evidence to suggest that older people and men are more susceptible to hypoglycaemia and less convincing evidence that the presence of diabetes per se and high intelligence also confer a greater predilection for cognitive impairment as glucose concentrations fall. With regard to individuals with Type 1 diabetes, duration of disease, age of onset and counterregulatory responses do not appear to be important determinants of cognitive decrements during hypoglycaemia, but there is evidence that prolonged exposure to hypoglycaemia results in cerebral adaptation and subsequent better cognitive performance.
The greatest controversy surrounds the impact of IAH, antecedent strict glycaemic control and antecedent hypoglycaemia. Some studies have supported the premise that glycaemic thresholds for cognitive dysfunction are shifted to lower glucose concentrations in people with IAH (and/or strict glycaemic control and/or exposure to recent antecedent hypoglycaemia), in a manner analogous to the well-recognised downward shift in glycaemic thresholds for counterregulatory responses and symptoms. However, other studies have not demonstrated any shift in glycaemic thresholds for cognitive dysfunction in these patient groups. It is probable that methodological differences between the studies, particularly small sample sizes, are responsible for these discrepant results. It has been noted that, after discounting investigations with methodological limitations, those studies that have demonstrated a shift in cognitive thresholds have utilised a battery of cognitive tests or the P300 evoked potential. By contrast, those studies that have not demonstrated any change in thresholds have often utilised only one or two cognitive tasks. This finding is open to two contrasting explanations. It is possible that not all cognitive tasks are affected to the same degree by IAH/strict glycaemic control/antecedent hypoglycaemia, thereby making small test batteries inadequate. Alternatively, the sympa-tho-adrenal response to hypoglycaemia may itself interfere with certain cognitive tasks, notably psychomotor tests, by its propensity to induce tremor and sweating, making large cognitive test batteries, in effect, over-sensitive.

Further studies are clearly required to address this important issue. If glycaemic thresholds for cognitive function are not altered in patients with impaired hypoglycaemia awareness, then this would imply that the upregulation of GLUT 1 glucose transporters, one of the mechanisms which may underpin the development of this phenomenon, is centred around only the glucose-sensing areas of the brain. By contrast, if glycaemic thresholds for cognitive dysfunction do shift to lower glucose concentrations, then this would imply that the upregulation of GLUT 1 occurs throughout widespread areas of the brain.
2.2 EFFECTS OF RECURRENT SEVERE HYPOGLYCAEMIA ON COGNITIVE FUNCTION

Concerns about the long term effects of hypoglycaemia on the brain first surfaced in the 1930's and 1940's following a series of case reports describing the development of permanent decrements of cognitive performance and personality in patients with insulin-treated diabetes who had experienced episodes of severe hypoglycaemia\(^4\)\(^3\)\(^2\)\(^8\)\(^2\(^1\)\(^1\). The impact of these reports was further heightened by the recognition that insulin-shock treatment for psychotic illnesses (in which severe hypoglycaemia was induced for prolonged periods) could also result in permanent neurological and cognitive dysfunction\(^2\(^1\)\(^2\)\(^1\(^5\). More recently, Gold et al described significant changes in cognitive and social function in a self-selected group of five patients with Type 1 diabetes of long duration (24-47 years), all of whom had experienced multiple episodes of severe hypoglycaemia\(^2\(^1\)\(^6\).

As a consequence of these observations, considerable research interest has focused on the impact of recurrent episodes of severe hypoglycaemia on cognitive function in both adults and children with Type 1 diabetes. This has considerable potential clinical significance. If a link between severe hypoglycaemia and cognitive decrements could be proven, then glycaemic targets for people at increased risk of severe hypoglycaemia would need re-evaluation in order to protect cerebral function. This research has, however, engendered considerable controversy as controlled studies have reached strikingly different conclusions\(^2\(^1\)\(^7\); this has served to further highlight the difficulties of assessing cognitive function in diabetes\(^9\)\(^1\(^4\)\(^6\). The following sections will provide a detailed description of the evidence for and against the hypothesis that recurrent severe hypoglycaemia causes cognitive decrements.

2.2.1 Methodological Considerations

Many of the methodological considerations that were raised in Section 2.1.1 regarding investigations of the effects of acute hypoglycaemia on cognitive function, particularly those concerning the limitations of cognitive testing, are relevant to the
literature examining the longer-term effects of hypoglycaemia. There are some additional methodological issues, however, that are pertinent to this area.

The literature examining the relationship between hypoglycaemia and cognitive performance in adults with Type 1 diabetes can be grouped into three main categories:

1. Case-control studies comparing cognitive function in diabetic and non-diabetic subjects, with subsequent attempts to correlate cognitive performance in the diabetic cohort with previous exposure to severe hypoglycaemia.

2. Case-control studies comparing diabetic subjects with and without previous exposure to recurrent episodes of severe hypoglycaemia.

3. Prospective studies.

Case-control studies are subject to a variety of different problems, none the least that the cross-sectional design does not allow direction of causality to be assumed. Moreover, cognitive function is subject to the influence of a large range of differing factors including age, gender, concurrent medical disorders (e.g. dementia, hypothyroidism and chronic obstructive airways disease), drugs with effects on the central nervous system (e.g. beta-adrenoreceptor blocking agents and antidepressants), alcohol consumption, smoking, and impaired sensory or motor function (e.g. poor eyesight, hearing or hand co-ordination)\textsuperscript{218}. In small case-control studies, these factors may potentially confound any potential association between cognitive function and recurrent severe hypoglycaemia, unless attempts are made to control for these in the matching of the subjects in the two groups. Foremost among potential confounding factors is pre-morbid intellectual ability, that is the highest level of ability attained prior to any cognitive decrements taking place\textsuperscript{219}. A variety of retrospective methods may be used to assess, and match, subjects' pre-morbid cognitive function. These include educational histories, academic achievement in standard examinations and occupational histories. These variables, however, are limited in that they may be influenced in individuals by factors such as lack of opportunity rather than lack of ability\textsuperscript{220}. An alternative is to use the National Adult
Reading Test (NART) as a more useful measure of pre-morbid IQ\textsuperscript{220}. In this task, subjects are asked to read aloud 50 standard English words using the correct pronunciation. The number of errors made correlates well with pre-morbid intellectual function, as word reading ability tends to be preserved even in generalised cognitive decline\textsuperscript{220}.

Another major potential problem with utilising a case-control design in an attempt to determine the impact of recurrent severe hypoglycaemia on cognitive function is that the assessment of previous severe hypoglycaemia history is likely to be retrospective. This may introduce a large element of error into the subsequent interpretation of results. The best way of overcoming this is to employ a prospective study design, which additionally allows causality to be determined. However, a potential difficulty with prospective studies is that the period of follow-up may be limited and not of sufficient duration to allow any cognitive decrements to develop.

2.2.2 Recurrent Hypoglycaemia in Adults with Type 1 Diabetes

Early Case-Control Studies

In one of the earliest reports, Bale administered the Walton-Black New Word Learning Test to 100 adults with Type 1 diabetes of relatively long duration (average 23.5 years)\textsuperscript{221}. Overall 17% of the diabetic subjects had scores which were classified in the 'brain damaged' range of the test, while no subjects in an age-matched, non-diabetic control group exhibited such scores. There appeared to be a correlation between cognitive performance and hypoglycaemia in that 30% of subjects who had been admitted to hospital in the past with an episode of hypoglycaemia had scores in the 'brain-damaged' range compared with 20% of subjects who had experienced severe hypoglycaemia, but had never required hospital admission, and 4% of subjects who had never experienced severe hypoglycaemia\textsuperscript{221}. Skenazy and Bigler also purported to demonstrate an association between severe hypoglycaemia and neuropsychological performance in 39 subjects with diabetes, 20 of whom were visually impaired secondary to diabetic retinopathy\textsuperscript{222}. However, the results of this study are open to question as, in contrast to most other studies, the authors found that those patients with the most severe diabetic complications actually
performed better on some measures of cognitive performance\textsuperscript{222}. In neither study were subjects matched for pre-morbid cognitive ability and little attention was paid to the impact of other potential confounding factors.

**Later Case-Control Studies**

Using a more sophisticated cognitive test battery, Wredling et al compared the neuropsychological function of 17 patients with Type 1 diabetes who had a self-reported history of repeated episodes of severe hypoglycaemia and 17 patients with Type 1 diabetes who had never experienced severe hypoglycaemia\textsuperscript{223}. Subjects were matched for age, sex, duration of diabetes, educational achievement, employment status and presence of microvascular complications; cognitive function was assessed using an extensive cognitive battery. Subjects with a previous history of severe hypoglycaemia scored lower on measures of motor ability, memory and visuospatial tasks assessing problem-solving ability\textsuperscript{223}. In another study, patients with Type 1 diabetes and a history of severe hypoglycaemia performed more poorly than diabetic control subjects, without previous severe hypoglycaemia, on only one test (word-list recall) from a seven-test battery\textsuperscript{224}. Subjects were not matched for pre-morbid intelligence and there were no controls for multiple statistical testing\textsuperscript{224}.

If the above studies are accepted at face value, then it is possible for their results to be interpreted in two ways: either recurrent severe hypoglycaemia causes cognitive impairment or that subjects with pre-existing cognitive impairment are prone to experiencing recurrent episodes of severe hypoglycaemia. Langan and colleagues attempted to refute this latter interpretation by including a measure of pre-morbid cognitive function (the NART) in an extensive cognitive test battery which was applied to 100 subjects with Type 1 diabetes\textsuperscript{225}. Only patients who had developed diabetes after adolescence were studied, to remove the potential deleterious impact of childhood diabetes on peak intellectual attainment, and rigorous entry criteria excluded patients with a history of severe alcoholism, cerebrovascular disease, head injury, epilepsy, psychiatric disorder or use of psychotropic medication. The authors also went to considerable lengths to corroborate the subjects' retrospective assessment of their experience of severe hypoglycaemia by checking case records
from hospitals and general practitioners and by interviewing family members of the subjects.

Frequency of severe hypoglycaemia correlated with current performance IQ level ($r = -0.32$) and IQ decrement (i.e. the difference between pre-morbid IQ and current performance IQ; $r = 0.33$), but not with pre-morbid IQ ($r = -0.09$). Severe hypoglycaemia was also associated with slower and more variable reaction times. To estimate the extent of intellectual impairment, two sub-groups were compared: 24 subjects with no previous hypoglycaemia and 23 diabetic subjects with five or more episodes of hypoglycaemia. The authors concluded that a history of five or more episodes of severe hypoglycaemia was associated with a modest IQ decrement of 5-6 points$^{225}$. The cognitive performance of the whole group of 100 diabetic subjects was subsequently compared with 100 health control subjects, individually matched for age, sex, education and social class$^{226}$. After adjustment for pre-morbid IQ, the diabetic group had lower verbal and performance IQs, as assessed by the Wechsler Adult Intelligence Scale, than the control group. The difference in performance IQ between the groups was eliminated after adjustment for previous hypoglycaemia history. The difference in verbal IQ, however, persisted and it was postulated that this may be the result of the social impact of diabetes$^{226}$. A different research group, employing similar methodology, also demonstrated a significant correlation between decline in performance IQ and frequency of previous episodes of severe hypoglycaemia ($r = -0.22$)$^{227}$.

More recently, Kramer et al failed to find any effect of severe hypoglycaemia on cognitive function employing a case-control study design$^{228}$. However, subjects were not matched for pre-morbid intelligence and the neuropsychological test battery was very limited, comprising only the Mini-Mental State Examination (MMSE) and Trail-Making Test, Part A (TMA). The scores from the MMSE exhibited a striking 'ceiling effect' in that the mean score ($\pm$ standard deviation) for patients with no history of hypoglycaemic coma was 29.5 ($\pm0.9$), and for those with a history of hypoglycaemic coma 29.6 ($\pm0.7$). However, the maximum score for this test is 30
and so it may not have been difficult enough to allow detection of differences in performance between the study groups.

**Prospective Studies**
The results of the majority of these cross-sectional, case-control studies have not, however, been replicated in two large studies in which exposure to severe hypoglycaemia was evaluated prospectively\(^{21,22}\). In the Stockholm Diabetes Intervention Study, there was no difference in cognitive function over 7.5 years between 102 diabetic subjects managed with either conventional or intensive treatment, despite the fact that the latter group experienced significantly more episodes of severe hypoglycaemia\(^{53}\). On its own, the study could be criticised for insufficient separation of the groups based on patients' experience of hypoglycaemia, the small sample size and resultant low statistical power, a brief neuropsychological test battery and a relatively short period of follow-up\(^{217}\). However, similar results were obtained in the 1441 participants of the DCCT over 6.5 years of follow up\(^{229}\). In this study, neuropsychological function was assessed with a large test battery at three time points (two, five and seven years) and attrition was minimal, with 98% of the expected test sessions completed. As has already been discussed though, the period of follow-up may have been insufficient, while the subjects were relatively young and had little or no exposure to hypoglycaemia before the study. In fact, only 23 subjects experienced more than five episodes of hypoglycaemic coma or seizure during the trial. Moreover, the data from the neuropsychological test battery was grouped arbitrarily into eight cognitive domains, none of which were directly comparable to performance or general IQ. This latter drawback was overcome by a re-analysis of the DCCT data using a factor analysis approach, in which the cognitive data was grouped into statistically robust cognitive domains; as in the initial analysis, no effect of hypoglycaemia on cognitive performance was demonstrated\(^{210}\).

**2.2.3 Recurrent Hypoglycaemia in Children with Type 1 Diabetes**
The majority of investigations on cognitive function in children with diabetes have been case-control studies. These studies have consistently demonstrated cognitive decrements in children and adolescents with diabetes, compared with non-diabetic
children, and have implied that one of the strongest predictors of impaired cognitive performance is early onset of diabetes (i.e. less than six years of age)\textsuperscript{231-236}. As a group, such children with early onset diabetes tend to have a higher prevalence of clinically significant impairment\textsuperscript{233} and are more likely to have repeated a year in school\textsuperscript{234,237}. Children or adults who develop diabetes after 5 or 6 years of age show a much less consistent level of cognitive impairment\textsuperscript{147}. Several studies have demonstrated no evidence of cognitive deficits in this late onset group\textsuperscript{234,236,238}, while others have demonstrated the presence of cognitive decrements\textsuperscript{224,239,240}.

\textbf{Case-Control Studies}

It has been postulated that recurrent exposure to severe hypoglycaemia may account for the cognitive decrements seen in children with early-onset diabetes\textsuperscript{241}. Young children have higher energy requirements to sustain optimal growth and brain development, and their immature brains appear to be particularly prone to any type of traumatic or neurotoxic insult\textsuperscript{242-244}. Indeed, the occurrence of hypoglycaemia in non-diabetic neonates is associated with subsequent retarded development and lower IQ\textsuperscript{245}. Moreover, very young children with diabetes are far more likely to experience severe hypoglycaemia than older children\textsuperscript{232-234}. This is presumably because episodes of hypoglycaemia may go unrecognised in non-verbal children (who lack the capacity to perceive and communicate early episodes) and because food intake and activity levels are unpredictable in young children, making it difficult for parents and physicians to prescribe appropriate insulin doses\textsuperscript{241}.

Fallstrom demonstrated that diabetic children had elevated levels of behavioural disturbance, anxiety and perceptual difficulties, relative to non-diabetic children, and that the severity of these abnormalities correlated with the frequency of previous hypoglycaemic convulsions\textsuperscript{246}. Impairment of psychomotor efficiency and attention was also demonstrated in a small cohort of children with early-onset diabetes who had experienced episodes of severe hypoglycaemia early in childhood, but not in children who had experienced severe hypoglycaemia only in late childhood\textsuperscript{238}.
Moreover, several studies have demonstrated electrophysiological abnormalities in children with diabetes and have linked these changes with previous exposure to severe hypoglycaemia. For example, in a large series of 300 diabetic children, those with a previous history of severe hypoglycaemia were nearly twice as likely to show abnormalities on electroencephalograms (typically a non-specific abnormality).

However, the major problem with all of the above studies is that it is difficult to see how accurate estimates of previous hypoglycaemia history could have been obtained retrospectively.

**Prospective Studies**

There have been several studies on cognitive function in children with diabetes in which hypoglycaemia history has been prospectively ascertained. However, most are too small to allow robust conclusions to be drawn. Golden et al followed 23 diabetic children (mean age 6) over a 6 to 72 month period and found that number of episodes of asymptomatic hypoglycaemia correlated with impaired performance on the abstraction and visual reasoning subtests of the Stanford-Binet task. The size of the cognitive decrements was, however, small and there were no instances of clinically significant impairment. Rovet and Ehrlich evaluated 16 children over a seven year period from diagnosis. A significant decline in verbal, but not in visuospatial, ability was apparent particularly in those children who had experienced seizures during hypoglycaemia. At the seven year assessment, those children with hypoglycaemic seizures exhibited deficits on perceptual, motor, memory and attention tasks. In another study, 25 older diabetic children (average age 12 years) were followed from diagnosis over approximately 2.5 years. Thirteen children were randomly allocated to intensive insulin therapy and 12 to conventional treatment. Those children receiving intensive insulin therapy had a threefold increased risk of hypoglycaemia and performed less well on a spatial declarative memory task. However, this test was part of a very large cognitive battery, which included several other tests of memory function. The other memory tests showed no statistically significant between-group differences, although there was a tendency for
intensively-treated children actually to perform better on several measures. Therefore, given that no adjustment was made for multiple statistical testing, it is likely that the significant result in the spatial declarative memory task was a type 1 error.

Interim data from a large prospective study of cognitive performance in children with diabetes has recently been reported\textsuperscript{239,240}. Neuropsychological testing was performed on 123 children with new-onset Type 1 diabetes, aged between 3 and 14 years, three months after diagnosis and again at two years. At comparable time points, 129 non-diabetic children, matched for age and sex, were also evaluated. There was no difference in the cognitive performance of the groups at the first testing session; however, at two years there was a non-significant trend for IQ to have fallen in the diabetic children overall. There was a significant main effect of age on change in performance IQ, with younger children (i.e. children who diabetes when aged five or less) exhibiting larger drops in IQ\textsuperscript{239}. Such children with early-onset diabetes experienced significantly more episodes of severe hypoglycaemia (defined in this study as a blood glucose concentration of less than 2.0 mmol/l). In older children, who were able to undergo more detailed cognitive testing, recurrent severe hypoglycaemia was associated with impaired performance of several short- and long-term memory tasks\textsuperscript{240}.

2.2.4 Summary of Effects of Recurrent Hypoglycaemia on Cognitive Function

In adults with Type 1 diabetes, data from case-control studies has suggested that exposure to recurrent episodes of hypoglycaemia in adulthood may be associated with the development of modest cognitive impairment. However, two prospective studies have failed to demonstrate any association. Even in the cross-sectional studies, the cognitive decrements reported were relatively mild and so it may be possible to offer a degree of reassurance, both to people with diabetes and to their medical attendants, that recurrent severe hypoglycaemia in most adults probably causes at worst only mild cerebral damage. However, in some of the studies there were large inter-individual differences in the effects of recurrent hypoglycaemia on cognitive performance. The cases reported by Gold et al should also act as a
reminder that in some adults recurrent exposure to severe hypoglycaemia may have devastating effects on cognitive function. The future challenge is to identify the factors that make individuals vulnerable to hypoglycaemia-induced cognitive impairment.

Children with early-onset diabetes perform less well on subtle measures of cognitive performance. There are good theoretical reasons to suspect that repeated exposure to hypoglycaemia may contribute to the development of these cognitive decrements and this has been supported by cross-sectional, case-control studies. However, ascertainment of hypoglycaemia histories retrospectively in children is a strategy of questionable accuracy. Prospective studies reported to date have mainly been small with very short follow-up periods, but have suggested that hypoglycaemia is associated with cognitive dysfunction in children with early-onset diabetes. However, the cognitive decrements that have been described are small, with diabetic children performing, in general, within 'normal' ranges.
CHAPTER 3

HYPOTHESES FOR STUDIES
The following chapters describe four studies that were performed to expand clinical knowledge of the impact and aetiology of hypoglycaemia in patients with Type 1 diabetes.

3.1 ACUTE HYPOGLYCAEMIA, THE PERIPHERAL NERVOUS SYSTEM AND INFORMATION PROCESSING

The primary aim of Study 1 was to investigate further the basic neuronal processes affected by acute hypoglycaemia, by attempting to determine if acute hypoglycaemia has any effects on the function of the peripheral nervous system, in comparison to its well established deleterious impact on the functions of the central nervous system. Few studies have considered the effects of acute hypoglycaemia on peripheral neural function, although a peripheral motor neuropathy has been described following profound hypoglycaemia of prolonged duration\textsuperscript{254}. By studying the effects of hypoglycaemia on peripheral neural function, insights may be afforded into the basic metabolic processes within peripheral neurones and may help determine if there is any evidence of a differential reliance on glucose as a metabolic fuel between the central and peripheral nervous systems.

A secondary issue that this study aimed to address was the validity of utilising peripheral nerve conduction velocities as a marker of speed of neuronal function within the central nervous system. This potential association was investigated on the basis of the premise that speed of neural function within the central nervous system may be one of the physiological determinants of speed of information processing and ultimately of intelligence\textsuperscript{255}. Some studies have suggested that peripheral nerve conduction velocities may be a surrogate marker of speed of information processing and intelligence\textsuperscript{256,257}. However, such a hypothesis would imply that any external factor (such as hypoglycaemia) that diminished intelligence should also have a similar impact on peripheral neural function. Therefore, by comparing multiple levels of information processing during hypoglycaemia in the same group of subjects, the lower level processes that may contribute to the decrements in higher-
level mental performance (which putatively include peripheral nerve conduction) can be investigated.

3.2 RECOVERY FROM SEVERE HYPOGLYCAEMIA

Severe hypoglycaemia, as has been described in Chapters 1 and 2, is one of the most feared consequences of insulin therapy for diabetes. The effects of milder levels of acute hypoglycaemia on cognitive function have been well documented, as these may readily be studied under experimental conditions. However, studies of the immediate (as opposed to long-term) cognitive and mood sequelae following severe hypoglycaemia have attracted little attention, presumably because of the difficulty of studying such phenomena under controlled conditions. The aim of Study 2 was to attempt to determine how long cognitive function and mood states took to recover following an episode of spontaneous severe hypoglycaemia in people with insulin-treated diabetes. There are unpublished anecdotal reports of cognitive function taking several days to weeks to recover following an episode of severe hypoglycaemia in which complete neurological recovery was apparent, but no previous studies have investigated this issue formally. The results of this study were anticipated to provide practical information for professionals who care for people with diabetes. For example, the results could potentially be useful in educating patients about the appropriate time to return to activities such as work or driving following an episode of severe hypoglycaemia.

3.3 DETERMINING PROGNOSIS AFTER SEVERE HYPOGLYCAEMIA

The availability of a simple blood test that could help predict clinical outcome in patients who are comatose following an episode of severe hypoglycaemia, in a manner analogous to the measurement of serum concentrations of creatine kinase and troponin following myocardial infarction, would be of considerable value to clinicians. Following acute stroke, the serum concentrations of two proteins,
Neurone-Specific Enolase (NSE) and Protein S-100 (S-100), have been shown to be elevated, and the levels correlate with clinical outcome\textsuperscript{258,259}. The purpose of Study 3 was to investigate whether these markers of neuronal damage could have any relevance in determining prognosis following severe hypoglycaemia. Therefore, it was planned to measure changes in serum concentrations of NSE and S-100 following episodes of severe hypoglycaemia in which either complete neurological recovery occurred, or in which the patient subsequently died.

3.4 INSULIN LISPRO AND POST-PRANDIAL HYPOGLYCAEMIA

Insulin lispro has been advocated as a therapy to reduce the risk of occurrence of hypoglycaemia, particularly episodes at night\textsuperscript{132}. Insulin lispro has a very rapid onset of action, so it is recommended that it is injected immediately before food\textsuperscript{125}. However, in theory, if the normal post-prandial rise in blood glucose concentrations after a meal was reduced or delayed in magnitude, the pre-prandial injection of insulin lispro could cause early post-prandial hypoglycaemia. Such a situation could occur following the ingestion of a meal rich in fat (as is often the case in Scotland), since fat exerts a retardant effect upon gastric emptying\textsuperscript{260}. Therefore, in Study 4, the differential effects of pre- and post-prandial insulin lispro therapy were compared following four test meals in which the relative proportions of fat and carbohydrate, and the relative proportion of solid and liquid phase components, were varied.
CHAPTER 4

METHODS: COGNITIVE FUNCTION TESTS AND NERVE CONDUCTION STUDIES
During the course of Studies 1 and 2, detailed cognitive and mood testing was performed. All of the cognitive and mood tests utilised were standard and well validated tasks in common usage in psychological research and clinical practice. To limit repetition, the theoretical basis and format of each cognitive and mood test are described in full in this chapter. Nerve conduction studies were also performed in Study 1. The theoretical basis and the potential sources of error of the nerve conduction measurements made in Study 1, along with the methods employed, are also described in this chapter.

4.1 COGNITIVE FUNCTION TESTS

4.1.1 National Adult Reading Test (NART)
The NART provides a measure of 'best' global cognitive function ever attained, irrespective of time. Subjects are asked to read out aloud a list of 50 irregular English words (such as chord, prelate and campanile) and are marked on their ability to pronounce the words correctly, according to a standardised scoring system. The number of errors made is recorded. The NART has been validated against the Wechsler Adult Intelligence Scale - Revised and so, using the error score obtained and standard tables, it is possible to estimate figures for 'best ever' full-scale, verbal and performance IQ.

4.1.2 Alice Heim 4 Test (AH4)
The AH4 test is a measure of current intellectual function and is divided into two parts. Each part is preceded by practice items and the subject is then allowed 10 minutes to complete as many questions as possible. A choice of potential answers is provided for each question. The first part of the test measures verbal and numerical skills, while the second part assesses visuospatial skills. The maximum score for each part is 65 and the total score provides a measure of global cognitive ability. The test does not provide an actual IQ score.
4.1.3 Wechsler Adult Intelligence Scale - Revised (WAIS-R)
The 'performance' subtests of the WAIS-R provide a measure of current intellectual level\textsuperscript{14}. The performance sub-tests of the WAIS-R are: Picture Completion, Picture Arrangement, Block Design, Object Assembly and Digit Symbol.

**Picture Completion**
Subjects are shown a set of 20 picture cards of everyday objects or situations. In each picture something is missing e.g. the door handles from a car, or the tuning peg of a violin. Subjects are given 20 seconds to identify the missing part of each card, and the total number of correct responses is recorded.

**Picture Arrangement**
Subjects are presented with a series of pictures in a random order and are asked to arrange them in an order (from left to right) that tells a logical story. There are 10 sets of cards of increasing difficulty and there is a time limit for arranging each card set. The number of correct arrangements is recorded.

**Block Design Task (BD)**
Subjects are asked to reproduce, in as short a time as possible, a series of two-dimensional patterns using a set of nine identical cubes. Marks are awarded on a structured scoring system dependent on the time taken to complete each pattern.

**Object Assembly**
Subjects are presented, in random order, with a series of pieces of card that can be assembled to create a recognisable object, such as a manikin. For each set of cards, subjects are asked to assemble the object within a time limit and are scored on the number of pieces that are correctly joined together.

**Digit Symbol (DS)**
Nine digits are represented by nine different symbols, and subjects are required to write down the appropriate symbol for each in a given array of numbers over 90 seconds. The number of correct responses is recorded.
4.1.4 Digit Span
Digit Span is one of the verbal IQ sub-tests of the Wechsler Adult Intelligence Scale - Revised\textsuperscript{44}. Subjects are asked to repeat strings of numbers in either the precise order in which they are read out (Forward Digit span, FDS), or in the reverse order (Backward Digit Span, BDS). For each test, the number of correct responses is recorded.

4.1.5 Trail-Making Test B (TMB)
TMB is a test of mental flexibility from the Halsted Reitan Neuropsychological Battery\textsuperscript{143}. The subject has to connect correctly an alternating series of numbers (1-13) and letters (A-L) - which are dispersed on a standard A4 page - in their respective orders, as quickly as possible. The time taken (in seconds) to complete the task correctly is recorded. To limit the effects of practice, nine different versions of this task are available and have been validated for equal degrees of difficulty. In the present studies, two versions of the test were administered to subjects at each testing interval; the mean scores for the two tests are reported. The individual test versions were administered in random order such that no subject performed the same trail more than once and all the tests were utilised equally and in different orders.

4.1.6 Logical Memory (LM)
LM, a subtest of the Wechsler Memory Scale, assesses verbal memory function\textsuperscript{262}. A paragraph of text is read at a standard rate to subjects who are asked to repeat the text verbatim, both immediately afterwards (immediate recall) and after a period of 30 minutes (delayed recall). Marks are awarded on the basis of a structured scoring system. To limit the effects of practice, three different versions of the test are available and have been validated for equal degrees of difficulty. In the present studies, a different version of the test was administered at each testing session; the order in which the different versions were utilised remained constant.

4.1.7 Figural Memory (FM)
FM, a subtest of the Wechsler Memory Scale, assesses non-verbal memory function\textsuperscript{262}. Subjects are asked to attempt to memorise a series of diagrams within a
set time period. Subjects are then shown another numerically larger set of diagrams and are instructed to select from these the diagrams that had been seen previously. The total number of correct responses is recorded.

4.1.8 Borkowski Verbal Fluency Task
Verbal fluency assesses executive functions, which are assumed to involve frontal lobe activity\textsuperscript{263}. Patients are given 60 seconds to state as many words as possible, beginning with a letter of the alphabet specified by the tester. In the present studies, each subject gave responses to four letters - 'J', 'S', 'U' and 'M'. The total number of words obtained, excluding repetitions, was noted.

4.1.9 Stroop Task
The Stroop distraction task provides a very sensitive measure of cognitive decrement\textsuperscript{264}. However, the precise mental abilities examined are unclear, but are thought to include attention and speed of information processing. The subject is provided with a series of eight cards each containing 10 rows of five words (or in two instances, blocks of asterisks). Each word is printed in one of four colours of ink. Subjects are asked to read out, as quickly as possible and from left to right and top to bottom, the colour of ink that the individual words are printed in, rather than the words themselves. There are four pairs of cards: 1) asterisks; 2) common-words (e.g. dog); 3) colour-associated words (e.g. grass, sky, blood) in incongruent colours; 4) incongruent colour names (e.g. RED written in yellow ink). A 'practice' card is also administered in which colour names are printed in black ink and the subject simply reads the colour names out aloud. The time taken to read the practice card is subtracted from the average time for each of the four pairs of test cards.

4.1.10 Paced Auditory Serial Addition Task (PASAT)
The PASAT assesses attention, concentration, and general intellectual ability\textsuperscript{265}. Subjects listen to a series of numbers that are added together according to the rule: 'add each number that you hear, to the number that preceded it'. After practice, two trials of 61 digits are performed, with four and two second intervals between
successive digits, respectively. The total number of correct responses for each test is recorded (the maximum is 60).

4.1.11 Speed of Information Processing Task (SIP)
The SIP task is a subtest of the British Ability Scales. Subjects are presented with a card on which 25 rows of five numbers are printed. The complexity of the numbers increases from the top to the bottom of the page. Subjects are asked to circle the highest number on each row as quickly as possible. Two cards are presented to each subject: one containing 'easy' numbers which are 1 to 4 digits in length, and the other containing 'difficult' numbers which are 3 to 6 digits in length. For each card, 'easy' and 'difficult', the time taken to complete the task is recorded, as is the number of errors made.

4.1.12 Inspection Time (IT)
IT is a two-alternative, forced-choice, discrimination task which assesses the efficiency of the early stages of visual information processing using psychophysical procedures. Subjects are instructed to view a screen composed of multiple light-emitting diodes on which numerous stimuli consisting of two parallel vertical lines, of markedly different lengths, are presented sequentially. Subjects have to indicate which of the two vertical lines is the longer. During the test, the experimenter varies the stimulus duration, with briefer durations being more difficult. Only correctness of response is recorded; response speed is not measured, and the subject is instructed to respond at leisure to achieve maximum accuracy. The IT test's adaptive staircase algorithm measures the stimulus presentation time (in milliseconds) required by each subject to achieve 85% accuracy in responding (50% representing chance responding).

4.1.13 Visual Change Detection (VCD)
VCD, like IT, assesses speed of early visual processing. The stimulus display consists of 49 small, non-adjacent rectangles scattered on a computer monitor screen to which, after a variable interval, a single (target) rectangle is added. The subject's task is to identify the additional rectangle. The time interval between the onset of the
49-rectangle array and the target is manipulated by the experimenter; tests with shorter intervals are more difficult. As with IT, response time is not measured. Each subject is tested on a random block of 50 presentations; 10 trials of each of five different stimulus durations are presented at random. A total score of accuracy of responding is obtained.

4.1.14 Reaction Time (RT)

Reaction time provides a measure of information processing at the cognitive-experimental level\textsuperscript{268}. In the present studies a Hick-type reaction time device was utilised. The device, by employing a 'home' button, eight equidistant response buttons (which may be individually covered to provide the desired level of choice) and two timers, allows the separation of the decision and movement components of reaction times. The subject is asked to press the home button with the dominant hand; when one of the response buttons is lit, the first timer (which records the decision time) begins and is stopped when the subject's hand leaves the home button. The second timer (which records the movement time) is started when the subject's hand leaves the home button and is stopped when the correct response button is hit by the same hand. In the present studies, measures were made of 1-, 2-, 4- and 8-choice reaction time. Forty trials were attempted at each level and the median Decision Time (DT) and median Movement Time (MT) were recorded in milliseconds. Median values of the two reaction time components were recorded because of the skewed distribution that repeat reaction time trials consistently demonstrate.

Decision times (and to a lesser extent movement times) increase with increasing complexity of the choice reaction task\textsuperscript{268}. This effect is known as the Hick Paradigm (Figure 4.1). Negative correlations exist between intelligence and mean decision time ($r=-0.32$), the y-axis intercept of the regression line of decision times against number of lights in the Hick Paradigm ($r=-0.25$) and mean movement time ($r=-0.30$)\textsuperscript{268}. Some investigators have also postulated that there is a weak correlation between intelligence and the slope of the Hick Paradigm regression line ($r=-0.28$)\textsuperscript{268,269}. That is, more intelligent people demonstrate a lower rate of increase in
reaction time with increasing levels of choice than less intelligent people. However, the validity of this relationship has been disputed.

### 4.1.15 UWIST Mood Adjective Checklist

The UWIST Mood Adjective Checklist is a self-rating assessment of current mood\(^\text{270}\). Subjects are provided with a list of 24 mood adjectives (e.g. cheerful, anxious, jittery) and are instructed to rate the applicability of each adjective to their present mood as 'definitely', 'slightly', 'slightly not' or 'definitely not'. Responses are scored 4 for 'definitely' to 1 for 'definitely not'. The scores for three groups of eight adjectives assessing 'Tense arousal', 'Energetic arousal' and 'Hedonic tone' (or pleasure-displeasure) are summated to give a maximum score of 32 (and a minimum score of 4). Higher scores represent greater levels of the particular mood state.

### 4.1.16 Hospital Anxiety and Depression Scale (HAD)

The HAD is self-rating mood scale which provides a measure of mood state in the week prior to testing\(^\text{271}\). Subjects are provided with 14 statements relating to mood (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 a selection of four possible alternatives. Seven statements assess anxiety levels, and seven assess depression levels. Individual responses are scored 0-3, and maximum summated anxiety and depression scores of 21 are obtainable (the minimum score is 0). Higher scores are indicative of greater levels of anxiety or depression.

### 4.1.17 Cognitive Function Self-Appraisal Scale (CFSA)

The CFSA provides a subjective measure of everyday cognitive function, with particular emphasis on memory\(^\text{272}\). The questionnaire has 35 items and participants are asked to indicate, for each item, the frequency of lapses in memory or concentration from 'never' to 'very often' on a five-point scale (0-4). The minimum score is 0 and the maximum was 140. Subjects are also asked to rate on a three-point scale whether the overall responses they have given are as 'good as they had ever been', 'a bit worse' or 'a lot worse'; this is known as the 'cognitive function change score'.

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FIGURE 4.1: The Hick Reaction Time Paradigm

The number of lights in the choice task (i.e. 1, 2, 4 and 8) is expressed as the logarithm to the base 2 (i.e. 0, 1, 2, and 3). There is a linear relationship between the Log₂ of the number of lights in the choice reaction time task (on the x-axis) and decision times (on the y-axis), i.e. the more complex the task, the greater the decision time. It is believed by some psychologists that the gradient of this regression line is a marker of intelligence, with less intelligent people having steeper slopes.
4.2 METHODOLOGICAL ISSUES IN THE MEASUREMENT OF PERIPHERAL MOTOR NERVE FUNCTION

In clinical practice, nerve conduction studies help to delineate the extent and distribution of peripheral neural lesions and distinguish two main categories of disease: demyelination and axonal degeneration\textsuperscript{271}. Nerves are stimulated electrically and the resultant impulse travels along motor, sensory or mixed nerves. The assessment of conduction characteristics depends on the analysis of compound evoked potentials recorded from muscle in the study of motor fibres, and from the nerve itself in the case of sensory fibres. Nerve conduction studies can be performed on a large number of peripheral nerves, including the median, radial, ulnar, common peroneal and sural nerves.

4.2.1 Principles and Practice

The study of motor conduction requires stimulation of the nerve at two or more points along its course. Bipolar nerve stimulators are most commonly used; both electrodes are placed over the nerve trunk, with the cathode (negative pole) closer to the recording site to avoid anodal conduction block of the propagated impulse. A pulse of moderate intensity is initially utilised to adjust the position of the nerve stimulus to the best stimulating site. At maximal intensity, increasing the magnitude of the electrical stimulus should result in no further change in the size of the muscle action potential. Supra-maximal stimuli (20-30 per cent above maximum) are generally employed to guarantee activation of all the nerve actions. To record action potentials, two skin surface electrodes are used: an active lead placed on the belly of the muscle and an indifferent lead sited on the tendon.

The usual measurements made in motor nerve conduction studies are of the amplitude of the motor action potential (which in clinical practice provides a measure of axonal density) and the onset latency of the action potential (which is a measure of the fastest conducting nerve fibres). The onset latency is a function of nerve conduction time, neuromuscular transmission time and propagation time along the muscle membrane. Stimulation of the motor nerve at two separate points elicits
two responses with different latencies, but the last two components are common to both. Thus the latency difference between the two represents the time necessary for the nerve impulse to travel from one stimulus point to the other. The nerve conduction velocity is therefore derived as the ratio of the distance from one point of stimulation to the next (D) and the difference between the proximal (Lp) and distal (Ld) latencies:

\[
\text{Conduction velocity (m/s)} = \frac{D \text{ (mm)}}{L_p - L_d \text{ (ms)}}
\]

The impulses of slow-conducting fibres lag increasingly behind those of fast-conducting fibres over a long conduction path. Therefore, a proximal stimulus may give rise to evoked potentials that are of slightly longer duration and lower amplitude than are observed following a distal stimulation. Larger discrepancies between the amplitudes of action potentials after distal and proximal stimulation are indicative of conduction block.

Lower limb nerves (such as the common peroneal) have substantially slower conduction velocities than upper limb nerves (such as the median). The factors possibly responsible for the difference include more abrupt axonal tapering, a more progressive reduction in axonal diameter, shorter internodal distances and lower distal temperatures in lower limb nerves.

### 4.2.2 Common Sources of Error

Although the method of nerve conduction studies is simple in theory, pitfalls abound in practice:

*Problems with the Surface Electrodes*

A major technical challenge is often posed by stimulus artefact. This can be improved by reducing skin impedance; techniques to facilitate this include removing skin moisture and grease with an alcohol wipe, gently abrading the skin with fine
sandpaper and, thereafter, applying high conductance jelly to the skin. A ground electrode (earth) placed between the stimulating and recording electrodes will also reduce stimulus artefact.

Unexpectedly small or absent action potentials may be due to a stimulus that is inappropriately low in intensity (i.e. the stimulus electrodes are in a sub-optimal position or are mis-directed) or the skin surface electrodes are mis-placed. Considerable time, therefore, needs to be spent at the outset of nerve conduction studies to ensure that the muscle action potentials are being optimally detected with the minimum of stimulus artefact.

**Supra-Maximal Stimulation**

As was intimated above, it is important to ensure that nerve conduction studies are performed with supra-maximal electrical stimuli. Sub-maximal stimuli will not activate all of the axons contained in a nerve and will result in onset latencies that fluctuate from one trial to another. The use of supra-maximal stimuli, which activate all axons, circumvents this uncertainty.

**Effects of Limb Temperature**

Nerve impulses propagate faster at higher body temperatures, with velocities increasing by approximately 2.4 m/s per degree from 29 to 38°C. Therefore, in experimental studies it is crucial to control for limb temperature, ideally by using a thermostatically-regulated limb warmer. If skin temperature falls below 34°C, indicating a muscle temperature of less than 37°C, it is necessary to warm the limb. If such facilities are not available, it is theoretically possible to mathematically adjust for limb temperature by adding 5% of the measured conduction velocity for each degree below 34°C.

**4.2.3 Methods for Nerve Conduction Studies Performed in Study 1**

Motor nerve conduction studies were performed by the same investigator, according to the principles described in Section 4.2.1, on the dominant-side median and common peroneal nerves using a Medelec Sapphire® 2ME electromyograph
In the case of the median nerve studies, the nerve was stimulated with a bipolar electrode proximally in the antecubital fossa and distally on the volar aspect of the wrist; the skin surface electrodes were placed, after adequate skin preparation, over the muscles of the thenar eminence. For the common peroneal nerve studies, the nerve was stimulated proximally behind the head of the fibula and distally over the anterior aspect of the shin, just above the ankle joint; surface electrodes were placed over the extensor digitorum brevis muscle in the foot. Skin surface electrodes were sited after appropriate skin preparation and remained in position during the course of each experimental study. Ground (earth) electrodes were utilised to reduce stimulus artefact. Nerves were stimulated orthodromically with supra-maximal electrical signals; the sites for nerve stimulation were marked at the outset of each study session and remained constant thereafter. Limb temperature was kept constant at 34°C using a DISA thermostatically-regulated heater (DISA, Bristol, UK).

Recordings were made of the median and common peroneal nerve motor conduction velocities (m/s) and of the amplitudes of the motor action potentials (mV). The ratio of the amplitudes of the motor action potentials after distal and proximal stimulations was also calculated as an index of acute conduction block. If acute conduction block occurred, then a ratio significantly greater than 1.00 would be expected.
CHAPTER 5

STUDY 1

EFFECTS OF ACUTE HYPOGLYCAEMIA ON THE PERIPHERAL NERVOUS SYSTEM AND THE INFORMATION PROCESSING FUNCTIONS OF THE CENTRAL NERVOUS SYSTEM
5.1 INTRODUCTION

5.1.1 Effects of Hypoglycaemia on the Peripheral Nervous System

The ability of acute hypoglycaemia to impair higher functions of the central nervous system has been described in detail in Chapter 2. Considerably less attention has focused on the effects of hypoglycaemia on the peripheral nervous system, but limited experimental evidence in animals and anecdotal evidence in humans supports the existence of a putative 'hypoglycaemic neuropathy' 254.

Several studies in diabetic and non-diabetic rats have demonstrated that profound hypoglycaemia (venous blood glucose below 2.5 mmol/l) of prolonged duration (at least 12 hours) resulted in axonal degeneration of peripheral nerves with consequent reduction of nerve conduction velocities and evoked muscle action potentials 274-276. In the spontaneously diabetic BB rat, prolonged hypoglycaemia (venous blood glucose less than 3.0 mmol/l for six days) was associated with the development of a motor peripheral neuropathy characterised by loss of anterior horn motor neurones, loss of large myelinated fibres and Wallerian degeneration 277.

In humans, peripheral neuropathy is a rare, but recognised, complication of insulinoma 278-280, where the characteristic findings are those of a predominantly motor peripheral neuropathy which is distal and symmetrical. Upper limb involvement is generally more frequent and severe, and neurophysiological studies suggest that the axon is the primary site of damage 278. Following resection of the insulinoma, weakness improves and sensory symptoms may resolve completely 278. Earlier observations of psychiatric patients undergoing insulin-coma therapy also suggested that peripheral sensory disturbances and, in some instances, sensory loss followed prolonged insulin-induced hypoglycaemia 281-283. In addition, anecdotal reports exist of peripheral neuropathy being precipitated by the onset of strict glycaemic control in diabetic patients 284, in whom recurrent episodes of moderate hypoglycaemia are common.
Few data are available on the effects of more moderate degrees and durations of hypoglycaemia on peripheral nerve function in humans. Tamburrano and colleagues could not identify any change in median nerve sensory conduction velocity in six non-diabetic human subjects in whom mean arterialised blood glucose was maintained at 2.4 mmol/l for 60 minutes using a hyperinsulinaemic glucose clamp technique\textsuperscript{285}. However, nerve conduction studies were not performed on nerves in the lower limb, and motor nerve conduction velocities were not measured. In a different study, acute hypoglycaemia (venous plasma glucose nadir 2.4 mmol/l induced by stopping dextrose infusions in fasting subjects) had no effect on sensory conduction velocities of the median nerve in seven patients with insulinoma\textsuperscript{286}. However, no information was provided on how, or if, hypoglycaemia was avoided in the period leading up to the experimental studies, the order of the study sessions (euglycaemia versus hypoglycaemia) was not randomised and, in the hypoglycaemia studies, the neurophysiological readings were performed immediately after the blood glucose nadir was reached, so that insufficient time may have elapsed for significant alterations in peripheral nerve function to have occurred.

5.1.2 Peripheral Neural Function and Intelligence

Speed of information processing correlates strongly with overall mental ability\textsuperscript{287-289}, and so there is a widely held view, within the field of contemporary intelligence research, that speed of information processing is a key component of intelligence\textsuperscript{289}. That is, an intelligent person is one who can quickly and effectively process incoming stimuli, with this efficient system additionally allowing increased information handling ability. In a reductionist scheme, from the most complex to the simplest level, efficiency of information processing in humans can be assessed using psychometric tests (e.g. the processing speed test of the British Ability Scales), cognitive-experimental tasks (e.g. choice reaction time procedures) and psychophysical tests (e.g. backward masking procedures such as visual inspection time\textsuperscript{267}). However, little is known about factors that might act as the neural substrate for speed of information processing, but an obvious and simplistic explanation is that the speed of nerve conduction may be responsible\textsuperscript{290}.
In an attempt to examine this hypothesis, several studies have examined the relationship between peripheral nerve conduction velocity, speed of information processing and intelligence\(^{256,257,290-292}\). Peripheral nerve conduction velocities have been studied because their function involves no cognitive component and so they represent a purely physiological assessment of speed of neuronal function. It has been asserted that the accurate assessment of pure nerve conduction velocities cannot be undertaken using brain evoked potentials (even though this would appear a more logical choice) since their generation also involves a degree of cognitive processing\(^{290}\).

Vernon and Mori found correlations of moderate size (\(r=-0.45\)) between median nerve sensory nerve conduction velocities and general IQ in two separate groups of undergraduate students (~85 students in each group)\(^{256}\). There were smaller correlations between nerve conduction velocities and reaction times (\(r=-0.23\)), and adjustments for age and sex had no impact upon the size of the correlation coefficients. Rijsdijk and Boomsma found a smaller, but statistically significant, correlation (\(r=0.15\)) between median nerve conduction velocities and intelligence in 159 Dutch twin pairs aged 18 years\(^{257}\). Twin analyses suggested that the relationship between nerve conduction and intelligence was entirely mediated by common genetic factors. However, these results were not replicated in three other studies\(^{290-292}\). Barrett et al studied 44 men and women and did not find a significant correlation between peripheral nerve conduction velocities and intelligence as assessed by the Advanced Raven Matrices task\(^{290}\). Similarly Reed and Jensen found no link between intelligence (measured by Standard and Advanced Raven Matrices) and peripheral nerve conduction velocities in 200 male university and college students\(^{291}\). However, there were methodological differences between the latter two studies and that of Vernon and Mori that could have accounted for the discrepant findings. The study by Barrett et al was small, with the attendant risk of a type 2 statistical error\(^{290}\). Moreover, the nerve conduction studies employed sub-maximal stimulation of the sensory component of the median nerve which, as was described in Chapter 4.2, could have resulted in an atypical nerve conduction velocities. Moreover, in the study by Reed and Jensen, the effect of limb temperature on nerve conduction
velocities was controlled statistically rather than directly using a heater\textsuperscript{291}. However, in a later study, Wickett and Veron employed comparable methodology to that of Vernon and Mori and did not demonstrate a correlation between peripheral nerve conduction velocities and intelligence in 38 females\textsuperscript{292}. Clearly, there was again a risk of a type 2 statistical error in this study but, on the basis of the results, the authors re-analysed the original data presented by Vernon and Mori\textsuperscript{256} to determine whether differences in sex distribution could have accounted for the discordant results. The repeat analysis was not entirely convincing in this regard, but did raise the prospect that the correlations between nerve conduction velocities, reaction times and intelligence were stronger for men than women\textsuperscript{292}.

5.1.3 Aims of the Study

Since moderate hypoglycaemia is known to have significant impact on functions of the central nervous system, any differential effect of hypoglycaemia on the peripheral nervous system may offer insights into the metabolic requirements of central and peripheral neurones. The primary aim of the present study was, therefore, to consider the impact of one hour of hypoglycaemia (arterialised blood glucose 2.6 mmol/l) on the motor function of the median and common peroneal nerves in non-diabetic humans.

A secondary aim of the investigation was, within a single study, to examine comprehensively the effects of controlled hypoglycaemia on different levels of human information processing. Since speed of processing, at all levels of description, is related in normal humans to mental ability, by testing all such levels during hypoglycaemia in the same group of subjects, the lower level processes that may contribute to the decrements in higher-level mental performance (which putatively include peripheral nerve conduction) can be investigated.
5.2 METHODS

5.2.1 Subjects
Sixteen non-diabetic human subjects (eight male) were recruited from staff at the Royal Infirmary of Edinburgh. None had any relevant previous medical history or a family history of diabetes, and none were taking regular medication (other than the oral contraceptive pill). All subjects had a corrected visual acuity of 6/6 or better, as measured with a Snellen chart. The mean age (± SD) was 31.1 (± 8.3) years and the mean body mass index (± SD) was 23.1 (± 2.2) kg/m². Written informed consent was obtained from all subjects and the study was approved by the local medical ethics advisory committee.

5.2.2 Experimental Procedure
Subjects attended for three study sessions, each separated by at least two weeks. The purpose of the initial visit was primarily to familiarise subjects with the tests that would be used in the experimental sessions, so as to reduce the effects of practice on test results. During this initial visit, intellectual ability was assessed by the National Adult Reading Test (NART) and the Alice Heim 4 Test (AH4). In the following two study sessions, the participants underwent the experimental procedures during conditions of either hypoglycaemia or euglycaemia (Figure 5.1). The subjects were not informed which condition was being studied at each visit. The subjects completed both experimental conditions in a counterbalanced fashion, i.e. half of the subjects underwent the euglycaemia study first followed by the hypoglycaemia study and the other half underwent the studies in the reverse order.

During the two experimental visits, subjects underwent a glucose clamp procedure. Following a light breakfast at 07.00 hours, subjects were asked to attend the department at midday. A teflon cannula was inserted into an ante-cubital vein in the subject's non-dominant arm under local anaesthetic (1% lignocaine). This cannula was used to infuse human soluble insulin (Actrapid, Novo Nordisk Pharmaceuticals, Crawley, UK) and 10% dextrose. A second cannula was inserted in a retrograde direction into a vein on the dorsum of the same hand, which was then placed in a
heated box (Plexiglass) at 60°C to arterialise the venous blood. Arterialised blood samples were obtained throughout the study for the measurement of whole blood glucose at the bedside using a glucose oxidase method (Yellow Springs Instrument 2300 Stat, Yellow Springs, OH, USA). To maintain patency, both cannulae were flushed frequently with heparinised saline.

A modified hyperinsulinaemic glucose clamp technique was used to maintain the blood glucose at predetermined levels. After a brief priming regimen, insulin was infused at a steady rate (based on whole-body surface area) of 60mU/m²/min using an IMED Gemini PC1 pump; 10% dextrose was infused, using an IVAC Site Saver pump, at a variable rate according to the blood glucose value. Arterialised blood glucose was initially measured every three minutes, until a stable level had been achieved, and then at five minute intervals. Counterregulatory hormone concentrations were not estimated.

At each laboratory session, the arterialised blood glucose concentration was initially stabilised at 5.0 mmol/l for a period of one hour during which baseline peripheral nerve conduction studies were performed and the subject completed the mental test battery (baseline; Figure 5.1). Following this, the blood glucose concentration was either maintained at 5.0 mmol/l (euglycaemia; Figure 5.1) or lowered to 2.6 mmol/l (hypoglycaemia; Figure 5.1) and maintained at this level for one hour, during which peripheral nerve conduction studies were performed every 30 minutes and the neuropsychological test battery was repeated. The subjects were not informed about their blood glucose concentration during any phase of the study. A period of 20 minutes was allowed to elapse between the baseline and the attainment of euglycaemia or hypoglycaemia to allow the blood glucose concentration to stabilise. The target glucose concentration was maintained for a further ten minutes before the tests were administered.
FIGURE 5.1: Schematic Representation of Study Design

I G Baseline Study

↓↓ Study 1 Euglycaemia

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<tr>
<th>Euglycaemia</th>
<th>Test Battery</th>
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<td>Test Battery</td>
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Study 2 Euglycaemia

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<th>Euglycaemia</th>
<th>Test Battery</th>
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<table>
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<tr>
<th>Hypoglycaemia</th>
<th>Test Battery</th>
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Time (mins)
Footnote to Figure 5.1
Each subject attended for two study sessions, which comprised 'baseline' (euglycaemia) and 'experimental' (euglycaemia or hypoglycaemia) conditions. The durations of each condition are illustrated on the time scale. I and G represent the times at which insulin and glucose infusions respectively were commenced. The test battery included the cognitive and nerve conduction studies and lasted approximately one hour; to ensure stability of the blood glucose concentrations, the battery was preceded by a 10 minute period in which no tests were performed.
5.2.3 Nerve Conduction Studies
Motor nerve conduction studies were performed by the same investigator, according to the principles described in Chapter 4.2, on the dominant-side median and common peroneal nerves. Recordings were made of the motor nerve conduction velocities (m/s) and of the amplitudes of the motor action potentials (mV). The ratio of the amplitudes of the motor action potentials after distal and proximal stimulations was also calculated as an index of acute conduction block. If acute conduction block occurred, then a ratio significantly greater than 1.00 would have been expected. Measurements were made once during the baseline, at the beginning (T0) of the experimental session (hypoglycaemia or euglycaemia) and after 30 (T30) and 60 (T60) minutes.

5.2.4 Cognitive Test Battery
A combined psychometric, cognitive-experimental and psychophysical test battery was applied during the baseline and experimental phases of the euglycaemia and hypoglycaemia conditions. The order of the tests (as listed below) remained the same during each phase of the visit, and the battery was interrupted only to allow measurement of peripheral nerve conduction velocities at pre-requisite time points. The cognitive tests were described in detail in Chapter 4.1.

Tests of General Cognitive Ability
Two complex psychometric tests were used to assess general cognitive function:
- Digit Symbol (DS)
- Trail-Making Test B (TMB):

Tests of Information Processing
Three tasks were employed to assess the efficiency of information processing at the psychometric, psychophysical and cognitive-experimental levels:
- Speed of Information Processing (SIP)
- Inspection Time (IT)
- 1-, 2-, 4-, and 8-Choice Reaction Time (RT)
5.2.5 Edinburgh Hypoglycaemia Scale (EHS)
This is a subjective self-rating scale in which subjects are presented with a list of symptoms of hypoglycaemia and are asked to score the severity of each symptom from 1 (not present) to 7 (very intense). The scale was administered during the baseline and experimental stages of the clamp studies. Symptoms scores were summated and classified into three groups: autonomic (e.g. sweating, tremor, hunger), neuroglycopenic (e.g. confusion, drowsiness, inability to concentrate) and non-specific (nausea and headache).

5.2.6 Statistical Analyses
All data are presented as mean ± standard deviation (SD). Each measure of cognitive and peripheral nerve function was analysed independently.

The effects of acute hypoglycaemia on symptom scores and cognitive performance were assessed using a mixed-model Analysis of Variance (ANOVA). 'Order of session' was used as a between-subjects factor with two levels (euglycaemia-hypoglycaemia or hypoglycaemia-euglycaemia). There were two within-subjects factors, each with two levels: 'experimental condition' (euglycaemia versus hypoglycaemia) and 'time' (baseline versus experimental). The principal outcome statistic was any ['experimental condition' by 'time'] interaction as this indicated the effects of hypoglycaemia on cognitive and symptom parameters and controlled for day-to-day variation by including baseline scores. It was anticipated that the principal outcome statistic would show a significant effect of hypoglycaemia on the individual parameters and so significant main effects for 'experimental condition' are not reported, as subjects were euglycaemic at baseline during both the euglycaemia and hypoglycaemia condition days, making this effect non-meaningful. Significant main effects of 'time' are also not relevant in this context and are not reported.

The effects of hypoglycaemia on the movement and decision time components of the individual 1-, 2-, 4- and 8-choice reaction time tasks were compared using the above mixed-model ANOVA. In addition the effects of hypoglycaemia on the Hick Paradigm (i.e. the linear relation between decision time [and movement time] and the
logarithm of the number of lights in the choice task) were compared using the data from the euglycaemia and hypoglycaemia experimental conditions in a mixed model ANOVA. 'Order of session' was used as a between-subjects factor with two levels (euglycaemia-hypoglycaemia or hypoglycaemia-euglycaemia). There were two within-subjects factors: factor one, 'experimental condition', had two levels (euglycaemia versus hypoglycaemia) and factor two, 'number of lights', had four levels (1-choice reaction time versus 2-choice, versus 4-choice, versus 8-choice). The main effects of 'number of lights' and 'experimental condition' indicated the effects of increasing complexity of the reaction time task and of hypoglycaemia respectively on the decision and movement time parameters. The interaction of ['experimental condition' by 'number of lights'] indicated whether hypoglycaemia had a differential effect on decision and movement times depending on the complexity of the reaction time task.

A mixed-model ANOVA was used to assess the effects of acute hypoglycaemia on the median and common peroneal nerve conduction parameters. 'Order of session' was used as a between-subjects factor with two levels (euglycaemia-hypoglycaemia or hypoglycaemia-euglycaemia). There were two within-subjects factors: factor one, 'experimental condition', had two levels (euglycaemia versus hypoglycaemia) and factor two, 'time', had four levels (baseline versus T0, versus T30, versus T60). In addition to the principal outcome statistic of any ['experimental condition' by 'time'] interaction, the main effects of 'time' and 'experimental condition' are also reported. Direct comparisons between the median and common peroneal nerve conduction parameters during euglycaemia were also made using a mixed-model ANOVA. 'Order of session' was used as a between-subjects factor with two levels (euglycaemia-hypoglycaemia or hypoglycaemia-euglycaemia). There were two within-subjects factors: factor one, 'nerve', had two levels (median versus common peroneal) and factor two, 'time', had four levels (baseline versus T0, versus T30, versus T60).
A p-value of <0.05 was considered to be significant. All analyses were performed using SPSS version 7.5.1 for Windows 95. Reported p-values were rounded to two decimal places; thus p=0.00 signifies that the p-value was ≤0.005.
5.3 RESULTS

5.3.1 Intellectual Ability of the Study Participants
All but one of the subjects had above average intellectual ability. The mean (± SD) AH4 score for all 16 subjects was 98.8 (± 17.0). The mean (± SD) NART error score for 15 of the 16 subjects was 15.7 (± 5.6), which predicted a mean (± SD) intelligence quotient (IQ) of 111.2 (± 6.9). The NART was not administered to one of the subjects who, although fluent in English, did not learn English as his first language. However, this subject scored highly on the AH4 task. The subject with below average IQ made 28 errors on the NART, which predicted a full-scale IQ of 96.

5.3.2 Blood Glucose Profiles for the Clamp Studies
Stable glycaemic plateaux were achieved for every subject in each condition of the study (Figure 5.2). The whole blood glucose (mean ± SD) during the baseline euglycaemic clamps was 4.95 ± 0.15 mmol/l. During the experimental conditions of euglycaemia and hypoglycaemia, blood glucose values were 5.03 ± 0.07 mmol/l and 2.63 ± 0.08 mmol/l respectively.

There were no statistically significant effects of order on any of the outcome variables (p>0.05).

5.3.3 Symptoms
During the hypoglycaemic condition of the study, significant increments occurred both in autonomic (p=0.00) and neuroglycopenic symptom scores (p=0.01), but no significant change was observed in the non-specific symptoms of malaise (p=1.00; Table 5.1).

5.3.4 General Cognitive Function Tests
Moderate hypoglycaemia resulted in a marked deterioration in performance of the Digit Symbol (p=0.01) and Trail Making B tasks (p=0.04; Table 5.1).
5.3.5 Tests of Information Processing

SIP and Inspection Time

The time taken to complete the 'difficult' subset of the SIP test was prolonged by acute hypoglycaemia (p=0.00; Table 5.1), but the time taken to complete the 'easy' task failed to reach statistical significance (p=0.09). There was no change in the number of errors made, which was low in each subtest. Moderate hypoglycaemia also resulted in a deterioration in performance of the Inspection Time task (p=0.00; Table 5.1).

Reaction Time

Acute hypoglycaemia caused significant deterioration in the decision time and movement time components of the 1-, 2-, 4- and 8-choice reaction time tasks (all p<0.05; Table 5.2), with the exception of the movement time component of 2-choice reaction time which just failed to reach statistical significance (p=0.05).

With regard to the Hick Paradigm (Figures 5.3 and 5.4), decision times increased with increasing complexity of the reaction time task, i.e. the main effect of 'number of lights' was significant (p=0.00). Movement times also increased (p=0.00), but the proportional increment was smaller. In keeping with the results for the individual reaction time tasks, the main effect of 'experimental condition' revealed a significantly deleterious effect of hypoglycaemia on decision (p=0.00) and movement times (p=0.00). The interaction between ['number of lights' and 'experimental condition'] on decision (p=0.88) and movement times (p=0.67) was non-significant indicating that there was no differential effect of acute hypoglycaemia on any of the individual choice reaction time tasks.

5.3.6 Nerve Conduction Studies

Common Peroneal versus Median Nerve Conduction Parameters

Mean nerve conduction velocities (during euglycaemia) were significantly faster in the median than the common peroneal nerve (p=0.00) and similarly the mean amplitudes of the proximal (p=0.00) and distal (p=0.00) evoked potentials were greater in the median nerve. The mean ratio of distal to proximal amplitudes for the
median nerve were slightly greater than 1.0 at most time points, but approached 1.0 for most time points of common peroneal nerve stimulation. The inter-subject variability in amplitude measurements (as indicated by the standard deviation values) was considerably greater than that observed with the nerve conduction velocities for both nerves.

Effects of Interaction of 'Experimental Condition' and 'Time' on Nerve Conduction Parameters
Acute hypoglycaemia had no significant effect on the motor nerve conduction velocities of the median and common peroneal nerves (p>0.05; Table 5.3). Similarly, acute hypoglycaemia did not alter the amplitudes of the evoked action potentials of either nerve after proximal or distal stimulation, nor did it affect the ratio of the distal to proximal amplitudes (p>0.05; Table 5.3).

Main Effects of 'Experimental Condition' on Nerve Conduction Parameters
The amplitudes of median nerve evoked potentials after proximal stimulation were smaller during the hypoglycaemia study day than the euglycaemia study day (p=0.02) and there was also a non-significant trend for the amplitudes after distal stimulation to be smaller during the hypoglycaemia study day (p=0.11). There were no significant main effects of 'experimental condition' on median nerve conduction velocities or on any of the common peroneal nerve conduction parameters (p>0.05).

Main Effects of 'Time' on Nerve Conduction Parameters
There was a significant main effect of 'time' on common peroneal nerve conduction velocities (p=0.00), i.e. nerve conduction velocities in both experimental conditions declined with time. No main effect of 'time' was observed on the common peroneal amplitude data, or on any of the median nerve neurophysiological parameters (p>0.05).
FIGURE 5.2: Blood Glucose Profiles during the Euglycaemic and Hypoglycaemic Clamp Studies

Mean blood glucose concentrations (±SD) during the baseline and experimental conditions of both the euglycaemic (Eug) and hypoglycaemic (Hypo) glucose clamps. There was no difference in blood glucose concentrations during the baseline conditions. Blood glucose concentration was approximately 5.0 mmol/l during the euglycaemia experimental condition and 2.6 mmol/l during the hypoglycaemia experimental condition.
TABLE 5.1: Results of Cognitive Function Tests and Symptom Scores during Euglycaemia and Hypoglycaemia

<table>
<thead>
<tr>
<th></th>
<th>Euglycaemia</th>
<th>Hypoglycaemia</th>
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<th>p-value</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>Experimental</td>
<td>Baseline</td>
<td>Experimental</td>
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<tr>
<td><strong>Digit Symbol (score)</strong>*</td>
<td>72.5 ± 10.8</td>
<td>77.2 ± 10.2</td>
<td>72.5 ± 10.7</td>
<td>67.0 ± 13.1</td>
</tr>
<tr>
<td><strong>Trail Making B (s)</strong></td>
<td>26.5 ± 10.1</td>
<td>24.6 ± 8.0</td>
<td>27.0 ± 9.7</td>
<td>33.0 ± 17.2</td>
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<tr>
<td><strong>Speed of Information Processing</strong></td>
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<tr>
<td><em>Easy Numbers (s)</em></td>
<td>48.1 ± 8.2</td>
<td>49.1 ± 9.5</td>
<td>51.4 ± 14.8</td>
<td>57.6 ± 16.8</td>
</tr>
<tr>
<td><em>Easy Errors (no.)</em></td>
<td>1.0 ± 1.5</td>
<td>1.2 ± 1.3</td>
<td>0.9 ± 1.7</td>
<td>1.1 ± 1.2</td>
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<tr>
<td><em>Difficult Numbers (s)</em></td>
<td>69.7 ± 12.3</td>
<td>65.6 ± 13.0</td>
<td>72.5 ± 18.4</td>
<td>83.6 ± 20.8</td>
</tr>
<tr>
<td><em>Difficult Errors (no.)</em></td>
<td>1.7 ± 1.8</td>
<td>2.1 ± 1.8</td>
<td>1.4 ± 1.3</td>
<td>1.9 ± 2.5</td>
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<tr>
<td><strong>Inspection Time (ms)</strong></td>
<td>47.6 ± 19.1</td>
<td>47.3 ± 16.9</td>
<td>50.2 ± 20.3</td>
<td>63.4 ± 24.1</td>
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<tr>
<td><strong>Symptom Scores</strong></td>
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<tr>
<td><em>Autonomic</em></td>
<td>10.3 ± 2.0</td>
<td>10.4 ± 1.5</td>
<td>11.1 ± 2.5</td>
<td>21.1 ± 7.6</td>
</tr>
<tr>
<td><em>Neuroglycopenic</em></td>
<td>10.4 ± 1.5</td>
<td>10.8 ± 3.3</td>
<td>10.4 ± 1.3</td>
<td>19.9 ± 12.0</td>
</tr>
<tr>
<td><em>Malaise</em></td>
<td>2.0 ± 0.0</td>
<td>2.1 ± 0.3</td>
<td>2.4 ± 1.0</td>
<td>2.5 ± 1.1</td>
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</tbody>
</table>

Values are mean ± SD.

In the Digit Symbol task*, a higher score represents better performance; in all other cognitive tasks, a lower score indicates better performance. Higher Symptoms scores represent greater levels of hypoglycaemia symptomatology.

p-values are for the interaction of 'time' versus 'experimental condition' in ANOVA analyses (hypoglycaemia/euglycaemia).
### TABLE 5.2: Choice Reaction Times during Euglycaemia and Hypoglycaemia

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<th>Euglycaemia</th>
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<tr>
<td><strong>1-Choice Reaction Time</strong></td>
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<tr>
<td>Decision Time (ms)</td>
<td>259.8 ± 20.2</td>
<td>257.8 ± 22.2</td>
<td>259.4 ± 21.4</td>
<td>291.4 ± 43.1</td>
<td></td>
<td></td>
<td>12.2</td>
<td>0.00</td>
</tr>
<tr>
<td>Movement Time (ms)</td>
<td>103.6 ± 24.1</td>
<td>101.6 ± 26.7</td>
<td>104.0 ± 22.6</td>
<td>119.0 ± 32.4</td>
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<td></td>
<td>11.0</td>
<td>0.01</td>
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<tr>
<td><strong>2-Choice Reaction Time</strong></td>
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<tr>
<td>Decision Time (ms)</td>
<td>282.5 ± 18.6</td>
<td>280.6 ± 16.0</td>
<td>287.2 ± 23.8</td>
<td>312.7 ± 40.1</td>
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<td>6.6</td>
<td>0.02</td>
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<tr>
<td>Movement Time (ms)</td>
<td>110.2 ± 25.6</td>
<td>110.4 ± 28.1</td>
<td>113.5 ± 24.1</td>
<td>126.7 ± 36.4</td>
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<td>4.4</td>
<td>0.05</td>
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<td><strong>4-Choice Reaction Time</strong></td>
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<tr>
<td>Decision Time (ms)</td>
<td>300.9 ± 22.1</td>
<td>298.5 ± 20.8</td>
<td>304.4 ± 30.1</td>
<td>328.9 ± 46.5</td>
<td></td>
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<td>7.0</td>
<td>0.02</td>
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<tr>
<td>Movement Time (ms)</td>
<td>115.1 ± 26.5</td>
<td>109.2 ± 24.4</td>
<td>117.4 ± 24.5</td>
<td>128.9 ± 34.3</td>
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<td></td>
<td>9.6</td>
<td>0.01</td>
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<tr>
<td><strong>8-Choice Reaction Time</strong></td>
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<td></td>
</tr>
<tr>
<td>Decision Time (ms)</td>
<td>316.8 ± 27.1</td>
<td>322.9 ± 26.3</td>
<td>320.3 ± 30.7</td>
<td>356.6 ± 40.8</td>
<td></td>
<td></td>
<td>9.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Movement Time (ms)</td>
<td>118.3 ± 26.5</td>
<td>113.2 ± 28.9</td>
<td>121.6 ± 26.6</td>
<td>133.1 ± 37.8</td>
<td></td>
<td></td>
<td>7.1</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

p-values are for the interaction of 'time' versus 'experimental condition' in ANOVA analyses (hypoglycaemia/euglycaemia).
TABLE 5.3: Median and Common Peroneal Motor Nerve Conduction Velocities and Amplitudes

<table>
<thead>
<tr>
<th></th>
<th>Euglycaemia</th>
<th></th>
<th></th>
<th>Hypoglycaemia</th>
<th></th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>T0</td>
<td>T30</td>
<td>T60</td>
<td></td>
<td></td>
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<tr>
<td>Median Nerve</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Conduction Velocities (m/s)</td>
<td>55.4 ±5.4</td>
<td>56.6 ±5.4</td>
<td>55.2 ±4.9</td>
<td>56.7 ±5.4</td>
<td>54.0 ±4.9</td>
<td>55.7 ±5.4</td>
<td>54.9 ±6.1</td>
</tr>
<tr>
<td>Amplitudes (mV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Proximal</td>
<td>7.3 ±3.4</td>
<td>7.7 ±3.6</td>
<td>6.4 ±3.7</td>
<td>6.6 ±3.2</td>
<td>5.9 ±3.2</td>
<td>4.8 ±3.1</td>
<td>5.3 ±3.0</td>
</tr>
<tr>
<td>Distal</td>
<td>8.6 ±3.2</td>
<td>8.2 ±3.3</td>
<td>7.4 ±3.9</td>
<td>7.2 ±3.2</td>
<td>6.4 ±3.1</td>
<td>5.0 ±3.2</td>
<td>5.5 ±2.6</td>
</tr>
<tr>
<td>Ratio (D/P)</td>
<td>1.4 ±0.8</td>
<td>1.2 ±0.6</td>
<td>1.4 ±1.4</td>
<td>1.2 ±0.4</td>
<td>1.2 ±0.4</td>
<td>1.1 ±0.4</td>
<td>1.2 ±0.8</td>
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<tr>
<td>Common Peroneal Nerve</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conduction Velocities (m/s)</td>
<td>49.3 ±3.5</td>
<td>46.9 ±3.6</td>
<td>46.2 ±3.3</td>
<td>46.7 ±4.2</td>
<td>48.9 ±3.8</td>
<td>45.9 ±5.9</td>
<td>44.9 ±6.6</td>
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<tr>
<td>Amplitudes (mV)</td>
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<td></td>
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<tr>
<td>Proximal</td>
<td>2.4 ±1.6</td>
<td>2.9 ±1.7</td>
<td>3.1 ±1.8</td>
<td>2.9 ±1.8</td>
<td>2.9 ±1.6</td>
<td>2.9 ±2.8</td>
<td>2.7 ±1.5</td>
</tr>
<tr>
<td>Distal</td>
<td>2.7 ±1.8</td>
<td>3.1 ±2.3</td>
<td>3.1 ±2.2</td>
<td>3.0 ±2.2</td>
<td>2.9 ±2.2</td>
<td>2.9 ±2.7</td>
<td>2.6 ±1.6</td>
</tr>
<tr>
<td>Ratio (D/P)</td>
<td>1.1 ±0.6</td>
<td>1.0 ±0.6</td>
<td>1.0 ±0.3</td>
<td>1.0 ±0.4</td>
<td>1.0 ±0.3</td>
<td>1.3 ±1.0</td>
<td>1.0 ±0.5</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

The ratio of distal (D) to proximal (P) amplitudes is a measure of acute conduction block.

p-values are for the interaction of 'time' versus 'experimental condition' in ANOVA analyses (hypoglycaemia/euglycaemia)
Mean of median decision time components (±SD) of choice reaction time during the experimental conditions of both the euglycaemic (Eug) and hypoglycaemic (Hypo) glucose clamps. The number of lights in the choice task (i.e. 1, 2, 4 and 8) is expressed as the logarithm to the base 2 (i.e. 0, 1, 2, and 3). There were significant main effects of 'number of lights' and 'experimental condition' (both p=0.00), but the interaction of the main effects was not significant (p=0.88). Thus, hypoglycaemia had a deleterious effect on decision times, but the proportional size of the effect was not influenced by the complexity of the choice task.
Mean of median movement time components (±SD) of choice reaction time during the experimental conditions of both the euglycaemic (Eug) and hypoglycaemic (Hypo) glucose clamps. The number of lights in the choice task (i.e. 1, 2, 4 and 8) is expressed as the logarithm to the base 2 (i.e. 0, 1, 2, and 3). There were significant main effects of 'number of lights' and 'experimental condition' (both p=0.00), but the interaction of the main effects was not significant (p=0.67). Thus, hypoglycaemia had a deleterious effect on movement times, but the proportional size of the effect was not influenced by the complexity of the choice task.
5.4 DISCUSSION

5.4.1 Differences Between the Median and Common Peroneal Nerve Conduction Study Data
The differences observed in the present study between the median and common peroneal nerve conduction velocities and in the amplitudes of the evoked action potentials were entirely predictable on the basis of normative data from nerve conduction studies in healthy people. Factors that may be responsible for differences between upper and lower limb nerves include more abrupt distal axonal tapering, a more progressive reduction in axonal diameter, shorter inter-nodal distances and lower distal temperatures in lower limb nerves. The tendency for the ratio of median nerve distal to proximal amplitudes to be greater than 1.00 was again predictable since proximal stimulation may give rise to evoked potentials that are of slightly longer duration and lower amplitude than those observed following distal stimulation. This is because the impulses of slow-conducting fibres lag behind those of fast-conducting fibres over a long conduction path. It is also recognised that the variability of amplitude data may be substantially greater than that of nerve conduction velocity data, because of the technical vagaries involved in achieving optimum evoked potential amplitudes (see section 4.2).

5.4.2 Effects of Acute Hypoglycaemia on Peripheral Neural Function
Neurones of the central nervous system are exquisitely sensitive to the prevailing blood glucose concentration, with even moderate hypoglycaemia resulting in significant impairment of neuronal function, as demonstrated by the development of cognitive dysfunction. Experimental studies in animals and anecdotal reports in humans have also suggested that profound and protracted hypoglycaemia adversely affects peripheral nerve function, and may precipitate the development of a peripheral neuropathy. The effects of more moderate degrees and durations of hypoglycaemia on peripheral nerve function have not been definitively ascertained.
Previous studies demonstrated that acute moderate hypoglycaemia had no impact on median nerve sensory function in both non-diabetic subjects\textsuperscript{286} and in patients with insulinoma\textsuperscript{285}. However, these studies had significant methodological limitations, which were described in section 5.1, and which included very small sample sizes. The present study has supported and extended these earlier observations by demonstrating that acute hypoglycaemia had no effect on the motor conduction velocities of the dominant-side median and common peroneal nerves. The amplitudes of the action potentials were similarly unaffected and there was no evidence of acute conduction block. The differences observed between the median nerve evoked potential amplitudes measured on the euglycaemia and hypoglycaemia study days (which included the baseline euglycaemic measurements during both days) do not have an obvious physiological basis and presumably represent a chance occurrence (i.e. a type 1 statistical error) because of the high variability of the amplitude measurements\textsuperscript{293}.

It is reasonable to speculate whether any methodological issues such as sample size, the depth of hypoglycaemia attained, the nature of the nerves chosen for neurophysiological study or the nerve conduction studies themselves could have accounted for this null result.

- The present study had ample power to detect differences in cognitive function during acute hypoglycaemia. Moreover, because subjects acted as their own controls the sample size provided approximately 80% power to detect a 0.75 standard deviation change in median nerve conduction velocities with a p-value set at 0.05. Median and common peroneal nerve conduction velocities did appear to be consistently 1.0 - 1.5m/s slower during hypoglycaemia compared with euglycaemia, but these differences were non-significant. It is just conceivable that these differences could have become statistically significant in a larger study, but the magnitude of such a difference would be of minimal clinical relevance.

- An arterialised blood glucose concentration of 2.6 mmol/l was chosen for the hypoglycaemia phase of the investigations as numerous previous studies (see
Chapter 2.1) have demonstrated that this blood glucose level is associated with significant impairment of cognitive functions. Therefore, if peripheral nerves had a similar reliance on glucose as a metabolic fuel as central nervous system neurones, then this level of hypoglycaemia should have been sufficient to demonstrate a decrement in function. Lower blood glucose concentrations are associated with increased physical discomfort for subjects and it was not ethically justifiable to induce more profound hypoglycaemia.

The common peroneal and median nerves were chosen for several reasons. Both nerves are readily accessible for performing neurophysiological studies, and their stimulation causes minimal discomfort to subjects. Peripheral neuropathies tend to affect longer nerves rather than shorter nerves, therefore the common peroneal nerve was chosen on the theoretical basis that its long length may have made it more susceptible to the effects of moderate hypoglycaemia. The median nerve was chosen since animal and human studies suggested that 'hypoglycaemic neuropathy' affects predominantly upper limb nerves. Sensory neural function was not assessed in this study on the basis that 'hypoglycaemic neuropathy' primarily affects motor nerves and two previous studies, albeit of limited methodological quality, had previously shown no susceptibility of peripheral sensory nerves to acute hypoglycaemia. In addition, the measurement of sensory nerve function causes considerably more discomfort to subjects and is open to greater technical errors because the amplitudes of the evoked potentials are considerably smaller (leading to a reduced signal-to-noise ratio). There was a limited amount of time available in the context of each clamp study to perform nerve conduction studies and it was felt that this would best be utilised measuring motor nerve function. During the clamp studies, the intravenous cannulae were sited in the non-dominant hand so that the dominant hand was free for performance of the psychometric tests. Therefore, nerve conduction studies were performed on the dominant side because it would not have been possible to guarantee optimum placement of the electrodes for the nerve conduction studies on the non-dominant hand.
In current clinical practice, nerve conduction studies, and in particular the measurement of nerve conduction velocities and the amplitudes of the evoked potentials, represent the most effective methodology for assessing the functions of peripheral nerves. It is possible to measure other electrophysiological parameters, such as F-waves (which measure conduction time from the peripheral nerve to the spinal cord and back) and nerve refractory period (i.e. the shortest time interval between two sequential nerve stimuli that generate two sequential evoked potentials). It was not felt that the measurement of these parameters would have added considerably to the assessment of peripheral neural function. In particular, the measurement of refractory period is a time-consuming process and in a previous pilot study of four male Sprague-Dawley rats, acute hypoglycaemia (arterial blood glucose nadir of 1.66 mmol/l induced by insulin infusion) had no effect on the refractory period of the right femoral nerve.

The foregoing paragraphs, therefore, suggest that methodological issues do not account for the results of the present study and that the inability to detect any significant change in the neurophysiological parameters of peripheral nerves during hypoglycaemia indicates resistance of peripheral neural function to neuroglycopenia.

The significant change in common peroneal nerve conduction velocities that occurred with time during the hypoglycaemia and euglycaemia studies was unexpected and was not observed in any of the other peripheral nerve neurophysiological measurements. Tamburrano et al did not report any impact of hyperinsulinaemic euglycaemia on median sensory nerve conduction velocities in five non-diabetic subjects, and in the later study on nerve conduction parameters in patients with insulinoma no time course data was provided. While this observation could have resulted by chance, it should be noted that nerve conduction velocities are generally regarded as being highly reproducible within individual subjects (in contrast to amplitude measurements). It is, therefore, tempting to speculate that hyperinsulinaemia per se affected the common peroneal nerve conduction velocities, although the reason for a lack of effect on median nerve conduction velocities would require explanation. The institution of intensive insulin
therapy in diabetic patients with poor glycaemic control can promote the development of a peripheral neuropathy but, of course, in observational studies such as this, and others that have looked at 'hypoglycaemic neuropathy', it is not possible to separate a potential direct toxic effect of insulin itself from its hypoglycaemic actions. Greene and Winegrad, however, reported no direct toxic effects of insulin on isolated endoneurial preparations of rat peripheral nerve. Therefore, the observation that common peroneal motor nerve conduction velocities changed with time, and the speculation that this may be related to hyperinsulinaemia, must be re-assessed in future studies that are appropriately controlled.

5.4.3 Effects of Acute Hypoglycaemia on Symptoms of Hypoglycaemia and Cognitive Function

Acute hypoglycaemia predictably caused an increase in autonomic and neuroglycopenic symptoms of hypoglycaemia, but did not affect the 'non-specific' symptoms of nausea and malaise. The insensitivity of the 'non-specific' symptoms to hypoglycaemia has been identified in other studies. The three-factor model of hypoglycaemic symptoms, that is utilised in the EHS, is based on a multi-factor confirmatory factor analysis of symptoms of hypoglycaemia that were retrospectively recalled by two large groups of diabetic subjects. Therefore, the relative insensitivity of the 'non-specific' symptoms, in the present and previous studies, suggests that this model may not be appropriate for symptoms of hypoglycaemia induced during hyperinsulinaemic clamp studies. Further investigation of this possibility is clearly merited and would simply involve performing factor analysis on hypoglycaemia symptom data from a large number of clamp studies.

In contrast to its effects on the peripheral nervous system, acute hypoglycaemia caused significant disruption of central neural function as evidenced by impaired performance in the majority of tasks in the cognitive test battery. The deleterious effects of acute hypoglycaemia on general cognitive ability, as assessed by the Digit Symbol and Trail Making B tasks, and early stages of visual information processing, as assessed by the inspection time task, have been documented in other
studies and the results of the present study were entirely consistent with these.

No previous studies have assessed the effects of acute hypoglycaemia on performance of the SIP task from the British Ability Scales. Hypoglycaemia significantly reduced the time taken to complete the 'difficult' subtest, but the decreased time taken to complete the 'easy' subtest during hypoglycaemia just failed to reach statistical significance. Moderate hypoglycaemia had no significant effect on the number of errors made in either the 'easy' or the 'difficult' subtests of this task. These findings are consistent with previous studies that have suggested that hypoglycaemia causes greater impairment of complex cognitive tasks than simple tasks, but that accuracy of performance tends to be preserved at the expense of speed.

The present study was also the first to assess the impact of hypoglycaemia on 1-, 2-, 4-, and 8-choice reaction time within one experimental investigation. It is well established that increasing complexity of the choice task increases the decision time component of reaction time more markedly than the movement time component. The results of the present study support these observations. Acute hypoglycaemia caused significant impairment in performance of all the choice reaction time components, with the exception of the movement time component of 2-choice reaction time which just failed to reach statistical significance (p=0.05).

Correlations exist between intelligence and a variety of parameters that can be measured using the choice reaction time task (Chapter 4.1.14). Intelligence correlates with mean decision time ($r=-0.32$), the y-axis intercept of the regression line of decision times against number of lights in the Hick Paradigm ($r=-0.25$) and mean movement time ($r=-0.30$). Some investigators have also postulated that there is a weak correlation between intelligence and the slope of the Hick Paradigm regression line ($r=-0.28$). That is, more intelligent people demonstrate a lower rate of increase in reaction time, with increasing levels of choice, than less intelligent people. If such a relationship were valid, then an intervention that makes people less
intelligent (such as acute hypoglycaemia) would be expected to alter the gradient of the slope, such that the intervention would cause proportionately greater disruption in performance of the 8-choice reaction task, than the 1-choice task. However, acute hypoglycaemia did not alter the slope of the regression line of the Hick Paradigm and so the present study supports the observations of others who have failed to demonstrate a valid relationship between intelligence and the slope of the regression line\textsuperscript{255}. The present study, however, does support the validity of the correlations between intelligence and mean decision times, the y-axis intercept of the Hick Paradigm regression line, and mean movement times, since all were adversely affected by acute hypoglycaemia.

5.4.4 Implications for the Putative Relationship between Nerve Conduction Velocities and Intelligence

The hypothesis that differences in peripheral nerve conduction velocity form a partial basis for the association between psychometric intelligence and measures of information processing efficiency\textsuperscript{256,257} is based on the premise that central and peripheral nervous system neurones behave similarly in terms of neurophysiological function (i.e. rapid nerve conduction velocities peripherally reflect rapid nerve conduction velocities centrally). The present study was too small to allow meaningful correlations between intelligence, measures of speed of information processing and peripheral nerve conduction velocities to be made. However, if such a premise were correct then it would be reasonable to assume that central and peripheral neurones should also respond to external factors such as acute hypoglycaemia in similar ways. The present study, by demonstrating that multiple levels of higher information processing were altered in the central nervous system while peripheral nerve conduction velocities remained intact, therefore, supports others that have refuted this hypothesis\textsuperscript{290-292}. Of course, it remains perfectly feasible that speed of neuronal conduction within the central nervous system could still relate to speed of information processing and intelligence, but further studies are clearly required to investigate this in more detail.
5.4.5 Reasons for the Disparity in Central and Peripheral Neuronal Susceptibility to Acute Hypoglycaemia

The marked disparity in the effects of acute hypoglycaemia on peripheral and central neural system function implies that peripheral nerves do not have the same reliance on prevailing blood glucose concentrations as is demonstrated by neurones of the central nervous system. Neural components of peripheral nerves are separated from plasma and general extracellular fluid by endoneurial capillaries and the perineurial membrane, which form a 'blood-nerve' barrier that has similar properties to the blood-brain barrier in preventing the passive transfer of large molecules. As with neurones in the central nervous system, glucose is the major substrate for energy production in peripheral neural tissue, when plasma concentrations of ketone bodies are not elevated. Incubation of isolated nerve fascicles in a glucose-deficient medium produces a profound fall in glucose in nerve tissue, with reduced oxygen uptake and decrements in neural oxygen and ATP levels. Glucose utilisation in peripheral nerves does not appear to be regulated directly by insulin, which is also the case with neurones in the central nervous system. Peripheral neurones can, however, adapt to prolonged exposure to a low blood glucose. This was demonstrated by an elegant study of non-diabetic rats in which the anterograde fast component of axonal transport in peripheral neural tissue was reduced by 36% following the induction of acute hypoglycaemia (blood glucose ~1.5 mmol/l for two hours). The reduction in axonal transport was prevented, however, by pretreatment of the animals for three days with insulin to induce prolonged moderate hypoglycaemia. The mechanisms behind this adaptive response to hypoglycaemia are not clear, but may be similar to that observed in the rat central nervous system. Here, chronic hypoglycaemia over a period of days causes upregulation of the GLUT 1 blood-brain barrier glucose transport protein, which acts to increase extraction of glucose from the circulation during periods of subsequent hypoglycaemia.

There are, therefore, several possible explanations whereby peripheral neural tissue may be unaffected by moderate hypoglycaemia. Peripheral neurones have stores of glycogen, and these may provide sufficient energy to sustain neural metabolism during periods of short-lived, moderate hypoglycaemia. In addition, peripheral
neurones may be able to utilise other energy substrates, such as ketone bodies, fructose, lactate, amino acids and free fatty acids more efficiently than neurones within the brain. It is also possible that the 'blood-nerve' barrier may have a constitutively greater ability at extracting glucose from the circulation, or that peripheral neurones have a lower overall glucose requirement.

5.4.6 Summary and Conclusions
Acute hypoglycaemia caused predictable disruption of cognitive functions as assessed by tasks of general cognitive ability and multiple levels of speed of information processing. Hypoglycaemia did not have any differential effects on choice reaction time, as difficulty of the task increased, suggesting that the slope of the Hick Paradigm regression line may not be a valid marker of general intelligence.

Moreover, acute hypoglycaemia had no demonstrable effect on the function of peripheral nerves in healthy adult volunteers. This implies that neurones of the peripheral nervous system do not have the same obligate requirement for glucose as a metabolic fuel as neurones of the central nervous system. The data also partially refute the hypothesis that speed of conduction within the peripheral nervous system may be used as a marker of speed of nerve conduction within the central nervous system, and thus be a measure of speed of information processing and intelligence.

The effect of time on common peroneal conduction velocities may represent an effect of hyperinsulinaemia on peripheral neural function, but appropriately controlled studies are required to investigate this further.
CHAPTER 6

STUDY 2

RECOVERY OF COGNITIVE FUNCTION AND MOOD AFTER A SINGLE EPISODE OF SEVERE HYPOGLYCAEMIA
The cognitive decrements that occur during acute hypoglycaemia and the factors that moderate individual susceptibility to hypoglycaemia were discussed in detail in Chapter 2. Several investigators have also examined the time taken for cognitive function to recover to baseline levels following experimentally-induced, mild hypoglycaemia (blood glucose concentrations 2.5-3.0 mmol/l). Herold et al demonstrated that the recovery of simple reaction times was delayed for up to 40 minutes after the restoration of normoglycaemia and that there was considerable inter-individual variability in the recovery time, with the reaction times of some individuals returning to baseline within 10 minutes\textsuperscript{179}. These findings were supported by Blackman et al in studies of non-diabetic subjects\textsuperscript{171}, and people with Type 1 diabetes\textsuperscript{174}, in whom simple reaction times and latencies of the P300 event-related potential took between 45-75 minutes to return to baseline levels following correction of hypoglycaemia. Similarly, Lindgren et al reported that P300 amplitudes, which were reduced during hypoglycaemia, were restored approximately 40 minutes after the restitution of normoglycaemia\textsuperscript{175}.

The slight differences in recovery time between the above studies may be explicable by the fact that the duration and depth of hypoglycaemia was not consistent between the studies. Indeed, a recent preliminary report suggested that the recovery of cognitive function was dependent on the duration of antecedent hypoglycaemia\textsuperscript{300}. P300 latencies in 17 healthy volunteers took an average of 22 minutes to return to baseline following 60 minutes of hypoglycaemia (plasma glucose 2.8 mmol/l), but did not return to baseline values until 49 minutes after more prolonged hypoglycaemia (plasma glucose 2.8 mmol/l for 180 minutes)\textsuperscript{300}.

A delayed recovery in reaction times following acute hypoglycaemia was also observed in children with Type 1 diabetes by Ryan et al, who also noted that other cognitive tasks (Trail-Making B and the Stroop task) appeared to recover more quickly following the restitution of normoglycaemia\textsuperscript{301}. This latter observation was supported by a study of eight non-diabetic men, in whom the decrements in
performance of the Stroop task, induced by hypoglycaemia, returned to baseline within 20 minutes of restoration of normoglycaemia. However, 4-choice reaction times were still prolonged at this time.

By contrast, in non-diabetic subjects, Gold et al. found no delay in recovery of cognitive function following restoration of normoglycaemia. The reason for the discrepancy between this and the above studies that have examined the recovery of cognitive function after mild hypoglycaemia remains unclear, since a comprehensive battery of cognitive tasks was utilised and definite decrements in cognitive function were observed during the hypoglycaemic condition.

Two studies have considered the impact of nocturnal hypoglycaemia on cognitive function and well-being the following day. Bendtson et al. reported that nocturnal hypoglycaemia induced by insulin infusion (venous blood glucose nadir 1.5 mmol/l; blood glucose less than 3.0 mmol/l for 100 minutes) reduced the amount of deep sleep but had no impact on cognitive function, as assessed by a battery of tests, the following morning. These findings were replicated by King et al., who utilised a glucose clamp technique to maintain venous blood glucose at 2.3-2.7 mmol/l for one hour during the night. However, while cognitive function was not impaired the following morning, the subjects had a reduced sense of well-being and experienced greater fatigue during exercise.

The potential long-term effects of acute hypoglycaemia on mood have received less attention. Hepburn et al. in two separate studies of the effects of hypoglycaemia (induced by insulin infusion) on mood state, found that mood parameters, particularly tense arousal and hedonic tone, recovered in parallel with glucose concentrations. By contrast, Gold et al. demonstrated that the state of tense tiredness which developed in the context of more prolonged hypoglycaemia, induced using a glucose clamp technique, persisted for at least 30 minutes after restoration of normoglycaemia. Further assessment of mood status was not performed after this time period, so it is not known at what time the altered mood would have returned to baseline levels.
No previous studies have examined the timing of recovery of cognitive function and mood following a single episode of severe hypoglycaemia. It is quite conceivable that the more prolonged and profound neuroglycopenia that occurs in the context of an episode of severe hypoglycaemia, as opposed to an episode of experimentally-induced hypoglycaemia, could result in alterations in mood and cognitive function that persist for days or weeks. After all, the symptomatic and neuroendocrine responses to acute hypoglycaemia are diminished following antecedent (episodic) hypoglycaemia that has occurred within the previous 72 hours\textsuperscript{82,86,87,190,194} and the catecholamine response to hypoglycaemia, in particular, may take between six days and four weeks to return to a normal magnitude\textsuperscript{305}. Delayed recovery of cognitive function following severe hypoglycaemia could have potentially important effects on performance of activities such as work or driving. The purpose of the present study was, therefore, to examine the temporal changes in mood states and cognitive functions following a single, spontaneous episode of severe hypoglycaemia occurring in people with insulin-treated diabetes.
The study was approved by the local medical ethics advisory committee and all subjects gave written informed consent after the nature of the study was explained. Forty subjects with insulin-treated diabetes mellitus were studied, most of whom were attending the diabetes out-patient department at the Royal Infirmary of Edinburgh for regular review. Twenty subjects had experienced a recent episode of severe hypoglycaemia (the 'hypo' group) and twenty subjects had not experienced severe hypoglycaemia within the preceding year (the 'control' group).

### 6.2.1 Severe Hypoglycaemia

Severe hypoglycaemia was defined according to the criteria used in the DCCT, as an episode that required external assistance to aid recovery. In addition, the blood glucose concentration had to be documented at 2.8 mmol/l or lower and/or the clinical manifestations had to have been reversed with oral carbohydrate, intramuscular glucagon, or intravenous glucose.

Eighteen of the 'hypo' subjects were recruited following their presentation to the Accident and Emergency Departement of the Royal Infirmary of Edinburgh following an episode of severe hypoglycaemia. Only two of the subjects required overnight admission to hospital for observation, the remainder were discharged after treatment. The remaining two 'hypo' subjects were recruited following an advertising campaign about the study to both local general practitioners and patients attending the diabetes out-patient clinic at the Royal Infirmary of Edinburgh. Therefore, 'hypo' subjects received treatment for the severe hypoglycaemia either in the community or in the hospital emergency department; none had experienced persistent neurological dysfunction following restoration of normoglycaemia. Nine of the subjects were treated with intramuscular glucagon alone, two with intravenous dextrose alone, two with a combination of intravenous dextrose and intramuscular glucagon, and seven with oral carbohydrate alone. All subjects who required parenteral glucagon or dextrose reported a transient loss of consciousness at the time of hypoglycaemia. Where possible, the history of hypoglycaemia was verified by
scrutiny of contemporaneous notes made by the patient's general practitioner or the hospital case records, and by obtaining a direct account from a witness. The latter involved completion of a short questionnaire documenting the clinical features demonstrated by the affected patient, the nature of the treatment administered and their subsequent response. The history of previous severe hypoglycaemia in the 'control' group was confirmed by interview with the individual patients, by scrutiny of hospital case records and, where possible, by asking a relative or friend to complete a short questionnaire on when previous episodes of severe hypoglycaemia had been experienced by each patient. Hypoglycaemia history was graded on a four-point scale:

1 = no previous episodes of severe hypoglycaemia
2 = one to two previous episodes
3 = three to five previous episodes
4 = more than five previous episodes.

6.2.2 Patient Characteristics

One subject in each group had insulin-treated Type 2 diabetes, and all other subjects had Type 1 diabetes. All patients had received routine annual assessment in a diabetes out-patient clinic within the previous year and none had evidence of macrovascular disease. Few patients in either group had evidence of diabetic microangiopathy. Subjects were excluded if they had a history of cerebrovascular disease, previous head injury, epilepsy, serious systemic disease, major psychiatric illness, or if they were taking medication which could alter cognitive function, such as psychotropic agents or steroids. The corrected visual acuity of all subjects was 6/9 or better. Hypoglycaemia awareness was assessed using a visual analogue scale of 1 to 7 (1 = always aware of the onset of hypoglycaemia; 7 = never aware of the onset of hypoglycaemia). A score of 4 or greater has been shown to be associated with impairment of hypoglycaemia awareness\(^70\). Glycated haemoglobin (HbA1c) was measured at T1 using high-speed liquid chromatography based on an ion-exchange reverse-phase partition method (Hi Auto A1c HA 8121). The local non-diabetic range for HbA1c was 5.0-6.5%.
6.2.3 Neuropsychological Testing

Subjects underwent comprehensive neuropsychological testing at three time points (T1, T2 and T3) over a period of one month. To minimise any potential effect of sleep deprivation on the results of the cognitive tasks, all subjects had at least one night of undisturbed sleep prior to testing. For the 'hypo' subjects, this meant ensuring that they had obtained a complete night of sleep after the episode of severe hypoglycaemia. None of the 'hypo' subjects suffered further exposure to severe hypoglycaemia during the follow-up epoch of the study. Blood glucose was measured on three occasions during the cognitive testing sessions (beginning, middle and end); subjects were allowed to consume a snack, if necessary, to ensure that blood glucose concentrations did not fall below 4.0 mmol/l during testing.

The neuropsychological test battery provided a comprehensive assessment of the main cognitive domains, including fluid intelligence, memory, attention and concentration, frontal lobe function, information processing and mood state. Performance of many of the tests had been shown previously to be impaired during acute hypoglycemia in diabetic and non-diabetic subjects. The order of the tests (as listed below) remained constant for each session. Where appropriate, subjects underwent a brief practice session with each test prior to the recorded performance. The neuropsychological test battery included the following tests:

- **Cognitive Function Self-Appraisal Scale (CFSA)**
- **UWIST Mood Adjective Checklist**
- **Hospital Anxiety and Depression Scale (HAD)**
- **National Adult Reading Test (NART)** - administered at T1 only
- **Wechsler Adult Intelligence Scale - Revised (WAIS-R)** - Performance IQ subtests
- **Trail-Making Test B (TMB)**
- **Forward Digit Span (FDS)**
- **Backward Digit Span (BDS)**
- **Logical Memory (LM) - Immediate Recall**
- **Figural Memory (FM)**
- **Borkowski Verbal Fluency Task**
The cognitive tasks were described in detail in Chapter 4.1. Many of the Performance IQ subtests of the WAIS-R were subject to a practice effect, i.e. performance improved significantly following repeated administrations of the task. Therefore, Performance IQ was measured solely at T1; at the subsequent sessions, only the DS and BD tasks were administered, as these were subject to the least practice effect. An IQ Decrement Index was also calculated by subtracting the present IQ (as measured by the WAIS-R Performance IQ subtests) from the 'pre-morbid' IQ (as estimated by the NART).

6.2.4 Statistical Analyses
All data are presented as mean ± standard deviation (SD). Baseline characteristics of subjects were compared using Student's *t* tests for unpaired samples, and the Chi-squared test was used for categorical data. A test of sphericity revealed that there was insufficient correlation between the scores of individual cognitive tasks to allow employment of an omnibus, mixed-design, multivariate analysis of variance. Zero order correlation tables displaying the associations between the individual cognitive tasks at each time point are included in the Appendix. Therefore, differences in individual cognitive test scores between the 'hypo' and 'control' groups, at the three time points, were compared using multiple, mixed-model univariate analyses of variance (ANOVA) with 'group' ('hypo' or 'control') as a between-subjects factor and 'time' (T1, T2, or T3) as a repeated measure. Simple effects (unpaired *t* tests, two-tailed) at particular time points were examined only when there were significant group and/or interaction effects.
The principal outcome statistic was any ['group' by 'time'] interaction in the ANOVA analysis. This statistic had the potential to indicate evidence of 'catch-up' on behalf of the 'hypo' group who were hypothesised to demonstrate under-performance on the first and perhaps the second time points (Figure 6.1). Significant main effects for group indicated overall superiority of the 'hypo' or 'control' groups on the test. Significant effects of 'time' were expected and would indicate practice effects on the tasks.

Where significant simple effects were evident, cognitive and mood outcome scores were adjusted for individual differences in severe hypoglycaemia history (using the 4-point hypoglycaemia scoring system) using univariate linear regression. A multiple regression model was not utilised because of the repeated measures design. The group effect on the standardised residuals (Z-scores - with a mean value of 0 and a standard deviation of 1.0 for data across the three time points) was subsequently examined using Student's t-tests, at the appropriate time points. This was an attempt to test the hypothesis that any cognitive impairment was secondary to exposure to previous episodes of severe hypoglycaemia.

A p-value of less than 0.05 was considered to be significant, given the exploratory nature of this study. Two-tailed tests were used throughout to limit the occurrence of type 1 statistical errors. All analyses were performed using SPSS version 7.5.1 for Windows 95. Reported p-values were rounded to two decimal places; thus p=0.00 signifies that the p-value was ≤0.005.
Scores of 'control' and 'hypo' subjects from a theoretical cognitive task administered at three time points following an episode of severe hypoglycaemia (which occurred just before session 1 for the 'hypo' subjects). The scores of the 'control' subjects improved with time, indicating a slight practice effect. The scores of the 'hypo' subjects were lower than the 'controls' during sessions 1 and 2, but demonstrated evidence of a 'catch-up' effect, such that by session 3 the scores of the two groups were equivalent.
6.3 RESULTS

6.3.1 Baseline Clinical Characteristics
The 'hypo' subjects had a history of a significantly greater number of previous episodes of severe hypoglycaemia than the 'control' subjects (Table 6.1; p=0.00). The subjects in the two groups were well matched for age, sex, body mass index (BMI) and duration of diagnosis of diabetes. The 'hypo' subjects had a lower mean HbA1c at the outset of the study and a tendency towards a higher total insulin dose than the 'control' subjects (Table 6.2). The mean Hypoglycaemia Awareness Scores were also higher in the 'hypo' group than in the 'control' group, i.e. overall the 'hypo' subjects were less aware of hypoglycaemia (Table 6.2). Nine subjects in the 'hypo' group had a score of 4 or greater on the visual analogue scale, compared to only one patient in the 'control' group (p=0.00).

6.3.2 Timing of Cognitive Test Sessions
All subjects had a complete night of sleep prior to cognitive testing. For the 'hypo' subjects, as a consequence, T1 took place at a mean (SD) of 1.5 (0.6) days after the episode of severe hypoglycaemia. T2 took place a mean of 8.9 (2.0) days, and T3 30.2 (2.9) days after severe hypoglycaemia. The 'control' subjects were tested at comparable time intervals - the mean (SD) interval between T1 and T2 was 7.3 (0.7) days, and that between T2 and T3 was 29.0 (2.3) days (the comparable intervals for the 'hypo' subjects were 7.4 and 28.7 days respectively; p>0.05).

6.3.3 Baseline Cognitive Function and IQ Decrement Index
No significant difference in mean NART error scores was observed between the 'hypo' subjects (20.9±9.1) and the 'control' subjects (19.9±5.0; p=0.65), indicating that the two groups were well matched for pre-morbid IQ. The actual performance IQ of the 'hypo' subjects, measured at T1, was on average 6.8±10.8 points lower than would have been predicted by the NART score (the IQ Decrement Index). By contrast, the actual performance IQ of the 'control' subjects was 1.7±10.2 points higher than predicted. The difference in IQ Decrement Indices between the two groups was statistically significant (p=0.02).
The means and standard deviations of the scores of the remaining cognitive tests, mood scales and memory questionnaire are shown in Tables 6.3, 6.4 and 6.5.

**6.3.4 Effects of 'Group' by 'Time' Interactions on Cognitive and Mood Tasks**

Significant 'group' by 'time' interactions were found with the BD (p=0.01), PASAT 4 second (p=0.02) and VCD (p=0.03) tasks. In the cases of the BD and PASAT tasks, the interaction reflected significant differences between the two groups at T1 that were not present at T2 or T3. The interaction for the VCD task represented an apparently anomalous difference between the groups at T2, with the 'hypo' subjects actually attaining better scores, that was not statistically significant (p=0.07), and that was not present at T1 or T3. The interactions for the Hedonic Tone (p=0.08) and Energetic Arousal (p=0.05) sub-scales of the UWIST Mood Adjective Checklist approached statistical significance, although none of the simple effects were significant. No other cognitive or mood tests demonstrated a statistically significant 'group' by 'time' interaction (p>0.05).

**6.3.5 Main Effects of 'Group' on Cognitive Tasks**

The 'hypo' group was significantly poorer overall on the DS (p=0.01), PASAT 4 second (p=0.04), FDS (p=0.01), and BDS (p=0.02) tasks relative to 'control' subjects. In addition, the 'hypo' subjects performed more poorly than controls on the 'colour-associated words' (p=0.01) and 'incongruent colours' (p=0.01) subtests of the Stroop task. Post-hoc simple effects analysis demonstrated that performance in the DS and the 'incongruent colours' Stroop tasks was poorer in the 'hypo' subjects at all three time points (p<0.05), while that in the 'colour-associated words' Stroop subtest was significant at T1 and T2 (p<0.05), but did not reach statistical significance at T3 (p=0.06). The FDS and BDS scores were significantly poorer in the 'hypo' subjects only at T2 and T3 (p<0.05). The main effect of 'group' on the PASAT 2 second (p=0.07) and BD (p=0.06) tasks just failed to reach conventional levels of statistical significance. No significant main effects of 'group' were demonstrated on the information processing (IT, VCD, RT), memory (FM, LM), TMB and verbal fluency tasks (p>0.05).
6.3.6 Main Effects of 'Group' on Mood Scales

Significant main effects of 'group' ('hypos' versus 'control') were observed on the HAD-depression (p=0.01) and HAD-anxiety (p<0.05) scales. The 'hypo' subjects had significantly higher HAD-depression scores at all three time points (p<0.05) and significantly higher HAD-anxiety levels at T1 (p=0.02). No significant 'group' effects were recorded with any of the UWIST mood scores (p>0.05).

6.3.7 Main Effects of 'Group' on Self-Rated Cognitive Ability

A significant main effect of 'group' ('hypos' versus 'control') was noted for the CFSA 'total' score (p=0.00) with the 'hypo' subjects having significantly lower levels of self-rated cognitive ability at all three time points (p<0.05). At each time point, higher numbers of 'hypo' subjects indicated that they had noticed either a large or slight change in cognitive function with time (Table 6.5; p<0.05).

6.3.8 Main Effects of 'Time' on Cognitive and Mood Tasks

All cognitive tasks, with the exception of the FDS (p=0.74) and BDS tests (p=0.23), showed significant learning effects (as indicated by a statistically significant main effect of time; p<0.05). The UWIST Energetic Arousal, and the HAD depression scores did not show a significant effect of 'time' (p>0.05), while a time effect was seen with the UWIST Hedonic Tone (p=0.01) and Tense Arousal (p=0.01) scores, and the HAD-anxiety scores (p=0.00). Hedonic Tone increased with time, while Tense Arousal and HAD-anxiety decreased with time. These results do not affect the interpretability of the effect of the experimental variable ('hypo' versus 'control') because they apply to both groups.

6.3.9 Effects of Previous Episodes of Severe Hypoglycaemia on Cognitive and Mood Tasks

Following adjustment for history of severe hypoglycaemia, differences in DS, BD, Stroop (colour-associated words and incongruent colours), FDS, BDS, PASAT 4 second, HAD-anxiety, HAD-depression and CFSA scores (at time points where significant simple effects were obtained) became non-significant (Table 6.6; p>0.05).
### TABLE 6.1: History of Severe Hypoglycaemia

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>1-2</th>
<th>3-5</th>
<th>&gt;5</th>
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<td>4</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>'Controls'</td>
<td>13</td>
<td>3</td>
<td>2</td>
<td>2</td>
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</table>

Data represents the number of subjects who experienced no previous episodes of severe hypoglycaemia, 1-2 episodes, 3-5 episodes or greater than 5 episodes.
TABLE 6.2: Clinical Characteristics of Subjects

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<th></th>
<th>'Hypos'</th>
<th>'Controls'</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>36.4±14.7</td>
<td>33.7±12.5</td>
<td>0.54</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>13/7</td>
<td>12/8</td>
<td>0.74</td>
</tr>
<tr>
<td>Duration of Diabetes (yrs)</td>
<td>15.5±7.6</td>
<td>13.0±8.2</td>
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<td>BMI (kg/m²)</td>
<td>25.8±4.0</td>
<td>25.4±3.1</td>
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<td>Insulin Dose (U/kg)</td>
<td>0.82±0.31</td>
<td>0.71±0.18</td>
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<td>HbA1c (%)</td>
<td>8.3±1.3</td>
<td>10.0±1.7</td>
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<tr>
<td>Hypo Awareness Score</td>
<td>3.35±1.7</td>
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</table>

Data are means±SD
TABLE 6.3: Cognitive Test Results

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<th>T3</th>
<th>p-value</th>
<th>Simple Effects</th>
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<td>'Hypo'</td>
<td>'Control'</td>
<td>'Hypo' vs. 'Control'</td>
<td>'Time'</td>
<td>'Group' x 'Time' Interaction</td>
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<td><strong>Digit Symbol</strong></td>
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<td>0.00</td>
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<td>26.6 (11.8)</td>
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<td>'Control'</td>
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<td>0.02</td>
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</table>

Data are means (±SD)

* = cognitive test where a lower score indicates better performance; in the remaining tasks a higher score indicates better performance.
<table>
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<tr>
<th></th>
<th>T1</th>
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<th>T3</th>
<th>p-value</th>
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Data are means (±SD)

* = cognitive test where a lower score indicates better performance; in the remaining tasks a higher score indicates better performance.
TABLE 6.3: Cognitive Test Results (Continued)

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<th>'Hypo' vs. 'Control'</th>
<th>'Time'</th>
<th>'Group' x 'Time' Interaction</th>
<th>Simple Effects</th>
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<td></td>
<td>T1</td>
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<td>T3</td>
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</tr>
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<tr>
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<td>25.8 (7.4)</td>
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<td>27.1 (6.9)</td>
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<td>- Self-Appraisal</td>
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<td>'Hypo'</td>
<td>50.3 (20.8)</td>
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<td>0.01</td>
<td>0.45</td>
<td>0.00</td>
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<tr>
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<td>28.2 (12.2)</td>
<td>27.2 (12.5)</td>
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Data are means (±SD)

* = cognitive test where a lower score indicates better performance; in the remaining tasks a higher score indicates better performance.
### TABLE 6.3: Cognitive Test Results (Continued)

<table>
<thead>
<tr>
<th>Simple Effects</th>
<th>Time 1</th>
<th>Time 2</th>
<th>Time 3</th>
<th>p-value</th>
<th>p-value</th>
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<td>'Time'</td>
<td>'Group' x 'Time' Interaction</td>
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<td>1-Choice Reaction Time - Median Decision Time</td>
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</tr>
<tr>
<td>Hypo</td>
<td>273.6 (26.8)</td>
<td>268.7 (28.9)</td>
<td>267.8 (30.2)</td>
<td>0.15</td>
<td>0.12</td>
<td>0.29</td>
</tr>
<tr>
<td>Control</td>
<td>259.5 (25.4)</td>
<td>255.0 (21.1)</td>
<td>260.1 (26.0)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1-Choice Reaction Time - Median Movement Time</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Hypo</td>
<td>141.7 (34.3)</td>
<td>132.7 (44.2)</td>
<td>129.2 (47.2)</td>
<td>0.65</td>
<td>0.02</td>
<td>0.27</td>
</tr>
<tr>
<td>Control</td>
<td>131.8 (23.5)</td>
<td>128.3 (28.2)</td>
<td>128.6 (30.8)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2-Choice Reaction Time - Median Decision Time</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Hypo</td>
<td>307.3 (39.8)</td>
<td>295.1 (37.0)</td>
<td>289.3 (33.4)</td>
<td>0.41</td>
<td>0.00</td>
<td>0.47</td>
</tr>
<tr>
<td>Control</td>
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<td>284.2 (34.5)</td>
<td>285.6 (34.9)</td>
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</tr>
<tr>
<td>2-Choice Reaction Time - Median Movement Time</td>
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</tr>
<tr>
<td>Hypo</td>
<td>151.5 (39.2)</td>
<td>141.1 (49.6)</td>
<td>139.3 (50.5)</td>
<td>0.57</td>
<td>0.02</td>
<td>0.44</td>
</tr>
<tr>
<td>Control</td>
<td>140.7 (27.3)</td>
<td>133.7 (31.6)</td>
<td>137.2 (32.5)</td>
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<tr>
<td>4-Choice Reaction Time - Median Decision Time</td>
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<td></td>
</tr>
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<td>Hypo</td>
<td>324.2 (41.1)</td>
<td>311.9 (39.5)</td>
<td>307.0 (38.7)</td>
<td>0.49</td>
<td>0.00</td>
<td>0.77</td>
</tr>
<tr>
<td>Control</td>
<td>313.3 (41.7)</td>
<td>303.8 (35.2)</td>
<td>301.6 (39.5)</td>
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<tr>
<td>4-Choice Reaction Time - Median Movement Time</td>
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</tr>
<tr>
<td>Hypo</td>
<td>156.2 (41.9)</td>
<td>145.7 (52.5)</td>
<td>143.3 (52.4)</td>
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<td>0.00</td>
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<td>Control</td>
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<td>136.2 (33.0)</td>
<td>135.3 (27.6)</td>
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<td>8-Choice Reaction Time - Median Decision Time</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypo</td>
<td>351.8 (43.7)</td>
<td>337.0 (43.5)</td>
<td>330.0 (37.4)</td>
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<td>0.00</td>
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<td>Control</td>
<td>344.3 (49.8)</td>
<td>328.0 (40.8)</td>
<td>328.7 (41.8)</td>
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<tr>
<td>8-Choice Reaction Time - Median Movement Time</td>
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<td></td>
</tr>
<tr>
<td>Hypo</td>
<td>159.5 (38.4)</td>
<td>150.8 (51.6)</td>
<td>143.6 (52.0)</td>
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<td>0.00</td>
<td>0.80</td>
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<tr>
<td>Control</td>
<td>147.6 (26.1)</td>
<td>140.8 (34.0)</td>
<td>135.7 (28.4)</td>
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</table>

Data are means (±SD)

Higher scores represent slower reaction times.
### TABLE 6.4: Mood Scale Results

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<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>p-value</th>
<th>Simple Effects</th>
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<tr>
<td><strong>UWIST Hedonic Tone</strong></td>
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<tr>
<td>'Hypo'</td>
<td>26.6</td>
<td>28.5</td>
<td>28.3</td>
<td>0.17</td>
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</tr>
<tr>
<td>'Control'</td>
<td>29.2</td>
<td>30.2</td>
<td>28.7</td>
<td>0.01</td>
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<td>0.08</td>
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<tr>
<td><strong>UWIST Tense Arousal</strong></td>
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<td>'Hypo'</td>
<td>16.1</td>
<td>14.2</td>
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<td><strong>UWIST Energetic Arousal</strong></td>
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<td>23.0</td>
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<td>25.0</td>
<td>24.0</td>
<td>0.81</td>
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</tr>
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<td></td>
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<td>0.05</td>
<td></td>
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<tr>
<td><strong>HAD - Anxiety</strong></td>
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<td>'Hypo'</td>
<td>7.8</td>
<td>6.1</td>
<td>5.8</td>
<td>0.05</td>
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<td>4.4</td>
<td>4.2</td>
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<td>0.18</td>
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<td><strong>HAD - Depression</strong></td>
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<td>3.2</td>
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</table>

Data are means (±SD)

Higher scores represent greater levels of the mood parameter.
TABLE 6.5: Cognitive Function Self-Appraisal Scale - 'Change' Scores

<table>
<thead>
<tr>
<th></th>
<th>T1</th>
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<tbody>
<tr>
<td>'Hypo'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Change</td>
<td>9</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Slight Change</td>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Large Change</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>'Control'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Change</td>
<td>18</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Slight Change</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Large Change</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

p-values: 0.01, 0.04, 0.04 ('Hypo' v 'Control')

Data are number of subjects
TABLE 6.6: Standardised Residuals (Z-score format) of Significant Cognitive Tasks and Mood Scales after Adjustment for Severe Hypoglycaemia History

<table>
<thead>
<tr>
<th></th>
<th>Time 1</th>
<th>Time 2</th>
<th>Time 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z-score</td>
<td>p-value</td>
<td>Z-score</td>
</tr>
<tr>
<td><strong>Digit Symbol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Hypo'</td>
<td>-0.27 (0.87)</td>
<td>0.50</td>
<td>0.07 (0.90)</td>
</tr>
<tr>
<td>'Control'</td>
<td>-0.47 (0.98)</td>
<td></td>
<td>-0.00 (1.13)</td>
</tr>
<tr>
<td><strong>Block Design</strong></td>
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</tr>
<tr>
<td>'Hypo'</td>
<td>-0.16 (0.92)</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>'Control'</td>
<td>-0.53 (0.81)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Stroop Test - Colour-Associated Words</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Hypo'</td>
<td>0.16 (0.81)</td>
<td>0.17</td>
<td>-0.25 (0.84)</td>
</tr>
<tr>
<td>'Control'</td>
<td>0.69 (1.40)</td>
<td></td>
<td>-0.03 (0.93)</td>
</tr>
<tr>
<td><strong>Stroop Test - Incongruous Colours</strong></td>
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<tr>
<td>'Hypo'</td>
<td>0.34 (0.63)</td>
<td>0.17</td>
<td>-0.26 (0.80)</td>
</tr>
<tr>
<td>'Control'</td>
<td>0.86 (1.46)</td>
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<td>-0.09 (0.89)</td>
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<td><strong>PASAT 4 Second</strong></td>
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<td>'Hypo'</td>
<td>-0.12 (0.85)</td>
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<tr>
<td>'Control'</td>
<td>-0.58 (1.52)</td>
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</table>

Standardised residuals are in Z-score format (mean of 0 and standard deviation of 1.0 for data across the three time points).

p-value is the level of significance for the between group comparisons of the Z-scores.

N/A - statistical comparison not appropriate as the cognitive task or mood scale was not significant, at this time point, in the initial post hoc analyses.
TABLE 6.6: Standardised Residuals (Z-score format) of Significant Cognitive Tasks and Mood Scales after Adjustment for Severe Hypoglycaemia History (Continued)

<table>
<thead>
<tr>
<th></th>
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<td>p-value</td>
<td>Z-score</td>
<td>p-value</td>
<td>Z-score</td>
<td>p-value</td>
</tr>
<tr>
<td><strong>Forward Digit Span</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Hypo'</td>
<td>N/A</td>
<td>N/A</td>
<td>0.07 (0.87)</td>
<td>0.62</td>
<td>0.00 (0.88)</td>
<td>0.75</td>
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<tr>
<td>'Control'</td>
<td>-0.10 (1.20)</td>
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<td>-0.10 (1.16)</td>
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<td></td>
</tr>
<tr>
<td><strong>Backward Digit Span</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>'Hypo'</td>
<td>N/A</td>
<td>N/A</td>
<td>-0.03 (0.92)</td>
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<td>0.25 (1.10)</td>
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<td>0.02 (0.79)</td>
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<td><strong>Cognitive Function - Self-Appraisal</strong></td>
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<tr>
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<td>0.00 (0.53)</td>
<td>0.20</td>
<td>-0.16 (0.60)</td>
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<td>-0.22 (0.66)</td>
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<td>0.40 (1.24)</td>
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<td>0.09 (1.26)</td>
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</tr>
<tr>
<td>'Hypo'</td>
<td>0.06 (0.64)</td>
<td>0.26</td>
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<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<td>0.42 (1.28)</td>
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<tr>
<td><strong>HAD - Depression</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Hypo'</td>
<td>0.00 (0.93)</td>
<td>0.71</td>
<td>-0.05 (0.95)</td>
<td>0.68</td>
<td>-0.14 (0.88)</td>
<td>0.73</td>
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<tr>
<td>'Control'</td>
<td>0.11 (0.84)</td>
<td></td>
<td>0.08 (0.98)</td>
<td></td>
<td>-0.00 (1.40)</td>
<td></td>
</tr>
</tbody>
</table>

Standardised residuals are in Z-score format (mean of 0 and standard deviation of 1.0 for data across the three time points).

p-value is the level of significance for the between group comparisons of the Z-scores.

N/A - statistical comparison not appropriate as the cognitive task or mood scale was not significant, at this time point, in the initial post hoc analyses.
6.4 DISCUSSION

6.4.1 Interpretation of Results

The present study attempted to evaluate changes in mood and cognitive function prospectively in patients with insulin-treated diabetes who had experienced a spontaneous episode of acute, severe hypoglycaemia. It was appreciated during the initial phases of study design that not all episodes of severe hypoglycaemia are the same in terms of the depth and duration of neuroglycopenia and, ultimately, in the magnitude of the 'hypoglycaemic insult' experienced by the brain. The overwhelming majority of such episodes occur in the community where accurate measurements of variables such as blood glucose nadir and duration of loss of consciousness are often not available or are not estimated with accuracy. Therefore, to have attempted to achieve a greater degree of uniformity in terms of the magnitude of the 'hypoglycaemic insult' would have been of dubious validity and would have imposed enormous logistical strain on the study with regard to the recruitment of subjects. As a consequence, a pragmatic approach was taken to utilise the definition of severe hypoglycaemia employed in the DCCT55.

In the statistical analyses, it was anticipated that a significant interaction between 'group' and 'time' effects might be indicative of a 'hangover' effect of severe hypoglycaemia on the cognitive parameter measured, with performance improving with time in the 'hypo' subjects more than in the 'controls' (Figure 6.1). Such an interaction was statistically significant in only two cognitive tasks - BD and PASAT 4 second. The results for the PASAT 4 second task can be interpreted as showing a 'ceiling effect'. The performance of the 'control' subjects at T1 was very close to maximum and consequently there was little scope for their scores to improve with time. By contrast, the scores of the 'hypo' subjects at T1 were significantly lower, allowing the opportunity for improvement to occur over time. Thus, it may not be appropriate to attribute the significant result for the PASAT 4 second interaction as evidence of a prolonged effect of severe hypoglycaemia on cognitive function.
Therefore, in only one (BD) of the 14 cognitive tasks employed was convincing evidence obtained of improvement in the 'hypo' subjects relative to 'controls' over time. This implies that, in the main, any cognitive decrement that resulted as a consequence of the acute episode of severe hypoglycaemia had recovered by the time of the initial testing session at 1.5 days. The 'group' by 'time' interactions for UWIST Hedonic Tone and Energetic Arousal approached statistical significance, with evidence that 'happiness' and 'energy' increased with time in the 'hypo' subjects, with respect to 'controls'. There was no evidence of a ceiling effect with regards these mood parameters, and while such mood alterations in response to severe hypoglycaemia might be predictable, the 'simple effects' for the statistical interactions were not significant. Therefore, the interpretation that acute severe hypoglycaemia had a 'hangover' effect on mood must be made with caution.

The two study groups were well matched for standard demographic characteristics and pre-morbid IQ, but subjects in the 'hypo' group had a previous history of significantly more episodes of severe hypoglycaemia. Significant main effects of 'group' ('hypo' versus 'control') were observed with several of the psychometric tests measuring fluid-type intelligence and attention and concentration skills. In addition, the 'hypo' subjects had higher levels of HAD-depression and HAD-anxiety, and exhibited higher self-rated levels of cognitive impairment (CFSA). No significant 'group' effects were observed with the memory or information processing tasks. Several previous cross-sectional studies have suggested that recurrent exposure to episodes of severe hypoglycaemia is associated with cumulative cognitive impairment217,223,225-227. In the present study, the differences in cognitive performance and mood between the two groups was abolished when statistical adjustment was made for the number of previous episodes of severe hypoglycaemia experienced by subjects. In addition, the difference in mean IQ decrement index scores (i.e. the difference in IQ scores as predicted by the NART scale and as measured by the WAIS-R scale) for the 'hypo' and 'control' subjects was 8.5 points. This is comparable to the mean difference in IQ decrement scores of 5.8 points observed by Langan and colleagues225 when comparing diabetic subjects with a history of multiple episodes of severe hypoglycaemia with those who had no history.
of previous severe hypoglycaemia. It is possible, therefore, that the difference in cognitive ability noted in the current study between the 'hypo' subjects and 'controls' was a consequence of the previous exposure of the former group to many more episodes of severe hypoglycaemia. However, the study was not powered to specifically investigate this issue and the statistical adjustment for the effects of exposure to previous severe hypoglycaemia should be interpreted with caution because severe hypoglycaemia was itself a significant variable between the study groups. The small sample size precluded a sufficiently powerful correlational analysis between cognitive performance and previous severe hypoglycaemia history in the 'hypo' subjects alone.

The elevated anxiety and depression scores recorded in the 'hypo' subjects have been noted previously in diabetic patients exposed to recurrent severe hypoglycaemia\(^\text{216,307}\) and could be either a consequence or a cause of the previous severe hypoglycaemia. Unfortunately, it is not possible to perform a surrogate 'retrospective' assessment of mood in a similar manner to the use of the NART for intellectual ability. Gonder-Frederick et al\(^\text{308}\) prospectively measured symptoms of depression (using the Beck Depression Inventory) in 20 subjects who experienced two or more episodes of severe hypoglycaemia over a six month period and in 20 control subjects who experienced no severe hypoglycaemia during that time. Depression scores were comparable between the groups at baseline, but at six months were significantly higher in the subjects exposed to severe hypoglycaemia\(^\text{308}\).

The other main differences between the 'hypo' and 'control' subjects was that the former group had a greater frequency of impaired awareness of hypoglycaemia and had lower mean glycated haemoglobin concentrations. Impaired awareness of hypoglycaemia\(^\text{70,71}\) and strict glycaemic control\(^\text{55}\) are associated with an increased risk of severe hypoglycaemia and so their increased prevalence in the 'hypo' group was predictable and probably unavoidable. The controversy surrounding the impact of impaired awareness and strict glycaemic control on cognitive function during acute hypoglycaemia is discussed in detail in Chapter 2. The majority of studies have suggested that impaired awareness of hypoglycaemia and strict glycaemic
control are associated with either no effect on cognitive function or reduced cognitive decrements during acute hypoglycaemia. The possibility that either factor per se was associated with the 'group' effects noted in the present study cannot be excluded, but seems unlikely.

Most of the cognitive tasks showed a significant main effect of 'time', which was presumably indicative of a practice effect. The practice effect was evident despite the fact that subjects undertook training in the individual cognitive tasks before the formal test performance and clearly highlights the importance of a parallel, longitudinal 'control' group in studies of this type. The UWIST Tense Arousal and Hedonic Tone, and the HAD-anxiety scores also showed a significant main effect of 'time', with levels of tense arousal and anxiety decreasing with time and levels of hedonic tone or 'happiness' increasing with time. This may reflect the natural initial apprehension of subjects participating in a clinical study.

6.4.2 Limitations of the Study
The present study has some limitations. These include the relatively small sample size, the absence of pre-hypoglycaemia cognitive and mood data, and the need for multiple statistical testing.

The study had approximately 80% power to detect a 0.9 standard deviation difference in Digit Symbol scores (a task that is particularly sensitive to the effects of acute hypoglycaemia) with a p-value set at 0.05. The sample size meant that a small 'hangover' effect of severe hypoglycaemia on cognitive function may not have been detected and precluded meaningful analysis to determine if there were large individual differences in the impact of severe hypoglycaemia. The recruitment of insulin-treated diabetic patients following severe hypoglycaemia proved to be very difficult, despite the high frequency of this problem in clinical practice. As indicated previously, the majority of episodes of severe hypoglycaemia are treated effectively in the community, and only a small, selected group requires hospitalisation. Attempts to improve recruitment by advertising the study in advance to the patients attending our clinic, and to all local general practitioners, were fruitless. In addition,
as observed previously in a local survey of acute, severe hypoglycaemia\textsuperscript{310}, recruitment was hampered by the fact that many of the diabetic patients attending hospital for emergency treatment were either elderly, had concurrent medical disorders or were infrequent attendees at the diabetic clinic. Furthermore, many patients who were eligible for inclusion were unable to participate in the study because of the short notice or the time constraints necessitated by the study design, with the requirement for multiple testing sessions.

The comparability of the study groups in terms of NART scores provides a degree of reassurance about the matching for pre-morbid cognitive ability. The collection of more detailed cognitive and mood data on the 'hypo' subjects, prior to the index episode of severe hypoglycaemia, would have necessitated testing a large number of diabetic subjects and a relatively long period of follow-up to identify subsequent hypoglycaemic episodes. Future studies could reduce the number of subjects that would undergo cognitive pre-testing by studying subjects who are at high risk of severe hypoglycaemia, e.g. patients who have had an episode of severe hypoglycaemia in the preceding year or who have impaired awareness of hypoglycaemia.

The large number of statistical tests that were employed in analysing the data generated by this study, increased the risk of type 1 statistical errors and, indeed, the significant interaction between the 'group' and 'time' effects for the BD task may represent such an error. The risk of a type 1 error could have been reduced by applying the Bonferroni correction, in which a p-value of less than $\sim 0.004$ would strictly be accepted as evidence of statistical significance for the 12 main cognitive parameters measured. However, at the outset of the study, it was unclear which specific areas of cognitive function and mood might exhibit a 'hangover' effect and clearly a balance had to be made between testing a wide number of cognitive functions and risking type 1 errors, and including a limited number of cognitive measures and potentially missing an effect in area not tested. Given the exploratory/preliminary nature of the present study the former approach, utilising a conventional level of statistical significance, was adopted. The pattern of cognitive
and mood data provided should allow future investigators to select a smaller test battery, that is more specifically targeted towards potentially sensitive cognitive domains.

6.4.3 Conclusions

The results of this study imply that a single episode of acute, severe hypoglycaemia, from which recovery appears complete, does not have a prolonged effect on cognitive function. People with insulin-treated diabetes may be reassured that performance of activities such as work or driving are unlikely to be impaired 36 hours after an episode. The evidence of persistent impairment of cognitive performance and the mood disturbance demonstrated by the 'hypo' subjects may be a long-term consequence of repeated exposure to previous episodes of severe hypoglycaemia.
CHAPTER 7

STUDY 3

EVALUATION OF SERUM MARKERS OF NEURONAL DAMAGE FOLLOWING SEVERE HYPOGLYCAEMIA
7.1 INTRODUCTION

The ability of severe hypoglycaemia to induce both transient and permanent neurological abnormalities, and rarely death, was described in Chapter 1. Clinicians often have difficulty in determining the prognosis of comatose patients following exposure to severe hypoglycaemia, in terms of both survival and risk of permanent neurological dysfunction. The identification of serum markers that could predict the degree of neuronal damage and prognosis of patients after severe hypoglycaemia, analogous to the measurement of creatine kinase or troponin after myocardial infarction, would therefore have considerable clinical value.

Two proteins, Neuron-Specific Enolase (NSE) and Protein S-100 (S-100), have shown promising results as prognostic agents in the context of other acute neurological syndromes. NSE is a 78kD glycolytic enzyme that originates predominantly from the cytoplasm of neurones and neuroendocrine cells\textsuperscript{311}. Increased NSE concentrations are found in patients with small-cell lung cancer, neuroblastoma and neuroendocrine tumours\textsuperscript{311}. Furthermore, the concentrations of NSE, both in cerebrospinal fluid (CSF) and in serum, are elevated in various neurological disorders\textsuperscript{312-325}. In particular, concentrations of serum NSE, measured in venous blood within 48-72 hours of an acute stroke, correlate significantly with clinical outcome\textsuperscript{258,326,327}.

S-100 is a calcium-binding protein which exists as homo- or heterodimers of two subunits - $\alpha$ (10.4kD) and $\beta$ (10.5kD)\textsuperscript{328,329}. The $\alpha\alpha$-S-100 form is found exclusively in neurones, $\alpha\beta$-S-100 is present in glial cells, and $\beta\beta$-S-100 is found in glial cells and in Schwann cells\textsuperscript{329}. S-100 has also been detected in some non-neural tissues, such as melanocytes\textsuperscript{330} and adipose tissue\textsuperscript{331}, and elevated serum concentrations of S-100 have been reported in patients with malignant melanoma\textsuperscript{332}. As with NSE, acute neurological injury causes concentrations of S-100 to rise in CSF and in serum\textsuperscript{259,314,320,321,324,333-340}, and concentrations of S-100 measured in serum samples taken within 48 hours of an acute stroke correlate significantly with clinical outcome\textsuperscript{259}.
At present, no biological markers are available which predict clinical outcome with accuracy after an episode of severe hypoglycaemia. The effects of severe hypoglycaemia on serum concentrations of NSE and S-100 have not been evaluated previously. Serum concentrations of NSE and S-100 were, therefore, estimated (acutely) in 16 diabetic patients who experienced a single episode of severe hypoglycaemia without permanent neurological injury, in three diabetic patients who sustained significant brain damage (and who subsequently died) following an episode of severe hypoglycaemia, and in ten diabetic patients who had not experienced an episode of severe hypoglycaemia for at least one year.
7.2 METHODS

7.2.1 Subjects
The study was performed in conjunction with Study 2, reported in Chapter 6, and was approved by the local medical ethics advisory committee. Twenty six of the 29 subjects studied participated in Study 2: 16 subjects were in the 'hypo' group (i.e. were recruited shortly after experiencing an episode of severe hypoglycaemia) and 10 were in the 'control' group (i.e. had not experienced an episode of severe hypoglycaemia for at least one year). The methods of recruitment of the subjects, the definition of severe hypoglycaemia employed, the verification of the subjects' previous history of hypoglycaemia and the method of HbA1c estimation were described in Chapter 6. With regard to the 'hypo' subjects, nine of the subjects were treated with intramuscular glucagon, two with intravenous dextrose and five with oral carbohydrate. None of the subjects had a previous history of any neoplastic or neurological disorder that is known to elevate serum NSE or S-100 concentrations. In addition, three diabetic subjects who died after an episode of severe hypoglycaemia were studied as individual cases and their case histories are described in Chapter 7.2.5.

7.2.2 Timing of Blood Samples
Venous blood samples were taken for measurement of NSE and S-100 concentrations at two time points designated T1 and T2. In the case of the 'hypo' group, T1 was approximately 36 hours after the onset of the hypoglycaemic event (Table 7.1), while in the 'control' group, T1 represented the time of taking the first blood sample. In both groups, the second blood sample (T2) was taken approximately 7 days after the first blood sample (i.e. approximately 8.5 days after the hypoglycaemic event in the case of the 'hypo' group).

7.2.3 Measurements of Serum NSE and S-100 Concentrations
Blood samples were centrifuged within four hours of venepuncture at 1500g for 10 minutes and sera were stored at -20°C. Haemolysed samples were not analysed for
NSE because this enzyme is present within erythrocytes and platelets. All biochemical analyses were performed blinded to patient status.

NSE was measured by an enzyme immunomological test (Enzyme-Test NSE, Boehringer Mannheim Immunodiagnostics, Lewes, United Kingdom). The lower limit of detection of the assay was 0.5µg/l; the intra-assay coefficient of variation (CV) was 2.2% at 4.9µg/l and 1.5% at 13.6µg/l. The interassay CV was 5.8% at 23.1µg/l.

S-100 was measured by immunoradiometric assay (IRMA Sangtec, Bromma, Sweden). The lower limit of detection was 0.05µg/l; the intra-assay CV's were 2.8% at 0.18µg/l, 0.7% at 1.5µg/l and 0.8% at 5.3µg/l. The inter-assay CV's were 3.3%, 4.4% and 1.5% respectively.

7.2.4 Statistical Analyses
All demographic data (which was not normally distributed) are presented as median (interquartile range), while the NSE and S-100 data are presented as mean ± standard deviation (SD). Baseline characteristics of subjects were compared using the Mann-Whitney U test, and the Chi-squared test was used for categorical data. Differences in NSE and S-100 concentrations between the 'hypo' and 'control' groups, at the two time points, were compared using a mixed model analysis of variance (ANOVA) with 'group' (hypo or control') as a between-subjects factor and 'time' (T1 or T2) as a repeated measure. A p-value of less than 0.05 was considered to be significant. All analyses were performed using SPSS version 7.5.1 for Windows 95.

7.2.5 Case Histories
Patient 1
A 49 year old Caucasian male with Type 1 diabetes for four years, who had a previous history of frequent hypoglycaemia and alcohol dependence, was found unconscious at home. He had been drinking large amounts of alcohol in the previous two weeks, during which he had recorded frequent biochemical hypoglycaemia on his routine home blood glucose monitoring. He had last been seen awake 17 hours
earlier and, when found unconscious, a member of his family had injected a subcutaneous dose of insulin in the mistaken belief that he was suffering from hyperglycaemia and ketoacidosis. His general practitioner estimated a blood glucose that was less than 1.0 mmol/l and gave emergency treatment in the form of intramuscular glucagon and intravenous dextrose.

On admission to hospital, the patient was comatose, with a Glasgow Coma Scale (GCS) of 4. He was haemodynamically stable but had hypotonia and bilateral extensor plantar responses. A CT brain scan showed no evidence of cerebral oedema or raised intracranial pressure. The patient was intubated and ventilated and received infusions of mannitol and phenytoin to control mild epileptiform activity. He was extubated five days later, but his conscious level remained depressed with a GCS of 5. He subsequently developed bronchopneumonia and died 11 days after admission.

At autopsy the lungs showed extensive bronchopneumonic consolidation. The fresh brain weighed 1500 grams and showed an old contusion on the inferior surface of the left temporal lobe. Brain histology showed mild sulcal widening and generalised vascular congestion consistent with agonal hypoxia. There was a mild degree of white matter pallor and rarefaction with U-fibre sparing, indicative of mild cerebral oedema, but no mid-line shift or evidence of herniation was noted. The brunt of neuronal damage was borne by the hippocampus where there was severe neuronal depletion with corresponding gliosis, most marked in the CA4 and CA1 zones (Figure 7.1), with acidophilia of remaining neurones. Neurones of the dentate gyrus showed conspicuous acidophilia. The neocortex contained scattered acidophilic neurones, particularly in layers 2 and 3 and at the depths of sulci. These changes were most severe in the temporal neocortex, where similar changes were also seen in layer 4. Basal ganglia, hypothalamus, brain stem, cerebellum and spinal cord were unaffected and, in particular, cerebellar Purkinje cells were intact. There was no evidence of recent traumatic brain damage, inflammation, haemorrhage, regional infarction or Wernicke's encephalopathy.
Blood samples were obtained for estimation of NSE and S-100 concentrations approximately 24 hours after the onset of hypoglycaemia (T1) and seven days later (T2), although the time of onset of the severe hypoglycaemia could only be estimated.

**Patient 2**

A 27 year old Caucasian male with a four year history of Type 1 diabetes was found unconscious (GCS 3) at home, having last been seen awake 13 hours previously following a row with his girlfriend. Two empty vials of porcine soluble insulin and three insulin syringes were lying beside him, suggesting that he had self-administered a large dose of insulin. Blood glucose was estimated at less than 1.0 mmol/l by paramedical staff who attended the emergency call and intra-muscular glucagon was administered. On admission, the patient was tachypnoeic with extensor posturing and a CT brain scan showed evidence of cerebral oedema. He was intubated and ventilated for 24 hours without clinical improvement. All active intervention was subsequently withdrawn and the patient died shortly afterwards.

A post mortem revealed acute bronchitis and evidence of right lower lobe bronchopneumonia. The fresh brain weighed 1500 grams and externally showed a moderate degree of global swelling. Histological examination of the brain revealed widespread swelling and breakdown of the blood-brain barrier. There was global transcortical neuronal shrinkage and acidophilia (Figure 7.2). Acute inflammation was limited to the temporal neocortex and leptomeninges. Similar neuronal changes were present in the CA1 to CA4 zones of the hippocampus, proximal subiculum, dentate gyrus, head of the caudate nucleus, putamen and, to a lesser extent, globus pallidus. Relative neuronal sparing was observed in the distal subiculum, angle of the dentate gyrus, caudate tail and claustrum, as well as selected larger neurones in the basal ganglia. Selective superficial cortical damage was seen in the cingulate gyri. Thalamic neurones were distorted due to transtentorial herniation, whilst the mid-brain and pons showed oedema, patchy infarction and Duret haemorrhage due to terminal brain stem herniation and compression. Other significant features included
basal ganglia microcalcification and relative Purkinje cell preservation in the cerebellum.

Samples were obtained for estimation of NSE and S-100 concentrations at only one time point, approximately 18 hours after the presumed onset of hypoglycaemia (T1).

**Patient 3**
A 47 year old man with a 30 year history of poorly-controlled Type 1 diabetes was in hospital, seven days following an aortic valve replacement operation. He was found unconscious before breakfast, blood glucose was measured at less than 1.0 mmol/l and he was treated with intravenous dextrose. During this episode, the patient had a grand mal seizure that was complicated by aspiration of gastric contents and a respiratory arrest. He was intubated and ventilated and initial CT scan of the brain showed no intracerebral abnormality, although a repeat scan two days after the episode showed gross cerebral oedema. Despite treatment with mannitol and dexamethasone, his conscious level remained depressed and the patient died 33 days following the episode of severe hypoglycaemia. Permission was not obtained for an autopsy.

Blood samples were obtained, for estimation of S-100 concentration only, at serial time points between 1 and 17 days after the onset of severe hypoglycaemia.
7.3 RESULTS

7.3.1 Clinical Characteristics of the Subjects and Timing of Blood Samples
The clinical characteristics of the subjects are shown in Table 7.1. The subjects in the two groups were matched for age, sex and duration of diabetes. The 'hypo' group had a significantly lower HbA1c (p=0.02). The differences in the timing of the T2 samples were not statistically significant (p>0.05).

7.3.2 NSE and S-100 Concentrations Following Severe Hypoglycaemia
The NSE and S-100 data for the 'hypo', and 'control' patients are presented in Figures 7.3 and 7.4. There were no significant main effects of 'group' (F=0.09, p=0.77), or 'time' (F=4.09, p=0.05) on mean serum NSE concentrations. The 'group' by 'time' interaction was also not significant (F=0.75, p=0.39). Similarly, the main effects of 'group' (F=1.09, p=0.31), 'time' (F=2.12, p=0.16) and the interaction of 'group' by 'time' (F=2.37, p=0.14) did not reach statistical significance for mean serum S-100 concentrations.

The NSE and S-100 concentrations of Patient 1 were comparable with the 'control' patients at both time points (Figures 7.3 and 7.4). In Patient 2, however, NSE and S-100 concentrations were markedly elevated, at approximately 8 and 130 times, respectively, the levels noted in the 'control' subjects (Figures 7.3 and 7.4). In Patient 3, there was a large rise in S-100 concentrations that peaked at 24 hours after the severe hypoglycaemia; S-100 concentrations fell thereafter, but were still above baseline levels at 17 days (Figure 7.5).
TABLE 7.1: Clinical Characteristics of Subjects and Timing of Blood Samples

<table>
<thead>
<tr>
<th></th>
<th>'Hypo'</th>
<th>'Control'</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40.0 (32.3-54.3)</td>
<td>30.0 (26.0-47.3)</td>
<td>0.18</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>10/6</td>
<td>5/5</td>
<td>0.69</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>15.0 (10.3-23.5)</td>
<td>12.5 (8.0-27.0)</td>
<td>0.20</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.2 (7.6-9.4)</td>
<td>10.1 (8.5-11.7)</td>
<td>0.02</td>
</tr>
<tr>
<td>T1: Time to 1st Blood Sample (days)</td>
<td>1.5 (1.1-1.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2: Time between 1st and 2nd Blood Samples (days)</td>
<td>7.1 (6.6-7.4)</td>
<td>7.0 (6.9-7.1)</td>
<td>0.96</td>
</tr>
</tbody>
</table>

All continuous variables are median (inter-quartile range).
FIGURE 7.1: Section of CA4 Zone of the Hippocampus from Patient 1

There is complete absence of neurones and numerous reactive gemistocytic astrocytes (arrows). (Haematoxylin and eosin x360)

FIGURE 7.2: Section of the Superficial Cortex (Layer 2) from Patient 2

There is neuronal acidophilia and shrinkage in the cingulate gyrus. (Haematoxylin and eosin x360)
Mean (± SD) NSE concentrations at T1 and T2. There were no significant differences between 'hypo' and 'control' subjects in NSE at either T1 or T2 (p>0.05). NSE concentrations did not rise appreciably in Patient 1, but were markedly elevated in Patient 2 at T1.
Mean (± SD) NSE concentrations at T1 and T2. There were no significant differences between 'hypo' and 'control' subjects in S-100 at either T1 or T2 (p>0.05). S-100 concentrations did not rise appreciably in Patient 1, but were markedly elevated in Patient 2 at T1.
Time course of serum concentrations of S-100 in Patient 3 from the day of the episode of severe hypoglycaemia (Day 1) to 17 days afterwards. The S-100 concentrations peaked 24 hours after the detection of severe hypoglycaemia, but remained elevated up to Day 17.
7.4 DISCUSSION

The possible clinical sequelae of an episode of severe hypoglycaemia vary widely, ranging from complete recovery to permanent neurological disability or, rarely, death. Even when complete neurological recovery is apparent, recurrent exposure to severe hypoglycaemia may result in cumulative cognitive decrements, possibly because of chronic, low-level neuronal damage. When significant neuronal destruction does occur during acute hypoglycaemia, it may be very difficult in the early stages to predict the likely clinical outcome. The clinical picture may be affected by intercurrent disease, pharmacologically-induced sedation, or the need for artificial ventilation. CT scanning of the brain, while providing valuable information, may show no intracerebral abnormality initially (as in Patient 3), despite significant neuronal damage. Therefore, any biological index that could assist with prediction of the likely clinical outcome after an episode of severe hypoglycaemia would be of value in planning the optimal medical management for such patients. NSE and S-100 offer such a possibility, because serum concentrations of both proteins are elevated following neuronal damage from other causes and correlate with clinical outcome after stroke. However, changes in the serum concentrations of these markers have not been reported previously in diabetic patients exposed to severe hypoglycaemia.

In the first part of the present study, serum concentrations of NSE and S-100 were measured following severe hypoglycaemia that was not associated with permanent neurological impairment. This part of the investigation was performed concurrently with Study 2, but did not include all of the subjects who participated in this study. There were practical reasons for the discrepancy in subject numbers. The decision to measure NSE and S-100 was not made until after recruitment for Study 2 had commenced and there was an additional element of delay while an extension to the ethical permission for that study was obtained to allow blood samples to be taken for NSE and S-100 concentrations. A sample size of 10 'control' subjects was considered to be adequate to establish baseline values in people with diabetes and the subjects were selected at random from those who participated in Study 2. In the
'control' subjects, the serum NSE concentrations at T1 and T2 and the S-100 concentrations at T2 were comparable with those measured in healthy non-diabetic subjects in other studies\textsuperscript{259,327}. However, the 'control' S-100 levels at T1 were somewhat lower than that previously observed, although the difference between the S-100 concentrations at T1 and T2 was not statistically different. Given that there was no reason why the S-100 concentrations should have changed with time in the 'control' subjects, the apparent difference is likely to be spurious.

Severe hypoglycaemia, without subsequent permanent neurological sequelae, was not associated with any significant alteration in serum NSE and S-100 concentrations, either 36 hours or 8.5 days after the event. It is important, however, to consider whether the null result of this aspect of the study may have been a consequence of methodological limitations with regard to sample size, the timing of the blood samples or the sensitivity of the assays:

- The present study had somewhat less than 80\% power to detect a one standard deviation difference in NSE and S-100 concentrations with a p-value set at 0.05. However, despite the lack of power, it is unlikely that a type 2 statistical error occurred, as perusal of the data presented in Figures 7.3 and 7.4 reveals (with the exception of the S-100 concentrations at T1) no evidence of a trend for the 'hypo' patients to have higher NSE or S-100 levels. The apparent difference in the S-100 concentrations at T1 was more a reflection of spuriously low S-100 concentrations in the 'control' subjects, rather than high S-100 concentrations in the 'hypo' subjects.

- Previous studies in patients with various cerebral disorders, particularly stroke, have shown consistently that serum concentrations of NSE and S-100 become elevated within 48 hours of the acute insult and remain so for several days, or even weeks, afterwards\textsuperscript{258,259,324,326,327}. In fact, such a profile of S-100 concentrations was seen with Patient 3. Therefore, in the present study, it is unlikely that a transient rise in serum NSE and S-100 occurred in the 'hypo' subjects outwith the times of the blood sampling.
Following acute stroke, elevations in serum concentrations of NSE and S-100 are related to the size of the cerebral infarction\textsuperscript{258,259}. Thus, NSE and S-100 concentrations may not rise following small strokes in which only modest degrees of acute neuronal injury have occurred, indicating a relative lack of sensitivity of these markers. Therefore, the results from the patients who made a complete neurological recovery following severe hypoglycaemia should not be interpreted as implying that no neuronal damage occurred. Neuronal injury could have taken place, but of a degree that was below the detection of the NSE and S-100 assays.

Episodes of severe hypoglycaemia which result in permanent neurological abnormality are relatively rare in clinical practice, but changes in serum concentrations of NSE and S-100 were measured before death in three fatal cases. The serum concentrations of NSE and S-100 were low in the diabetic patient who died nearly two weeks following an episode of severe hypoglycaemia (Patient 1) and whose neuronal damage was centred primarily on the hippocampus. The significant CA1 and end-folial hippocampal damage may at least partly have pre-dated the fatal illness, and would be consistent with damage from previous episodes of hypoglycaemia\textsuperscript{37,43,45}. However, the acidophilia of the remaining neurones in the hippocampus implies that acute neuronal damage also occurred at the time of the episode of severe hypoglycaemia that preceded death. The patient's seizures were mild and promptly treated and, while they may have contributed to the hippocampal damage, are unlikely to have been the principal cause.

By contrast, serum NSE and S-100 concentrations were markedly elevated in a young diabetic patient (Patient 2) who died within 48 hours of the development of profound neuroglycopenia, after a probable deliberate insulin overdose. This patient suffered very extensive brain damage. The involvement of the dentate gyrus and the relative preservation of Purkinje cells and of larger basal ganglia and deep cingulate cortex neurones are consistent with the effects of hypoglycaemia\textsuperscript{37,43,45}. However, it must be stressed that there is considerable overlap between the neuropathological
features of hypoglycaemia, hypoxia/ischaemia and seizures\textsuperscript{38,39} and it is quite possible that hypoxia and seizure activity could have developed in Patient 2 when he was lying unconscious at home. Serum concentrations of S-100 were also significantly elevated in a middle-aged patient (Patient 3) who had a prolonged episode of severe hypoglycaemia, seven days following cardiac surgery. Initial concentrations of S-100 were low and a CT scan of the brain was normal. However, within 24 hours of the event, S-100 concentrations had risen significantly and a repeat CT scan of the brain confirmed gross cerebral oedema. Serum S-100 concentrations remained elevated, albeit at lower levels, for several days afterwards. This patient had a grand mal seizure and a respiratory arrest which were treated promptly, but clearly both events may have contributed to the neurological damage and the subsequent rise in S-100 concentrations. In both the above cases, the serum concentrations of NSE and S-100 were elevated to levels equivalent to those observed after a large ischaemic cerebral infarction\textsuperscript{258,259}. Neither patient had a previous history of any neurological or neoplastic condition that could have caused elevation of these serum proteins, and no evidence of an alternative cause was found at the autopsy of Patient 2.

Secondary neurological insults (such as hypoxia and seizure activity) are common in patients who have experienced profound hypoglycaemia. Therefore, in future studies of comatose patients, it may not be possible to separate out the impact of severe hypoglycaemia \textit{per se} on serum concentrations of NSE and S-100. However, the fact that the serum concentrations of both neuronal markers rose in two of the three patients who subsequently died following an episode of severe hypoglycaemia, but did not rise in patients who made a complete neurological recovery, raises the prospect that NSE and S-100 could have a future role in predicting both the severity of neuronal damage and the clinical outcome of patients who remain comatose following prolonged severe hypoglycaemia. The potential value of using such markers is that they are easy to measure, relatively inexpensive and show a rapid response following acute neurological insult that causes neurological damage. Therefore prognostic information could be available to clinicians at an early stage of management, and possibly before any abnormality is detectable by neuroimaging.
techniques. However, the results of the present study are preliminary and require validation in a larger series of patients experiencing hypoglycaemic coma, with or without cerebral oedema, relating the increments in these serum markers to initial clinical parameters (such as initial GCS, other neurological abnormalities and the results of neuroimaging), final clinical outcome and neuropathological findings.
CHAPTER 8

STUDY 4

DETERMINATION OF THE OPTIMAL TIME FOR ADMINISTRATION OF INSULIN LISPRO: THE IMPORTANCE OF MEAL COMPOSITION
8.1 INTRODUCTION

The studies presented in Chapters 5, 6 and 7 of this thesis have examined the effects of hypoglycaemia on the peripheral and central nervous systems. In this Chapter, the ability of a new therapy for diabetes, insulin lispro, to *induce* hypoglycaemia following certain meal types was specifically examined. Insulin lispro is a genetically engineered analogue of human insulin whose chemical and glucodynamic properties were described in detail in Chapter 1.8.2. Several studies have suggested that therapy with insulin lispro is associated with a reduced frequency of hypoglycaemia, particularly nocturnal episodes. The rapid onset of action of insulin lispro means that it should be administered shortly before meals to avoid pre-prandial hypoglycaemia. However, if for any reason the normal rise in post-prandial blood glucose was reduced in magnitude, or was substantially delayed, the rapid onset of action of insulin lispro could theoretically promote an increased risk of early post-prandial hypoglycaemia.

Gastric emptying is an important determinant of the timing of the rise in post-prandial blood glucose concentrations. In turn, meal composition has a significant influence on gastric emptying. Liquids pass through the stomach more rapidly than solids, and gastric emptying is slower in the context of a high fat content meal. It is, therefore, possible that the use of pre-prandial insulin lispro therapy in conjunction with a high fat diet (such as that which predominates in many Northern European countries, including Scotland) could be associated with an increased risk of early post-prandial hypoglycaemia. One approach to minimising this risk would be to inject insulin lispro after the meal, so as to delay its peak hypoglycaemic action.

The aim of the present study was, therefore, to compare the glucodynamics associated with pre- and post-prandial administration of insulin lispro following the consumption of test meals in which the relative proportions of carbohydrate and fat were varied. In particular, evidence was sought to support the premise that administration of insulin lispro before meals with a high content of fat may be
associated with an increased risk of early post-prandial hypoglycaemia. The role of the solid and liquid constituents of test meals was also evaluated.
8.2 METHODS

8.2.1 Subjects
The study was approved by the local medical ethics advisory committee and all subjects gave written informed consent before participation. Twenty subjects with Type 1 diabetes were recruited from the diabetes out-patient clinic of the Royal Infirmary of Edinburgh. All subjects had diabetes for more than one year and had moderately good glycaemic control, with a HbA1c level of less than 9.0% (non-diabetic range 4.5-5.8%). No subjects had significant microvascular complications of diabetes (other than background retinopathy) or other medical disorders, and none had a history of dyspepsia or gastric fullness after meals. All subjects administered human insulin in a multiple injection (basal-bolus) regimen. Autonomic neural function was assessed using standard tests of cardiovascular reflexes.\(^{351}\)

The subjects were allocated to two groups which were matched for age, body mass index, duration of diabetes, glycated haemoglobin concentration (HbA1c) and total daily insulin dose (Table 8.1). Eighteen subjects had normal tests of cardiac autonomic function; one subject in Group 2 had an abnormal result on the Valsalva manoeuvre alone. In another subject from Group 2, the full battery of tests of autonomic function could not be completed for technical reasons. This individual had no symptoms suggestive of autonomic neuropathy or gastroparesis and did not have postural hypotension.

8.2.2 Test Meal Protocol
Four isocaloric test meals were used in the study (Table 8.2). Two meals (designated F) had a high fat content and a relatively low carbohydrate content. Two meals (designated C) had a high carbohydrate content and a low fat content. One meal of each type had a pronounced liquid (L) content and one meal of each type had a prominent solid (S) component. The meals were designated as CL (carbohydrate, liquid), CS (carbohydrate, solid), FL (fat, liquid) and FS (fat, solid).
All subjects underwent four separate study sessions. On two occasions a high fat meal was consumed and on two occasions a high carbohydrate meal was consumed. Subjects in Group 1 were given the test meals with a pronounced liquid component (CL and FL), while subjects in Group 2 received the meals with a prominent solid phase (CS and FS). For each meal type, lispro was administered pre-prandially (10 minutes before the start of the test meal) on one occasion and post-prandially (20 minutes after the start of the test meal) on the other. The order of meal type and the timing of the lispro injection were randomised. There were four possible sequences of meal type and timing of the lispro injection:

**Sequence 1:**
- pre-prandial lispro/high carbohydrate meal
- post-prandial lispro/high carbohydrate meal
- post-prandial lispro/high fat meal
- pre-prandial lispro/high fat meal

**Sequence 2:**
- post-prandial lispro/high carbohydrate meal
- pre-prandial lispro/high fat meal
- pre-prandial lispro/high carbohydrate meal
- post-prandial lispro/high fat meal

**Sequence 3:**
- pre-prandial lispro/high fat meal
- post-prandial lispro/high fat meal
- post-prandial lispro/high carbohydrate meal
- pre-prandial lispro/high carbohydrate meal

**Sequence 4:**
- post-prandial lispro/high fat meal
- pre-prandial lispro/high carbohydrate meal
- pre-prandial lispro/high fat meal
- post-prandial lispro/high carbohydrate meal

The time taken to consume the different test meals was very similar. The dose of lispro administered was determined individually, after consultation with each subject, and was equivalent to the amount of soluble insulin that the individual would normally have taken before a breakfast of the nature, size and caloric content as used
in the study. The dose of lispro administered to each subject remained constant across the four study sessions.

The subjects fasted overnight and omitted their normal morning dose of soluble insulin before each study. The study session was postponed if fasting blood glucose was greater than 12.0 mmol/l or if hypoglycaemia had been experienced during the preceding night. An intravenous cannula was inserted for frequent blood sampling. Each dose of insulin lispro was administered subcutaneously into the anterior abdominal wall. Venous blood samples were collected before the meal and at 15 minute intervals after the start of the meal, for a period of two hours. At each time point, the blood glucose excursion was calculated as the difference in blood glucose concentration from that at baseline.

Significant biochemical hypoglycaemia was arbitrarily defined as a venous blood glucose concentration of less than 3.0 mmol/l. The study was discontinued immediately if the blood glucose declined below 2.5 mmol/l, or if symptoms of hypoglycaemia developed with blood glucose below 4.0 mmol/l.

8.2.3 Sample Analyses
Glycated haemoglobin (as HbA1c) was measured using high speed liquid chromatography based on an ion-exchange reverse-phase partition method (Hi Auto A1c HA 8121). Whole blood venous glucose was determined using a glucose oxidase method (Yellow Springs Instrument 2300 Stat).

8.2.4 Statistical Analyses
Data are presented as mean (±SD), with exception of the glucose profile data presented in Figures 8.1, 8.2, 8.3 and 8.4, which are presented, for purposes of clarity, as mean (±SEM). Baseline characteristics of subjects were compared using the Mann-Whitney U test and the Chi-squared test was used for categorical data. Baseline blood glucose concentrations were compared using Student’s t-test for paired data. Post-prandial blood glucose excursions for each of the four individual meal types were compared using a repeated-measures analysis of variance
(ANOVA). 'Order of session' was a between-subjects factor with four levels (sequence 1 versus sequence 2, versus sequence 3, versus sequence 4). There were two within-subjects factors: factor one, 'lispro timing', had two levels (pre-prandial lispro versus post-prandial lispro) and factor two, 'time' had eight levels (corresponding to the time points of the blood samples). The principal outcome statistic was any ['lispro timing' by 'time'] interaction, which indicated whether the timing of the lispro injection had any impact on the blood glucose profile for the test meal. Main effects of 'lispro timing' and 'time' are not reported. Post-hoc analyses were performed using Student's t-test for paired data. A p-value of less than 0.05 was considered significant for the Mann-Whitney U-test, Chi-squared test and ANOVA analyses. A p-value of less than 0.01 was considered significant for the paired t-test analyses, to reduce the risk of a type 1 statistical error. All analyses were performed using SPSS version 6.1.3 for Windows. Reported p-values were rounded to two decimal places; thus p=0.00 signifies that the p-value was <0.005.
TABLE 8.1: Clinical Characteristics of Subjects

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.3 (9.2)</td>
<td>27.6 (9.9)</td>
<td>0.60</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>7/3</td>
<td>4/6</td>
<td>0.37</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>11.5 (8.2)</td>
<td>13.9 (8.4)</td>
<td>0.68</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.0 (10.3)</td>
<td>75.7 (8.4)</td>
<td>0.79</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.5 (3.2)</td>
<td>26.4 (3.4)</td>
<td>0.54</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.3 (1.1)</td>
<td>7.2 (1.0)</td>
<td>0.71</td>
</tr>
<tr>
<td>Total daily insulin dose</td>
<td>0.71 (0.22)</td>
<td>0.78 (0.26)</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Values shown are mean (SD)
Non-diabetic range for HbA1c was 4.5-5.8%
<table>
<thead>
<tr>
<th>Food</th>
<th>Weight (g)</th>
<th>Energy (kcal)</th>
<th>CHO (g)</th>
<th>Fat (g)</th>
<th>Protein (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High Carbohydrate [Liquid] Breakfast (CL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wholemeal Bread</td>
<td>40</td>
<td>86</td>
<td>16.7</td>
<td>1.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Low Fat Spread</td>
<td>14</td>
<td>51</td>
<td>0.0</td>
<td>5.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Weetabix</td>
<td>35</td>
<td>119</td>
<td>24.6</td>
<td>1.2</td>
<td>4.0</td>
</tr>
<tr>
<td>Skimmed Milk</td>
<td>568</td>
<td>187</td>
<td>28.4</td>
<td>0.5</td>
<td>19.3</td>
</tr>
<tr>
<td>Skinned Banana</td>
<td>100</td>
<td>79</td>
<td>19.2</td>
<td>0.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Total (g)</td>
<td>88.9</td>
<td>8.5</td>
<td>27.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>522</td>
<td>356</td>
<td>79</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>% Energy</td>
<td>68</td>
<td>15</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>High Carbohydrate [Solid] Breakfast (CS)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wholemeal Bread</td>
<td>80</td>
<td>173</td>
<td>33.4</td>
<td>2.2</td>
<td>7.0</td>
</tr>
<tr>
<td>Low Fat Spread</td>
<td>14</td>
<td>51</td>
<td>0.0</td>
<td>5.7</td>
<td>0.0</td>
</tr>
<tr>
<td>Fruit Jam</td>
<td>15</td>
<td>167</td>
<td>10.3</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Weetabix</td>
<td>35</td>
<td>119</td>
<td>24.6</td>
<td>1.2</td>
<td>4.0</td>
</tr>
<tr>
<td>Skimmed Milk</td>
<td>180</td>
<td>59</td>
<td>9.0</td>
<td>0.2</td>
<td>6.1</td>
</tr>
<tr>
<td>Skinned Banana</td>
<td>100</td>
<td>81</td>
<td>19.9</td>
<td>0.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Total (g)</td>
<td>97.2</td>
<td>9.6</td>
<td>18.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>522</td>
<td>389</td>
<td>86</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>% Energy</td>
<td>74</td>
<td>17</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>High Fat [Liquid] Breakfast (FL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Bread</td>
<td>40</td>
<td>87</td>
<td>18.8</td>
<td>0.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Butter</td>
<td>14</td>
<td>104</td>
<td>0.0</td>
<td>11.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Whole Milk</td>
<td>568</td>
<td>369</td>
<td>26.7</td>
<td>21.6</td>
<td>18.7</td>
</tr>
<tr>
<td>Total (g)</td>
<td>45.5</td>
<td>33.6</td>
<td>21.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>560</td>
<td>182</td>
<td>302</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>% Energy</td>
<td>33</td>
<td>54</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>High Fat [Solid] Breakfast (FS)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Bread</td>
<td>40</td>
<td>87</td>
<td>18.8</td>
<td>0.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Butter</td>
<td>14</td>
<td>104</td>
<td>0.0</td>
<td>11.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Cornflakes</td>
<td>25</td>
<td>92</td>
<td>21.3</td>
<td>0.4</td>
<td>2.2</td>
</tr>
<tr>
<td>Cheese</td>
<td>45</td>
<td>183</td>
<td>0.0</td>
<td>15.1</td>
<td>11.7</td>
</tr>
<tr>
<td>Whole Milk</td>
<td>180</td>
<td>117</td>
<td>8.5</td>
<td>6.8</td>
<td>5.9</td>
</tr>
<tr>
<td>Total (g)</td>
<td>48.6</td>
<td>34.3</td>
<td>22.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>583</td>
<td>194</td>
<td>309</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>% Energy</td>
<td>33</td>
<td>53</td>
<td>16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
8.3 RESULTS

The baseline fasting blood glucose concentrations before each test meal did not differ whether lispro was administered pre- or post-prandially (Table 8.3). There were no significant between-subjects effects i.e. the sequence of administration of the meals and the pre- and post-prandial injections of insulin lispro had no effect on the resulting glycaemic excursions (all p>0.05). The mean dose of insulin lispro administered to subjects in Group 1 (12.7 ± 4.0 units) and Group 2 (13.4 ± 4.5 units) was not statistically different (p=0.72).

8.3.1 High Carbohydrate Liquid Meal (CL)
When insulin lispro was administered before the CL breakfast, the post-prandial glycaemic excursion was modest, with a mean peak rise in blood glucose of 2.0 mmol/l (Figure 8.1). By contrast, when lispro was administered after the test meal, a significantly larger blood glucose excursion was observed (p=0.00 for interaction of 'lispro timing' and 'time'), with the maximum mean rise in blood glucose of 5.6 mmol/l occurring at 45 minutes. The blood glucose excursions at the 30, 45, 60 and 75 minute time points were all significantly higher with post-meal administration of lispro (p<0.01). No subjects developed significant biochemical hypoglycaemia.

8.3.2 High Carbohydrate Solid Meal (CS)
Injection of insulin lispro before the CS meal produced a much smaller blood glucose excursion, compared with post-prandial administration of lispro (p=0.00 for interaction of 'lispro timing' and 'time'); Figure 8.2). Following pre-prandial administration, a small negative excursion of between 0.5 to 1.0 mmol/l was observed during the initial 30 minutes after the start of the breakfast; thereafter the blood glucose excursion rose, peaking at 1.0 mmol/l at 90 minutes. Significant asymptomatic, biochemical hypoglycaemia occurred in one subject whose fasting glucose concentration was 3.2 mmol/l. Blood glucose concentrations fell to a nadir of 2.8 mmol/l at 15 minutes, but rose to 4.7 mmol/l at 30 minutes and thereafter remained above 4.0 mmol/l. No other subject developed significant biochemical hypoglycaemia.
In the post-prandial lispro studies, the mean blood glucose excursion peaked at 3.5 mmol/l at 45 minutes, and was significantly higher than in the pre-prandial lispro studies at 15, 45 and 60 minutes after the test meal (p<0.01). No subjects developed significant biochemical hypoglycaemia.

8.3.3 High Fat Liquid Meal (FL)
Administration of insulin lispro before the FL breakfast was associated with a post-prandial glycaemic excursion in which the mean blood glucose varied little from baseline values (Figure 8.3). One subject developed persistent, biochemical hypoglycaemia which provoked symptoms and necessitated termination of the session after 60 minutes. This subject started the study session with a pre-meal blood glucose of 5.0 mmol/l (the lowest value recorded for any subject ingesting the FL meal) which declined progressively over 60 minutes to 2.7 mmol/l, at which point the study was terminated. Because a repeated-measures ANOVA can only be performed on complete data sets, the incomplete time course data from this subject was not included in the ANOVA and subsequent t-test analysis. The data presented in Figure 8.3 are derived from the remaining nine subjects, in whom blood glucose did not fall below 3.0 mmol/l at any time.

Administration of lispro after the FL breakfast produced a significantly larger blood glucose excursion (p=0.00 for interaction of 'lispro timing' and 'time'). No subjects developed significant hypoglycaemia. The blood glucose excursion peaked at 5.2 mmol/l at 45 minutes, and was significantly greater at the 30, 45, 60 and 75 minute time points, than that observed following pre-prandial administration of insulin lispro (p<0.01).

8.3.4 High Fat Solid Meal (FS)
Administration of insulin lispro before the FS meal provoked a continuous decline in blood glucose, so that by 120 minutes mean blood glucose was 3.4 mmol/l below baseline concentrations (Figure 8.4). One subject developed persistent, symptomatic hypoglycaemia that necessitated termination of the study session after 105 minutes. The subject’s pre-meal blood glucose was 6.4 mmol/l and this fell progressively to

187
2.0 mmol/l when the session was terminated. Data from this subject was not included in the ANOVA analysis or in Figure 8.4. Two other subjects developed significant, asymptomatic biochemical hypoglycaemia. In one individual, pre-meal blood glucose was 11.7 mmol/l and at 120 minutes was 2.8 mmol/l; in the other subject, pre-meal blood glucose was 7.4 mmol/l and at 120 minutes was 2.6 mmol/l. In all other subjects, the blood glucose remained above 3.0 mmol/l throughout the study.

Post-prandial administration of lispro resulted in significantly greater mean blood glucose concentrations than pre-prandial administration (p=0.00 for interaction of 'lispro timing' and 'time'), with a peak excursion of 3.6 mmol/l at 45 minutes. No subject developed significant hypoglycaemia. When lispro was administered post-prandially, the blood glucose excursions were significantly greater at all time points from 15 to 105 minutes inclusive (p<0.01).

The difference in the incidence of significant biochemical hypoglycaemia between the groups (3 versus 0 instances) was not statistically significant (p=0.06).
TABLE 8.3: Baseline Blood Glucose Concentrations

<table>
<thead>
<tr>
<th>Test Meal</th>
<th>Blood Glucose (mmol/l)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Prandial Lispro</td>
<td>Post-Prandial Lispro</td>
</tr>
<tr>
<td>CL</td>
<td>9.1 (3.0)</td>
<td>8.0 (2.7)</td>
</tr>
<tr>
<td>CS</td>
<td>8.1 (2.6)</td>
<td>8.1 (3.1)</td>
</tr>
<tr>
<td>FL</td>
<td>8.8 (2.8)</td>
<td>9.0 (2.7)</td>
</tr>
<tr>
<td>FS</td>
<td>8.6 (2.4)</td>
<td>7.7 (3.2)</td>
</tr>
</tbody>
</table>

Values shown are mean (SD)

CL: liquid high carbohydrate meal
CS: solid high carbohydrate meal
FL: liquid high fat meal
FS: solid high fat meal
FIGURE 8.1: Glucose Excursions Following the High Carbohydrate Liquid (CL) Meal

Mean (±SEM) post-prandial glycaemic excursions for two treatment arms, after ingestion meal CL. Time ‘0’ represents the start of the test breakfast. * p<0.01 (pre-prandial vs post-prandial insulin lispro). n=10 for both treatment arms. LP represents insulin lispro.
FIGURE 8.2: Glucose Excursions Following the High Carbohydrate Solid (CS) Meal

Mean (±SEM) post-prandial glycaemic excursions for two treatment arms, after ingestion meal CS. Time ‘0’ represents the start of the test breakfast. * p<0.01 (pre-prandial vs post-prandial insulin lispro). n=10 for both treatment arms. LP represents insulin lispro.
FIGURE 8.3: Glucose Excursions Following the High Fat Liquid (FL) Meal

Mean (±SEM) post-prandial glycaemic excursions for two treatment arms, after ingestion meal FL. Time ‘0’ represents the start of the test breakfast. * p<0.01 (pre-prandial vs post-prandial insulin lispro). n=9 for both treatment arms. LP represents insulin lispro.
Mean (±SEM) post-prandial glycaemic excursions for two treatment arms, after ingestion meal FS. Time ‘0’ represents the start of the test breakfast. * p<0.01 (pre-prandial vs post-prandial insulin lispro). n=9 for both treatment arms. LP represents insulin lispro.
8.4 DISCUSSION

8.4.1 Meal Composition, Insulin Lispro and Post-Prandial Hypoglycaemia

In clinical practice, the introduction of insulin lispro has offered potential benefits for patients with Type 1 diabetes in terms of convenience of time of administration before meals and a reduced frequency of, particularly nocturnal, hypoglycaemia\(^{124,125,131,132,136}\). However, if for any reason the rise in blood glucose after a meal was delayed or of low magnitude, the rapid onset of action of insulin lispro could potentially increase the risk of early post-prandial hypoglycaemia.

The potential importance of meal composition in determining the post-prandial glycaemic response was first raised in a study of 12 patients with Type 1 diabetes by Burge et al\(^{352}\). In this study, subjects attended for three sessions and consumed, on each occasion, one of three isocaloric test meals - the meals were designated as 'standard' (50% carbohydrate, 20% protein and 30% fat), 'high carbohydrate low fat' (80% carbohydrate, 10% protein, 10% fat) and 'low carbohydrate high fat' (20% carbohydrate, 20% protein and 60% fat). The subjects were divided into two groups of six - one group received injections of insulin lispro immediately before the meals and the other received injections of soluble insulin 30 minutes before the meals; both groups received insulin doses of 0.15 units/kg. Pre-prandial euglycaemia was established, if necessary, by a continuous infusion of soluble insulin for 90 minutes to attain a target glucose concentration of 5.0-7.8 mmol/l. Hypoglycaemia was defined as a plasma glucose of <2.8 mmol/l associated with typical symptoms, or a plasma glucose of <2.2 mmol/l, irrespective of whether symptoms were induced or not. Subjects injected with insulin lispro demonstrated significantly smaller glucose excursions over four hours for all three meal types. Overall, insulin lispro therapy was associated with an increased risk of post-prandial hypoglycaemia (p = 0.04). Hypoglycaemia was particularly common after the low carbohydrate, high fat meal in which five subjects receiving insulin lispro were affected compared with two subjects who received soluble insulin (p=0.09)\(^{352}\). The study did, however, have many drawbacks. The use of a between-subjects design considerably reduced the
power of the study and left open the possibility that individual differences between the subjects may have been responsible for the study results. For example, subjects receiving insulin lispro had a mean BMI of 22.4 kg/m², compared with 24.6 kg/m² for subjects receiving soluble insulin; this was not statistically different given the small sample size, but in view of the fact that subjects in both groups received the same dose of insulin, the potentially increased insulin sensitivity of the lispro subjects may have increased their risk of hypoglycaemia. The risk of hypoglycaemia may also have been over-estimated by the artificial lowering of plasma glucose concentrations before the study. Moreover, the glucose profile data was of doubtful validity, since complete data was obtained only on a proportion of the subjects, because of the high dropout rate caused by the development of post-prandial hypoglycaemia.

8.4.2 Methodological Considerations Regarding the Present Study
In the present study, the effects of pre- and post-prandial administration of insulin lispro were compared in relation to test meals of differing composition. The principal aim of the study was to assess the risk of early post-prandial hypoglycaemia, for which a two hour time course was thought to be adequate. A strength of the present study was that the effects of pre- and post-prandial lispro were compared within individual subjects, providing increased statistical power.

A major premise of the study was that meals with a high fat content would exhibit a delayed glycaemic response by retarding gastric emptying. However, gastric emptying is not influenced solely by meal composition. 'Gastroparesis diabeticorum' is a rare, but debilitating complication of diabetes associated with autonomic neuropathy. More subtle abnormalities of gastric emptying have been described in many individuals with diabetes of relatively long duration. Gastric emptying is also influenced by ambient blood glucose concentration: hyperglycaemia delays gastric emptying, while hypoglycaemia promotes gastric emptying. In the present study, the mean basal blood glucose concentrations did not differ between the pre- and post-prandial lispro arms. However, the subjects had a mean duration of diabetes of more than 10 years. None had any symptoms suggestive of
gastric stasis and the majority had normal cardiac autonomic neural function. Therefore, although the two subject groups were well matched demographically and in particular did not differ with respect to glycaemic control, total daily insulin dose and dose of insulin lispro administered, complete normality of gastric emptying cannot be asserted in all subjects, in the absence of formal studies of gastric emptying. Ideally, all test meals would have been administered to the entire cohort of subjects. However, this would have involved each subject attending for nine individual sessions which was considered to be an unrealistic demand on the subjects' time. Unrecognised differences between the groups do not affect the validity of the within-subjects comparisons (i.e. the effects of pre-prandial versus post-prandial insulin lispro for each meal type), but for this reason between-subjects comparisons (i.e. direct comparisons of the excursions for the different meal types) were not made.

The precise timing of the insulin lispro injections was determined in a pragmatic manner. It was hypothesised when designing the study that a difference of approximately 30 minutes between the insulin lispro injections would yield statistically significant results. It was felt that administration of the post-prandial injection a full 30 minutes after the test meal might result in unacceptably high post-prandial blood glucose concentrations, and as a compromise the 20 minute post-prandial time point was chosen. This required the pre-prandial injection to be given 10 minutes before food (to maintain the 30 minute gap). The insulin lispro data sheet recommends that lispro should be administered between zero and 15 minutes before food, so the 10 minute time period was well within the recommended schedule.

In contrast to the study by Burge et al\textsuperscript{152}, baseline blood glucose concentrations were not reduced artificially by insulin infusion prior to the administration of the test meals. There were no differences in baseline blood glucose concentrations for the pre- and post-prandial lispro study days for each test meal. The blood glucose concentrations were, inevitably, higher than those studied by Burge et al\textsuperscript{152}, but were perhaps more representative of clinical practice. Moreover, the higher baseline blood glucose concentrations resulted in fewer subjects failing to complete the protocols
because of intercurrent hypoglycaemia, thereby providing more accurate profile data.

It would have been of additional interest to have compared the impact of pre-prandial injections of soluble insulin on the blood glucose profiles following the test meals. Subjects were invited to participate in such an extension to the study, but an insufficient number was willing to continue to allow this proposed extension to be statistically meaningful.

The definition of biochemical hypoglycaemia employed in the present study was entirely arbitrary, as it is in any study of this type. The definition chosen was felt to be clinically relevant and yet did not pose a danger to the subjects participating.

8.4.3 Interpretation of Results and Comparisons with Other Studies

Irrespective of the composition of the test meal, the post-prandial excursions of blood glucose were lower when insulin lispro was administered before rather than after food. This resulted in better post-meal glycaemic control with high carbohydrate (low fat) meals. Administration of lispro before meals with a high fat (and low carbohydrate) content produced a significant fall in post-prandial blood glucose when the meal was predominantly in solid phase. When the meal was in a more liquid form, the blood glucose excursion was flat, presumably because the liquid traversed the stomach more rapidly. Complete data sets were not obtained for either form of the high fat meal, as one subject developed a level of hypoglycaemia that necessitated premature termination of the session during each pre-meal lispro study. If complete data sets had been available for analysis, the mean decrement in blood glucose might have been greater when lispro was administered before either type of high fat meal. Therefore, when lispro is injected before meals with a high fat content, particularly those with a prominent solid phase, the post-prandial fall in blood glucose which occurs may induce early post-prandial hypoglycaemia, especially if the pre-meal blood glucose is relatively 'low' (for example, less than 8 mmol/l). Indeed, there was a non-significant trend for pre-prandial lispro to be associated with an increased risk of significant biochemical
hypoglycaemia in the context of the meal with a high fat content that was predominantly in solid phase. However, if the pre-meal blood glucose is high, the post-prandial fall in blood glucose may be acceptable and indeed desirable. In the present study, when insulin lispro was administered after a high fat breakfast, post-prandial blood glucose rose in most subjects and the risk of post-prandial hypoglycemia was minimal. The increments in post-prandial blood glucose were, however, relatively high and might have been restricted by administering the insulin lispro immediately after the test meal, instead of after an interval of 20 minutes.

The efficacy of the post-prandial administration of insulin analogues has been examined in two recent studies. Injection of insulin lispro 15 minutes after the start of a test meal consisting of 350g of beef stroganoff (584.5kcal; 35g protein, 45.5g carbohydrate and 28g fat) produced a glucodynamic pattern over two hours which was comparable to soluble human insulin given, either 20 minutes before, or immediately before, the meal. The lowest post-prandial glucose excursion occurred when insulin lispro was injected 20 minutes before the meal, but this provoked early post-prandial hypoglycaemia (defined as a blood glucose of <2.78 mmol/l) in some subjects, consistent with the findings of the present study. Post-prandial injection of the newer insulin analogue, insulin aspart, has also been demonstrated to result in comparable glucodynamic profiles with human soluble insulin administered 15 minutes pre-prandially, and superior results to soluble insulin injected immediately before meals. Insulin aspart therapy (injected either immediately pre-prandially or 15 minutes after food) was also associated with a non-significant increased trend towards increasing early post-prandial hypoglycaemia (defined as a plasma glucose <2.8 mmol/l).

8.4.4 Other Factors which may Influence Post-Prandial Glucose Excursions

The present study has indicated that the optimal timing for administration of insulin lispro is determined, in part, by the composition (carbohydrate and fat content) of the meal being consumed, and also by its relative liquid and solid content. However, the high carbohydrate test breakfast contained not only larger monosaccharide,
disaccharide and starch components, relative to the high fat breakfasts, but also a greater proportion of insoluble (cereal) fibre. Variation in the insoluble fibre content of test meals generally has little effect on the overall glycaemic response. By contrast, meals with a large proportion of soluble fibre, as found in barley and legumes, generally exhibit blunted glycaemic responses. The test meals used in the present study contained only a small amount of soluble fibre, and an important area of further study would be to examine the glycaemic responses of meals with a high soluble fibre content in relation to the timing of administration of insulin lispro. All test meals used in the present study had a comparable protein content. The effects of variations in protein content on the glycaemic response to a test meal are more varied, and may also merit further investigation in relation to the use of insulin lispro.

The impact of insulin lispro on prevailing blood glucose could be affected by factors other than the timing of the insulin injection alone. Insulin is absorbed into the circulation from subcutaneous tissue at different rates depending on the site of injection. In the present study, lispro was injected subcutaneously into the anterior abdominal wall, a site which is associated with a rapid rate of absorption of insulin. Administration of insulin lispro into an alternative anatomical site, such as the arm or the thigh, is associated with a slower onset of glucose-lowering activity. Therefore, when a person with diabetes wishes to consume a high fat meal, an alternative strategy may be to inject insulin lispro into the thigh instead of considering a post-prandial time of administration. However, the absorption of insulin from any injection site is dependent on many variables (such as depth of injection, skin temperature and subcutaneous tissue blood flow) and so the use of an injection site from which the absorption of insulin is usually slow may be a less reliable method of avoiding hypoglycaemia than adjusting the time of injection in relation to meals. A further means of avoiding early post-prandial hypoglycaemia would be to reduce the dose of insulin lispro, but this may provoke late post-prandial hyperglycaemia.
8.4.5 Conclusions

When a person with Type 1 diabetes consumes a meal with a high carbohydrate content, the pre-prandial administration of insulin lispro provides better glycaemic control with little risk of early post-prandial hypoglycaemia. By contrast, when consuming meals that have a high content of fat, which is mainly in a solid phase, the risks of early post-prandial hypoglycaemia are markedly increased and so post-prandial administration of lispro may be more appropriate. Patients who are commencing therapy with insulin lispro should therefore be educated that the timing of insulin injections, in relation to food, may need to be varied according to prevailing blood glucose concentrations and the content of the meal. Patients should also be advised of the potential risk of early post-prandial hypoglycaemia.
CHAPTER 9

CONCLUSIONS AND FUTURE RESEARCH
The results of the DCCT\textsuperscript{6}, and to a more limited extent the United Kingdom Prospective Diabetes Study (UKPDS)\textsuperscript{375,376}, have demonstrated the importance of achieving strict glycaemic control in the prevention of microvascular complications of diabetes. However, strict glycaemic control comes at the price of an increased incidence of hypoglycaemia\textsuperscript{6}, and indeed hypoglycaemia represents the major barrier in preventing the majority of patients with insulin-treated diabetes achieving and maintaining blood glucose concentrations that are within the non-diabetic range\textsuperscript{8}. Therefore, research into the epidemiology, pathogenesis, clinical features and prevention of hypoglycaemia is of considerable importance and relevance. The data presented in this thesis have extended our understanding of the effects of hypoglycaemia on the peripheral and central nervous systems and have identified strategies that may reduce the occurrence of hypoglycaemia in patients treated with insulin lispro.

9.1 ACUTE HYPOGLYCAEMIA, THE PERIPHERAL NERVOUS SYSTEM AND INFORMATION PROCESSING

The primary aim of Study 1 was to determine the effects of hypoglycaemia on the function of the peripheral nervous system. Acute hypoglycaemia had no impact on the motor nerve conduction velocities, or on the amplitudes of the evoked action potentials, of either the median or common peroneal nerves. This study has provided clear evidence that the peripheral nervous system does not have the same reliance on glucose as a metabolic fuel as the central nervous system. This may be a reflection of the enhanced ability of peripheral neurones to use non-glucose fuels or to extract glucose more efficiently from the peripheral circulation. Future studies should, however, examine the effects of acute hypoglycaemia on peripheral neural function in people with diabetes, with and without peripheral neuropathy, as it is conceivable that peripheral nerves that are already dysfunctional may exhibit a greater degree of sensitivity to glucopenia.
It has been a widely held belief that insulin does not have any role in regulating the glucose metabolism or function of neural tissues. However, the discovery of insulin-sensitive glucose transport proteins within the central nervous system and of insulin receptors within the brain has forced a re-evaluation of this hypothesis. An unexpected finding of Study 1 was the potential for common peroneal nerve conduction velocities to decline throughout the course of both the euglycaemic and hypoglycaemic hyperinsulinaemic clamps. This raises the prospect that insulin, in supra-physiological concentrations, may be exerting a direct effect on peripheral neural function. Further studies, employing a euglycaemic/normoinsulinaemic control arm, are clearly required to investigate this in more detail. If the effect of hyperinsulinaemia on peripheral nerve function was confirmed, it would be important, once more, to study patients with diabetes, with and without peripheral neuropathy and to examine sensory, as well as motor, peripheral neural function.

As a secondary issue, Study 1 has also provided some fascinating insights into the field of contemporary intelligence research. Indices of speed of information processing correlate strongly with general intelligence. Foremost among these indices is the choice reaction time task. There is a linear relationship between the decision time component of reaction time and the logarithm of the number of lights from which the choice is made (the so-called Hick Paradigm). It has been suggested that the slope of this regression line correlates with intelligence, with less intelligent people having steeper slopes. However, the failure of the slope of the Hick Paradigm line to be altered by acute hypoglycaemia in Study 1 provides compelling evidence that this putative relationship with intelligence is not valid. Moreover, attempts by psychologists to determine a physiological basis for general intelligence by proposing a link between peripheral nerve conduction velocities and intelligence have been partially refuted by the inability of hypoglycaemia to impair peripheral neural function, despite its obvious deleterious effects on speed of information processing. The results of Study 1 do not discount the hypothesis that speed of central nervous system function may form part of the physiological basis of intelligence, merely that peripheral neural function cannot be used as a surrogate marker. This is not, however, entirely surprising given the differences in structure.
between peripheral and central neurones. Future investigators in this field may, therefore, wish to examine the link between auditory, visual and/or somatosensory evoked potentials (more direct measures of speed of central nervous system processing) and intelligence.

9.2 RECOVERY FROM SEVERE HYPOGLYCAEMIA

Study 2 has yielded important information in determining the time course of cognitive and mood alterations following severe hypoglycaemia in patients with insulin-treated diabetes. The absence of any significant 'hangover' effect of hypoglycaemia on cognitive function at the time of the first testing session, approximately 36 hours after the episode, suggests that patients can be reassured that it is safe to return to work or to drive after this time interval. However, further studies should examine the potential 'hangover' effect of severe hypoglycaemia on more relevant everyday tasks such as performance in driving simulators. Future studies that attempt to determine, more precisely, how quickly cognitive function recovers following severe hypoglycaemia will require that subjects are tested within 1.5 days following the severe hypoglycaemia. Given that most episodes of severe hypoglycaemia occur in the community, this will pose considerable ethical and practical difficulties. However, many cognitive tasks are now available in hand-held computer format. Thus, one potential study design would be to provide, prospectively, hand-held computers to subjects at high risk of severe hypoglycaemia, such as people with impaired hypoglycaemia awareness, and to ask them to perform the cognitive tasks repeatedly at fixed time intervals following recovery from an episodes of severe hypoglycaemia. Control subjects would clearly be required to adjust for practice effects, and the potential confounding influence of sleep deprivation would also have to be considered.

The impression that mood state took slightly longer to recover following severe hypoglycaemia was predictable and highlights the psychological impact that severe hypoglycaemia has on patients with diabetes. Study 2 also provided further evidence
to support the premise that recurrent episodes of severe hypoglycaemia are associated with cumulative cognitive decrements\textsuperscript{226}. This area of research has engendered considerable controversy\textsuperscript{217} and prolonged follow-up and evaluation of large cohorts of diabetic subjects, such as those who participated in the DCCT, are clearly essential.

9.3 DETERMINING PROGNOSIS AFTER SEVERE HYPOGLYCAEMIA

Clinicians often have difficulty in determining the clinical outlook of patients admitted in a coma following an episode of hypoglycaemia. The results of neuroimaging may be normal within the first few days following the episode, and the clinical picture may be altered by the need for sedation and artificial ventilation, and the presence of concurrent physiological abnormalities. The availability of a simple blood test that could aid clinicians in evaluating the prognosis of such patients would have considerable clinical value. Serum concentrations of NSE and S-100 have demonstrated such ability in the context of acute stroke\textsuperscript{258,259}. The preliminary results of Study 3 suggest that NSE and S-100 may have the potential to fill such a role following severe hypoglycaemia. Concentrations of the neural markers were elevated in two of three patients who died following severe hypoglycaemia, but not in patients who made a complete neurological recovery. Further detailed study is clearly required in large numbers of patients admitted in a coma with severe hypoglycaemia, correlating NSE and S-100 concentrations with clinical variables (such as the presence of neurological abnormalities and the results of neuroimaging) and final outcome. It must be stressed that elevated concentrations of NSE and S-100 following severe hypoglycaemia may not be caused solely by hypoglycaemic brain damage, but other factors may also be responsible, such as seizure activity and hypoxia, which commonly occur in this situation.
9.4 INSULIN LISPRO AND POST-PRANDIAL HYPOGLYCAEMIA

Insulin lispro has been advocated as a potential therapy for patients at increased risk of severe hypoglycaemia, because of trial evidence that suggests its use is associated with a decreased incidence of nocturnal hypoglycaemia$^{132}$ and, possibly, severe hypoglycaemia$^{138}$. However, the rapid onset of action of insulin lispro creates the theoretical risk that its administration may actually increase the risk of early post-prandial hypoglycaemia. Study 4 has confirmed this theoretical risk by demonstrating marked negative glucose excursions following the ingestion of meals with a high fat content. The fall in glucose concentrations was prevented by post-prandial administration of the insulin lispro. The results of this study imply that patients who are being commenced on insulin lispro therapy should be educated about the possibility of early post-prandial hypoglycaemia, particularly if pre-prandial blood glucose concentrations are low or if high fat meals are to be consumed. In such circumstances, post-prandial injection of the insulin lispro may be safer. Further studies are required with insulin lispro to determine the its impact on glucose concentrations following the ingestion of meals with high soluble fibre or protein contents. In addition, large-scale studies are required in patients who are at particular risk of hypoglycaemia, such as those with impaired hypoglycaemia awareness, to determine whether insulin lispro can be advocated as a therapy in such patients.

9.5 CONCLUSIONS

Much still remains to be done in the field of hypoglycaemia research to improve our understanding of its pathogenesis and, ultimately, to prevent its occurrence. The advent of sophisticated neuroimaging techniques such as functional MRI, positron emission tomography (PET) and single photon emission tomography (SPET), as well as the continued advances in molecular genetic techniques, are likely to herald significant progress in our understanding of the pathogenesis of hypoglycaemia and associated disorders such as impaired awareness of hypoglycaemia. There is also
considerable research activity into the use of neuroprotective agents, such as \( N \)-methyl-\( \beta \)-aspartate receptor antagonists, which are currently of particular interest in the context of acute stroke\(^{377} \), but which could ultimately be utilised to protect patients from the neurotoxic effects of severe hypoglycaemia. The advent of non-invasive blood glucose monitoring may one day allow patients with diabetes to continually monitor blood glucose concentrations and may provide early warning about the onset of hypoglycaemia\(^{378} \). The long-term survival of pancreatic and islet cell grafts in patients with Type 1 diabetes continues to improve\(^{379} \), while the introduction of new short- and long-acting insulin analogues may provide insulin therapy that mimics more closely the normal physiological pattern of insulin secretion\(^{380} \). The Holy Grail of specific treatments that prevent diabetes or return physiological insulin secretion remains elusive, but continued research effort will surely see the development, in the foreseeable future, of therapies that abolish the risk of hypoglycaemia in insulin-treated diabetes.
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APPENDIX 1

STUDY 2: CORRELATIONS BETWEEN THE COGNITIVE TASKS AT THE INDIVIDUAL TESTING POINTS
## CORRELATIONS BETWEEN COGNITIVE TESTS AT T1

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<th>PASAT4</th>
<th>FDS</th>
<th>FM</th>
<th>LMIR</th>
<th>LMDR</th>
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** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
## Correlations Between Cognitive Tests at T2

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<tr>
<td><strong>FM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMR</td>
<td>-0.346*</td>
<td>0.001</td>
</tr>
<tr>
<td>LMDR</td>
<td>-0.351**</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>VF</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IT</td>
<td>0.369**</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>VCD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RTDEC</td>
<td>-0.341*</td>
<td>0.001</td>
</tr>
<tr>
<td>RTMOV</td>
<td>-0.217</td>
<td>0.038</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).
## Correlations Between Cognitive Tests at T3

| Test | DS     | SD     | TMB    | STROOP  | PASAT2 | PASAT4 | FDS    | BDS    | FM     | LMR    | LMDR   | VRD    | IT     | VCD    | RTDEC  | RTMOV  |
|------|--------|--------|--------|---------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Sig. (2-tailed) | **.586** | -.612* | **-.532** | .217   | .549   | .145   | .565   | .472*  | .137   | .175   | .250   | -.318*  | -.402*  | -.379*  | -.576*  |
| Sig. (2-tailed) | .000   | .000   | .001   | .179   | .765   | .368   | .624   | .002   | .223   | .279   | .070   | .046   | .010   | .018   | .000   |
| Sig. (2-tailed) | **.586** | -.612* | **-.532** | .217   | .549   | .145   | .565   | .472*  | .137   | .175   | .250   | -.318*  | -.402*  | -.379*  | -.576*  |
| Sig. (2-tailed) | .000   | .000   | .001   | .179   | .765   | .368   | .624   | .002   | .223   | .279   | .070   | .046   | .010   | .018   | .000   |
| Sig. (2-tailed) | **.586** | -.612* | **-.532** | .217   | .549   | .145   | .565   | .472*  | .137   | .175   | .250   | -.318*  | -.402*  | -.379*  | -.576*  |
| Sig. (2-tailed) | .000   | .000   | .001   | .179   | .765   | .368   | .624   | .002   | .223   | .279   | .070   | .046   | .010   | .018   | .000   |
| Sig. (2-tailed) | **.586** | -.612* | **-.532** | .217   | .549   | .145   | .565   | .472*  | .137   | .175   | .250   | -.318*  | -.402*  | -.379*  | -.576*  |
| Sig. (2-tailed) | .000   | .000   | .001   | .179   | .765   | .368   | .624   | .002   | .223   | .279   | .070   | .046   | .010   | .018   | .000   |
| Sig. (2-tailed) | **.586** | -.612* | **-.532** | .217   | .549   | .145   | .565   | .472*  | .137   | .175   | .250   | -.318*  | -.402*  | -.379*  | -.576*  |
| Sig. (2-tailed) | .000   | .000   | .001   | .179   | .765   | .368   | .624   | .002   | .223   | .279   | .070   | .046   | .010   | .018   | .000   |

**Correlation is significant at the 0.01 level (2-tailed).**

*Correlation is significant at the 0.05 level (2-tailed).**
APPENDIX 2

PUBLICATIONS


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Cognitive Assessment in Diabetes: the Need for Consensus

Neuropsychological Assessment in Diabetes

The investigation of cognitive function in relation to diabetes has a long history which stretches back to the early 1920s. An extensive literature has accumulated describing the effects of insulin-dependent and non-insulin-dependent diabetes on cognitive ability. Studies have considered the impact of acute, transient metabolic derangement (principally hypoglycaemia and hyperglycaemia) on cognitive function, as well as the longer-term effects of chronic hyperglycaemia and recurrent severe hypoglycaemia on the brain. Several review articles have demonstrated that there is widespread inconsistency in the conclusions drawn from the individual studies in these areas. This discrepancy may be attributable to multiple factors, such as the use of non-comparable patient populations, insufficient sample sizes and inadequate controls. However, an additional and important cause of discrepancy has been the use of different cognitive test batteries.

Although much of the variance in human mental abilities is attributable to general intelligence, in clinical practice it is useful to employ tests to examine the various cognitive domains. These domains include attention and concentration, memory (both verbal and non-verbal), abstract thinking, constructional ability, and speed of information processing. Full assessment of cognitive function requires, therefore, the administration of a battery of psychometric tests, to ensure that sufficient cognitive domains are examined to detect any deficits or decrements. However, a bewildering range of psychometric tests is available. The format of individual tests is very variable, ranging from the relative simplicity of paper and pencil tasks, such as Trail-Making B (from the Halstead-Reitan battery) and Digit Symbol (from the Wechsler Adult Intelligence Scale), to the electronic complexity of driving simulators. The lack of gold standards for the validation of psychological tests means that multiple tests of differing nature and complexity are available to examine the parameters of any given cognitive domain. The situation is made more complex since many tests measure more than one aspect of cognitive ability, so that the results from two different tests purporting to examine, for example, verbal memory may not be directly comparable. Thus, comparisons between studies which have used different batteries of cognitive tests must be made with caution, even though they may have been attempting to examine the same nominal cognitive domains. This lack of common endpoints precludes the combination of study results by meta-analysis, thereby greatly diminishing the value of smaller studies. The practical reality of this situation has been illustrated by ourselves in a recent review on the effects of non-insulin-dependent diabetes on cognitive function; the heterogeneity of the cognitive testing performed in the 19 studies that were evaluated prevented a meta-analysis being conducted and allowed only qualitative comparisons to be made between the studies. This reduces the strength of conclusions that can be drawn from the body of work published on this topic.

Achieving a Consensus on Neuropsychological Assessment

This problem is not unique to diabetes. Investigators in other fields have addressed the matter of assessing cognitive function to facilitate comparisons across different studies. A recent meeting in Fort Lauderdale, USA, entitled 'CNS Dysfunction After Cardiac Surgery: Defining the Problem', was attended by leading investigators in cerebral physiology and in outcomes of cardiac surgery. This group agreed on a consensus statement which attempted to standardize the assessment of neuropsychological function after cardiac surgery. Among a list of 14 recommendations, the statement suggested a core battery of psychological tests to be employed in such an assessment. The tests recommended were the Rey Auditory Verbal Learning Test (assessing learning and immediate and delayed memory functions), the Trail-Making A and B Tests (assessing attention, concentration, and mental flexibility) and the Grooved Pegboard Test (assessing psychomotor skills, including dexterity). These tests are well standardized and validated and are not dependent on sophisticated computer technology. The Fort Lauderdale statement stressed that mood state assessment should be performed concurrently with neuropsychological testing, as performance of the latter can be influenced by mood state and its variation. While the suggested core test battery does not provide a comprehensive assessment of cognitive function, investigators retain the scientific freedom to add supplementary tests as appropriate. The key point, however, is that the core battery provides a basis for making rational comparisons between studies and should allow, if required, the combination of study results by meta-analysis.

A Diversity of Batteries?

We suggest that if significant scientific progress is to be made, the development and application of core batteries of neuropsychological tests are vital for future studies of cognitive function in diabetes. However, the situation in

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diabetes is especially complex because of the diversity of situations in which cognitive function is measured. Diabetes affects people of widely differing ages and cognitive function may be altered either by acute metabolic perturbation or by the longer-term effects of diabetes. Clearly it might not be reasonable to have the same core battery of tests for children, young adults, and elderly people. Similarly, it may be more appropriate to use different batteries of tests (and experimental designs) in studies examining acute metabolic effects as opposed to longer-term cognitive deficits. For example, where investigators wish to identify the precise glycaemic threshold for impairment in specific cognitive domains, a small battery of domain-sensitive tests, perhaps in conjunction with other physiological measurements, may be more appropriate than the larger battery of cognitive tests that would be required to identify cognitive impairment secondary to recurrent, severe hypoglycaemia. Thus, in diabetes, it is likely that several different core batteries of cognitive tests will be required to meet the requirement of investigators. Such investigators would of course be at liberty to supplement these batteries with extra tests where necessary.

We propose that an international forum of investigators with expertise in this field should be convened to agree on guidelines for core cognitive testing in diabetes, thereby advancing the study of this important area.

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Department of Psychology, University of Edinburgh

References

Optimal Time of Administration of Insulin Lispro

Importance of meal composition

MARK W.J. STRACHAN, MRCP
BRIAN M. FRIER, MD

OBJECTIVE — To compare the glucodynamics of pre- and postprandial administration of insulin lispro using test meals of differing composition.

RESEARCH DESIGN AND METHODS — Twenty subjects with IDDM were studied on four separate occasions. Ten subjects ingested high-carbohydrate and high-fat breakfasts with a large liquid component, and 10 subjects ingested high-carbohydrate and high-fat breakfasts in a more solid form. With each meal, insulin lispro was injected 10 min preprandially on one occasion and 20 min postprandially on another. The magnitude and temporal pattern of postprandial glucose excursions were observed.

RESULTS — With all meal types studied, postprandial blood glucose excursions were significantly smaller when insulin lispro was administered preprandially (P < 0.05). With both high-carbohydrate meals and the liquid high-fat meal, preprandial administration of lispro was associated with modest postprandial increments of blood glucose. With the solid high-fat meal, preprandial lispro produced a cumulative decline in postprandial blood glucose, whereas blood glucose rose when lispro was administered postprandially.

CONCLUSIONS — For meals with a high carbohydrate content, the optimal time of administration of lispro is preprandial. However, for meals with a high solid fat content, postprandial administration of lispro may be preferable.

Insulin lispro [Lys(B28), Pro(B29)] insulin is a genetically engineered analog of human insulin (1). Compared to conventional regular insulins, it is absorbed more rapidly and its glucose-lowering activity is both more rapid in onset and of shorter duration (1). To avoid preprandial hypoglycemia, it is recommended that lispro be administered shortly before meals (2,3). However, if the rise in postprandial blood glucose is reduced in magnitude or substantially delayed, the rapid onset of action of insulin lispro may promote early postprandial hypoglycemia.

Gastric emptying is an important determinant of the timing of the rise in postprandial blood glucose (4), and the composition of a meal has a significant influence on gastric emptying (5,6). Liquids pass through the stomach more rapidly than solids (7–9), and gastric emptying is slower when a meal has a high fat content (10–12). It is possible, therefore, that the administration of insulin lispro before a meal with a high fat content may promote early postprandial hypoglycemia. One approach to minimizing this risk would be to inject lispro after the meal. The aim of the present study was to compare the glucodynamics associated with pre- and postprandial administration of insulin lispro after the consumption of test meals in which the relative proportions of carbohydrate and fat were varied. The roles of the solid and liquid constituents of the meals were also evaluated.

RESEARCH DESIGN AND METHODS — Twenty subjects with IDDM were recruited from the diabetes outpatient clinic of the Royal Infirmary of Edinburgh. All had IDDM, diagnosed according to WHO criteria, for more than 1 year and had an HbA1c level of less than 9.0% (non-diabetic range 4.5–5.8%). No subjects had significant complications of diabetes (other than background retinopathy) or other medical disorders, and none had a history of dyspepsia or gastric fullness after meals. All subjects were administering human insulin in a multiple injection (basal-bolus) regimen. Autonomic neural function was assessed using standard tests of cardiovascular reflexes (13). The study was approved by the local medical ethics advisory committee, and all subjects gave written informed consent before participation.

The subjects were allocated to two groups matched for age, BMI, duration of diabetes, HbA1c, and total daily insulin dose (Table 1). Eighteen subjects had normal tests of cardiac autonomic function; one subject in Group 2 had an abnormal result on theValsalva maneuver alone. In another subject from Group 2, the full battery of tests of autonomic function could not be completed for technical reasons. This person had no symptoms suggestive of autonomic neuropathy or gastroparesis and did not have postural hypotension.

Four isocaloric test meals were used in the study (Table 2). Two meals (designated F) had a high fat content and a relatively low carbohydrate content. Two meals (designated C) had a high carbohydrate content and a low fat content. One meal of each type had a pronounced liquid (L) component, and one meal of each type had a prominent solid (S) component. The meals are designated as CL (carbohydrate and liquid), CS (carbohydrate and solid), FL (fat and liquid), FS (fat and solid).

All subjects underwent four separate study sessions. On two occasions, a high-fat
meal was consumed, and on two occasions, a high-carbohydrate meal was consumed. Subjects in Group 1 were given the test meals with a pronounced liquid component (CL and FL), and subjects in Group 2 received the meals with a prominent solid phase (CS and FS). For each meal type, lispro was administered preprandially on one occasion (10 min before the start of the test meal) and postprandially on the other (20 min after the start of the test meal). The order of meal type and the timing of the lispro injection were randomized. The time taken to consume the different test meals was very similar. The dose of lispro administered was determined individually after consultation with each subject, and the dose was equivalent to the amount of regular insulin that each subject would normally take before breakfasts of the nature, size, and caloric content used in the study. The agreed dose of lispro administered to each subject remained constant across the four study sessions.

The subjects fasted overnight and omitted their normal morning dose of regular insulin before each study. The study session was postponed if fasting blood glucose was >12.0 mmol/l or if hypoglycemia had been experienced during the preceding night. An intravenous cannula was inserted for frequent blood sampling. Each dose of insulin lispro was administered subcutaneously into the anterior abdominal wall. Venous blood samples were collected before the meal and at 15-min intervals after the start of the meal for a period of 2 h. At each time point, the blood glucose excursion was calculated as the difference in blood glucose concentration from that at baseline. The study was discontinued immediately if the blood glucose declined below 2.5 mmol/l or if symptoms of hypoglycemia developed with blood glucose below 4.0 mmol/l.

Sample analyses
Glycated hemoglobin (as HbA1c) was measured using high-speed liquid chromatography based on an ion-exchange reverse-phase partition method (HPLC HA 8121). Whole blood venous glucose was determined using a glucose oxidase method (Yellow Springs Instrument 2300 Stat).

Statistical analyses
Clinical characteristics of subjects were compared using the Mann-Whitney U test, and the χ² test was used for categorical data.

| Table 1—Clinical characteristics of subjects with IDDM |

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29.3 ± 2.9</td>
<td>27.6 ± 3.1</td>
<td>0.60</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>7/3</td>
<td>4/6</td>
<td>0.37</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>11.5 ± 2.6</td>
<td>13.9 ± 2.6</td>
<td>0.68</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.0 ± 3.3</td>
<td>75.7 ± 2.6</td>
<td>0.79</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.5 ± 1.0</td>
<td>26.4 ± 1.1</td>
<td>0.54</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.3 ± 0.4</td>
<td>7.2 ± 0.3</td>
<td>0.71</td>
</tr>
<tr>
<td>Total daily insulin dose (U/kg)</td>
<td>0.71 ± 0.07</td>
<td>0.78 ± 0.08</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Data are n and means ± SE.

Table 2—Constituents of test meals used in the study

<table>
<thead>
<tr>
<th>Food</th>
<th>Weight (g)</th>
<th>Energy (kcal)</th>
<th>Carbohydrate (g)</th>
<th>Fat (g)</th>
<th>Protein (g)</th>
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</thead>
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<tr>
<td>High-carbohydrate (liquid)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Breakfast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wholemeal bread</td>
<td>40</td>
<td>86</td>
<td>16.7</td>
<td>1.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Low-fat spread</td>
<td>14</td>
<td>91</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Whole milk</td>
<td>35</td>
<td>119</td>
<td>24.6</td>
<td>1.2</td>
<td>4.0</td>
</tr>
<tr>
<td>Skimmed milk</td>
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<td>187</td>
<td>28.4</td>
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<td>19.3</td>
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<tr>
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<td>19.2</td>
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<td>522</td>
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<td>% Energy</td>
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<td>15</td>
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<td>Fruit jam</td>
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<tr>
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<td>139</td>
<td>24.6</td>
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<td>4.0</td>
</tr>
<tr>
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<td>Skimmed banana</td>
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<td>9.6</td>
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<tr>
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</tr>
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<td>40</td>
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</tr>
<tr>
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<tr>
<td>Whole milk</td>
<td>568</td>
<td>369</td>
<td>26.7</td>
<td>21.6</td>
<td>18.7</td>
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<td>560</td>
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<td>34</td>
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<td>—</td>
<td>33</td>
<td>33</td>
<td>16</td>
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<tr>
<td>High-fat (solid) breakfast</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Breakfast</td>
<td></td>
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<tr>
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<td>3.0</td>
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<tr>
<td>Butter</td>
<td>14</td>
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<tr>
<td>Cornflakes</td>
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<td>Cheese</td>
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<td>—</td>
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<td>583</td>
<td>309</td>
<td>92</td>
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<tr>
<td>% Energy</td>
<td>—</td>
<td>—</td>
<td>33</td>
<td>33</td>
<td>16</td>
</tr>
</tbody>
</table>

Strachan and Frier
Baseline blood glucose concentrations were compared using Student's t test for paired data. Postprandial blood glucose excursions were compared using a repeated measures analysis of variance (ANOVA). Post hoc analyses were performed using Student's t test for paired data. A P value of <0.05 was considered significant for the Mann-Whitney U test, χ² test, and ANOVA; a P value of <0.01 was considered significant for the paired t test analyses, to reduce the risk of a type I statistical error occurring in view of the multiple analyses carried out. All analyses were performed using SPSS version 6.1.3 for Windows.

RESULTS — The baseline fasting blood glucose concentrations before each test meal did not differ whether lispro was administered pre- or postprandially (Table 3). Between-subjects ANOVA showed that neither the order in which the test meals were consumed nor the order of the timing of the injection of lispro had an effect on the resulting glycemic excursions (P > 0.05). No significant difference existed in the mean dose of lispro administered to subjects in Group 1 (12.7 ± 1.3 U) compared with subjects in Group 2 (13.4 ± 1.4 U).

High-carbohydrate liquid (CL) meal
When insulin lispro was administered before the CL breakfast, the postprandial glycemic excursion was modest, with a mean peak rise in blood glucose of 2.0 mmol/L (Fig. 1A). By contrast, when lispro was administered after the test meal, a significantly larger blood glucose excursion was observed (P < 0.05), with the maximum mean rise in blood glucose of 5.6 mmol/L occurring at 45 min. The blood glucose excursions at 30, 45, 60, and 75 min were all significantly higher with postmeal administration of lispro (P < 0.01). No subjects developed significant hypoglycemia.

High-carbohydrate solid (CS) meal
Injection of insulin lispro before the CS meal produced a much smaller blood glucose excursion compared with postprandial administration of lispro (P < 0.05; Fig. 1B). After postprandial administration, a small negative excursion of between 0.5 and 1.0 mmol/L was observed during the initial 30 min after the start of the breakfast; thereafter, the blood glucose excursion rose, peaking at 1.0 mmol/L at 90 min. In the postprandial lispro studies, the mean blood glucose excursion peaked at 3.5 mmol/L at 45 min and was significantly higher than in the postprandial lispro studies at 15, 45, and 60 min after the test meal (P < 0.01). No subjects developed significant hypoglycemia.

High-fat liquid (FL) meal
Administration of insulin lispro before the FL breakfast was associated with a postprandial glycemic excursion in which the mean blood glucose varied little from baseline values (Fig. 1C). One subject developed persistent, biochemical hypoglycemia that provoked symptoms and necessitated termination of the session after 60 min. This subject started the study session with a premeal blood glucose of 5.0 mmol/L (the lowest value recorded for any subject ingesting the FL meal), which declined progressively over 60 min to 2.7 mmol/L, at which point the study was terminated. Because a repeated-measures ANOVA can only be performed on complete data sets, the incomplete time course data from this subject has not been included in the ANOVA and subsequent t-test analysis. The data presented in Fig. 1C are derived from the remaining nine subjects, in whom blood glucose did not fall below 3.0 mmol/L at any time.

Administration of lispro after the FL breakfast produced a significantly larger blood glucose excursion (P < 0.05). No subjects developed significant hypoglycemia. The blood glucose excursion peaked at 5.2 mmol/L at 45 min and was significantly greater at 30, 45, 60, and 75 min than that observed following postprandial administration of insulin lispro (P < 0.01).

High-fat solid (FS) meal
Administration of insulin lispro before the FS meal provoked a continuous decline in blood glucose, so that by 120 min, mean blood glucose was 3.4 mmol/L below baseline concentrations (Fig. 1D). One subject developed persistent, symptomatic hypoglycemia that necessitated termination of the study session after 105 min. The subject's premeal blood glucose was 6.4 mmol/L, and it fell progressively to 2.0 mmol/L, when the session was terminated. Data from this subject have not been included in the ANOVA analysis or in Fig. 1D. In two other subjects, blood glucose fell below 3.0 mmol/L by 120 min. In one individual, premeal blood glucose was 11.7 mmol/L and at 120 min was 2.8 mmol/L; in the other subject, premeal blood glucose was 7.4 mmol/L and at 120 min was 2.6 mmol/L. In all other subjects, the blood glucose remained above 3.0 mmol/L throughout the study.

Postprandial administration of lispro resulted in a significant rise in mean blood glucose (P < 0.05), with a peak excursion of 3.6 mmol/L at 45 min. No subject developed significant hypoglycemia. When lispro was administered postprandially, the blood glucose excursions were significantly greater at all time points from 15 to 105 min inclusive (P < 0.01).

CONCLUSIONS — When the rise in blood glucose after a meal is delayed or of low magnitude, the rapid onset of action of insulin lispro may potentially increase the risk of early postprandial hypoglycemia. The importance of meal composition in determining the postprandial glycemic response was highlighted in a recent study of patients with IDDM in which the injection of lispro before the consumption of a test meal with a low carbohydrate and high fat content resulted in a higher frequency of early postprandial hypoglycemia (14).

In the present study, the effects of pre- and postprandial administration of insulin lispro were compared in relation to test meals of differing composition. The principal aim of the study was to assess the risk of early postprandial hypoglycemia, for
which a 2-h time course was thought to suffice. Administration of all test meals to the entire cohort of subjects (which would have required eight separate study sessions) was considered to be an unrealistic demand on the subjects' time. The two subject groups were well matched demographically, and, in particular, the groups did not differ with respect to glycemic control, total daily insulin dose, or dose of lispro administered.

Irrespective of the composition of the test meal, the postprandial excursions of blood glucose were lower when insulin lispro was administered before rather than after food. This resulted in better postmeal glycemic control with high-carbohydrate (low-fat) meals. Administration of lispro before meals with a high fat (and low carbohydrate) content produced a significant fall in postprandial blood glucose when the meal was predominantly in solid phase. When the meal was in a more liquid form, the blood glucose excursion was flat, presumably because the liquid traversed the stomach more rapidly (4,7,9). Complete data sets were not obtained for either form of the high-fat meal, as one subject developed hypoglycemia during each premeal lispro study. If complete data sets had been available for analysis, a greater mean decrement in blood glucose might have been observed when lispro was administered before either type of high-fat meal. Therefore, when lispro is injected before meals with a high fat content, particularly those with a prominent solid phase, the resultant postprandial fall in blood glucose may induce early postprandial hypoglycemia, especially if the premeal blood glucose is relatively low (for example, <8 mmol/l). However, if the premeal blood glucose is high, the postprandial fall in blood glucose may be acceptable and indeed desirable. In the present study, when insulin lispro was administered after a high-fat breakfast, postprandial blood glucose rose in most subjects, and the risk of postprandial hypoglycemia was minimal. The increments in postprandial blood glucose were, however, relatively high and might have been restricted by administering the insulin lispro immediately after the test meal, instead of after an interval of 20 min.

The efficacy of the postprandial administration of insulin lispro has been examined in a previous study (15). Injection of lispro 15 min after the start of a test meal consisting of beef stroganoff produced a glucodynamic pattern over 2 h that was comparable to the pattern of regular human insulin given either 20 min before or immediately before the meal. The lowest postprandial glucose excursion occurred when insulin lispro was injected 20 min before the meal, but this provoked early postprandial hypoglycemia in some subjects (15), consistent with the findings of the present study.

A major premise of the present study was that a meal with a high fat content may be associated with a delayed glycemic response by retarding gastric emptying. However, gastric emptying is not influenced solely by meal composition. Gastroparesis diabeticorum is a rare but debilitating complication of diabetes (5). More subtle abnormalities of gastric emptying have been described in many individuals with diabetes.
of relatively long duration (5, 9, 16-21). Gastric emptying is also influenced by ambient glucose concentration; hyperglycemia delays gastric emptying (22-24), whereas hypoglycemia promotes gastric emptying (25-27). In the present study, the mean basal glucose concentrations did not differ between the pre- and postprandial lispro arms. The subjects had a mean duration of IDDM of >10 years. None had any symptoms suggestive of gastric stasis, and the majority had normal cardiac autonomic neural function. However, in the absence of formal studies of gastric emptying, complete normality of gastric emptying cannot be ascertained in all subjects, some of whom might have had subclinical abnormalities of gastric function.

In the present study, lispro was injected subcutaneously into the anterior abdominal wall, a site that is associated with a rapid rate of absorption of insulin (28-30). Administration of insulin lispro into an alternative anatomical site, such as the arm or the thigh, is associated with a slower onset of glucose-lowering activity (28-30). Therefore, when a person with IDDM wishes to consume a high-fat meal, an alternative strategy may be to inject insulin lispro into the thigh instead of choosing a postprandial time of administration. The absorption of insulin from any injection site is dependent on many variables (31-33), however, and so the use of an injection site from which the absorption of insulin is usually slow may be a less reliable method of avoiding hypoglycemia than adjusting the time of injection in relation to meals. A further means of avoiding early postprandial hypoglycemia would be to reduce the dose of insulin lispro, but this may provoke late postprandial hypoglycemia (14).

In conclusion, when a person with IDDM consumes a meal with a high carbohydrate content, the preprandial administration of insulin lispro provides better glycemic control with little risk of early postprandial hypoglycemia. By contrast, when a person consumes a meal that has a high content of fat mainly in a solid phase, postprandial administration of lispro may be more appropriate.

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Evaluation of Serum Markers of Neuronal Damage Following Severe Hypoglycaemia in Adults with Insulin-treated Diabetes Mellitus

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Abstract

Background Neurone-specific enolase (NSE) and protein S-100 (S-100) may be used as markers of acute neuronal damage in humans with neurological disorders.

Method To evaluate their use following a single episode of severe hypoglycaemia (defined as an episode requiring external assistance to aid recovery), serum concentrations of NSE and S-100 were measured following hypoglycaemia which had not caused persistent neurological impairment in 16 patients with insulin-treated diabetes (the 'hypo' subjects), and in three diabetic patients who died following severe hypoglycaemia. The serum proteins were also measured in 10 subjects with insulin-treated diabetes who had not experienced an episode of severe hypoglycaemia within the preceding year (the 'control' subjects).

Results No differences in serum concentrations of NSE and S-100 were observed between the 'control' and the 'hypo' subjects at either 36 hours or seven days after the episode of severe hypoglycaemia (p>0.05). However, in two of the three subjects who died following hypoglycaemia, serum concentrations of the markers were markedly elevated.

Conclusions Any neuronal injury occurring during severe hypoglycaemia that is not associated with persistent neurological deficit is insufficient to provoke elevation of these serum markers. However, the measurement of serum concentrations of NSE and S-100 may have a prognostic role in evaluating clinical outcome following severe hypoglycaemia which is associated with neurological damage. Copyright © 1999 John Wiley & Sons, Ltd.

Key words neurone-specific enolase; protein S-100; hypoglycaemia; insulin-treated diabetes; neuroglycopenia

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Introduction

People with diabetes mellitus who receive treatment with insulin or oral hypoglycaemic agents may experience transient or occasionally permanent neurological abnormalities, such as hemiplegia, coma and convulsions, as a consequence of severe hypoglycaemia [1–3]. Rarely decerebration and brain death may supervene [1–3]. In addition, some studies have suggested that
repeated exposure to severe hypoglycaemia may have a cumulative effect, resulting in permanent, though mostly modest, neuropsychological decrements [4–7]. The identification of serum markers that could predict the degree of neuronal damage and prognosis of patients after neurological injury, analogous to the measurement of creatine kinase or troponin after myocardial infarction, would have considerable clinical value. Two proteins, neuron-specific enolase (NSE) and protein S-100 (S-100), have shown promising results in this role.

NSE is a 78 kD glycolytic enzyme which originates predominantly from the cytoplasm of neurones and neuroendocrine cells [8]. Increased serum concentrations of NSE are found in patients with small-cell lung cancer, neuroblastoma and neuroendocrine tumours [8]. Furthermore, the concentrations of NSE, both in cerebrospinal fluid (CSF) and in serum, are elevated in various neurological disorders [9–22]. In particular, serum concentrations of NSE, measured in venous blood within 48–72 hours of an acute stroke, correlate significantly with clinical outcome [23–25].

S-100 is a calcium-binding protein which exists as homo- or heterodimers of two subunits – α (10.4 kD) and β (10.5 kD) [26,27]. The αS-100 form is found exclusively in neurones, αβS-100 is present in glial cells, and ββS-100 is found in glial and in Schwann cells [27]. S-100 has also been detected in some non-neural tissues, such as melanocytes [28] and adipose tissue [29], and elevated serum concentrations of S-100 have been reported in patients with malignant melanoma [30]. As with NSE, acute neurological injury causes concentrations of S-100 to rise in CSF and in serum [11, 17,18,21,31–39] and concentrations of S-100 measured in serum samples taken within 48 hours of an acute stroke correlate significantly with clinical outcome [38].

At present, no biological markers are available which can predict clinical outcome with accuracy after an episode of severe hypoglycaemia. To our knowledge, the effects of severe hypoglycaemia on serum levels of NSE and S-100 have not been evaluated previously. Serum concentrations of NSE and S-100 were therefore estimated acutely in 16 diabetic patients experiencing a single episode of severe hypoglycaemia which was not associated with persistent neurological abnormality, in 3 diabetic patients who sustained profound brain damage and subsequently died following an episode of severe hypoglycaemia, and in 10 diabetic patients who had not experienced an episode of severe hypoglycaemia within the preceding year.

Patients and methods

Patients

The study was approved by the local medical ethics advisory committee. Sixteen subjects were recruited shortly after experiencing an episode of severe hypoglycaemia (the 'hypo' group), as part of a simultaneous investigation of cognitive function following hypoglycaemia. Severe hypoglycaemia was defined, according to the criteria used in the Diabetes Control and Complications Trial (DCCT), as an episode that required external assistance to aid recovery [40]. In addition, the blood glucose concentration had to be documented as 2.8 mmol L⁻¹ or less and/or the clinical manifestations had to have been reversed with oral carbohydrate, subcutaneous glucagon, or intravenous glucose [40].

The 'hypo' subjects received treatment for the severe hypoglycaemia either in the community or in the hospital emergency department; none had experienced persistent neurological dysfunction following restoration of normoglycaemia. Nine of the subjects were treated with intramuscular glucagon, two with intravenous dextrose and five with oral carbohydrate. The subjects treated with glucagon or dextrose all experienced transient loss of consciousness at the time of hypoglycaemia. Where possible the history of hypoglycaemia was verified by scrutiny of general practitioners' notes or the hospital records, and by obtaining a contemporaneous account from a witness through completion of a short questionnaire documenting the clinical signs demonstrated by the affected patient, the nature of the treatment administered and the subsequent response. Ten subjects, who had not experienced an episode of severe hypoglycaemia within the preceding year (the 'control' group), were studied. The subjects in the two groups were matched for age, sex and duration of diabetes (Table 1), and none had a previous history of any neoplastic or neurological disorder that is known to elevate serum NSE or S-100 concentrations. The mean glycated haemoglobin (HbA₁c) was lower in the 'hypo' subjects than in the 'controls' (the non-diabetic range for HbA₁c was 5.0–6.5%; Table 1). The history of previous severe hypoglycaemia in the control group was confirmed by interview with the individual patients, by scrutiny of hospital records and, where possible, by asking a relative or friend to complete a short questionnaire on when previous episodes of severe hypoglycaemia had been experienced by the patients. Blood glucose concentrations were measured concurrently to ensure that no subjects were hypoglycaemic at the time of measurement of serum NSE and S-100.

Table 1. Clinical characteristics of subjects with Type 1 diabetes and timing of blood samples

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>'Hypo'</th>
<th>'Control'</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 (1.1–1.5)</td>
<td>30.0 (26.0–47.3)</td>
<td>10.6 (5.0)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>1.3/1.5</td>
<td>5.7/6.8</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>15.0 (10.3–23.5)</td>
<td>12.5 (8.0–27.9)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>8.2 (7.0–9.0)</td>
<td>10.1 (8.5–11.7)</td>
<td>0.02</td>
</tr>
<tr>
<td>T1: Time to 1st blood sample (days)</td>
<td>3.1 (1.1–1.9)</td>
<td>7.1 (6.6–7.4)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>T2: Time between 1st and 2nd blood samples (days)</td>
<td>7.0 (6.9–7.1)</td>
<td>7.0 (6.9–7.1)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

All values are median (inter-quartile range).
Timing of blood samples

Venous blood samples for measurement of NSE and S-100 concentrations were taken at two time points designated T1 and T2. In the case of the 'hypo' group, T1 was approximately 36 hours after the onset of the hypoglycaemic event (Table 1), while in the 'control' group, T1 represented the time of taking the first blood sample. In both groups, the second blood sample (T2) was taken approximately 7 days after the first blood sample (i.e. approximately 8.5 days after the hypoglycaemic event in the case of the 'hypo' group).

Measurements of serum NSE and S-100 concentrations

Blood was centrifuged at 1500 g for 10 minutes within 4 hours of venepuncture and sera were stored at -20°C. Haemolysed samples were not analysed for NSE because of the presence of this enzyme within erythrocytes and platelets. All biochemical analyses were performed blind to each patient's status.

NSE was measured by an enzyme immunolodugstic test (Enzyme-Test NSE, Boehringer Mannheim Immunodiagnostics, Lewes, UK). The lower limit of detection of the assay was 0.5 μg l⁻¹; the intra-assay coefficient of variation (CV) was 2.2% at 4.9 μg l⁻¹ and 1.5% at 13.6 μg l⁻¹. The inter-assay CV was 5.8% at 23.1 μg l⁻¹. S-100 was measured by immunoradiometric assay (IRMA Sangtec, Bromma, Sweden). The lower limit of detection was 0.05 μg l⁻¹; the intra-assay CVs were 2.8% at 0.18 μg l⁻¹, 0.7% at 1.5 μg l⁻¹ and 0.8% at 5.3 μg l⁻¹. The inter-assay CVs were 3.3%, 4.4% and 1.5% respectively.

Statistical analyses

All demographic data are presented as median (inter-quartile range), while the NSE and S-100 data are presented as mean ± standard error of the mean. Baseline characteristics of subjects were compared using the Mann-Whitney U test, and the Chi-squared test was used for categorical data. Differences in NSE and S-100 concentrations between the 'hypo' and 'control' groups, at the two time points, were compared using a mixed model analysis of variance (ANOVA) with group ('hypo' or 'control') as a between subjects factor and time (T1 or T2) as a repeated measure. A p-value of less than 0.05 was considered to be significant. All analyses were performed using SPSS version 7.5.1 for Windows 95.

Case histories

Three diabetic subjects who died following severe hypoglycaemia were also studied and their histories are described below.

Patient 1

A 49-year-old Caucasian male with Type 1 diabetes for four years, who had a previous history of frequent hypoglycaemia and alcohol dependence, was found unconscious at home. He had been drinking heavily in the previous two weeks during which frequent biochemical hypoglycaemia had been recorded with routine home blood glucose monitoring. When found comatose, his blood glucose concentration was less than 1.0 mmol l⁻¹ and he was given intramuscular glucagon and intravenous dextrose. On admission to hospital, the patient was comatose, with a Glasgow Coma Scale (GCS) of 4, was haemodynamically stable, but had hypotonia and bilateral extensor plantar responses. A CT brain scan showed no evidence of cerebral oedema or raised intracranial pressure. The patient was intubated and ventilated and received infusions of mannitol and phenytoin to control mild epileptiform activity. He was extubated five days later, but his conscious level remained depressed with a GCS of 5. He subsequently developed bronchopneumonia and died 11 days after admission. Blood samples for NSE and S-100 level estimation were obtained approximately 24 hours after the onset of hypoglycaemia (T1) and seven days later (T2).

At autopsy, the lungs showed extensive bronchopneumonic consolidation. Brain histology showed mild sulcal widening and generalised vascular congestion consistent with agonal hypoxia. There was a mild degree of white matter pallor and rarefaction with U-fibre sparing, indicative of mild cerebral oedema, but no mid-line shift or evidence of herniation were noted. The brunt of neuronal damage was borne by the hippocampus where there was severe neuronal depletion with corresponding gliosis, most marked in the CA4 and CA1 zones (Figure 1), with acidophilia of remaining neurones. Neurones of the dentate gyrus showed conspicuous acidophilia. The neocortex contained scattered acidoophilic neurones, particularly in layers 2 and 3 and at the depths of sulci. These changes were most severe in temporal neocortex, where similar changes were also apparent.

Figure 1. Section of CA4 zone of the hippocampus from Patient 1. There are numerous reactive gemistocytic astrocytes (arrows) and neurones are completely absent. (Haematoxylin and eosin x 360)
seen in layer 4. The basal ganglia, hypothalamus, brain stem, cerebellum and spinal cord were unaffected and, in particular, cerebellar Purkinje cells were intact. There was no evidence of recent traumatic brain damage, inflammation, haemorrhage, regional infarction or Wernicke's encephalopathy.

Patient 2
A 27-year-old Caucasian male with a 4-year history of Type 1 diabetes for 4 years was found unconscious (GCS 3) at home, having last been seen awake 13 hours previously following a row with his girlfriend. Two empty vials of porcine soluble insulin and three insulin syringes were lying beside him, suggesting that he had self-administered a large dose of insulin. Blood glucose was less than 1 mmol L⁻¹ and intramuscular glucagon was administered. On admission, the patient was tachypnoeic with extensor posturing and a CT brain scan showed evidence of cerebral oedema. He was intubated and ventilated for 24 hours without clinical improvement. All active intervention was subsequently withdrawn and the patient died shortly after. Blood samples for NSE and S-100 level estimation were obtained approximately 18 hours after the onset of hypoglycaemia (T1).

A post mortem revealed acute bronchitis and right lower lobe bronchopneumonia. Histological examination of the brain revealed widespread swelling and breakdown of the blood-brain barrier. There was global transcortical neuronal shrinkage and acidophilia (Figure 2). Acute inflammation was limited to the temporal neocortex and leptomeninges. Similar neuronal changes were present in the CA1 to CA4 zones of the hippocampus, proximal subiculum, dentate gyrus, head of the caudate nucleus, putamen and, to a lesser extent, globus pallidus. Relative neuronal sparing was observed in the distal subiculum, angle of the dentate gyrus, caudate tail and claustrum, as well as selected larger neurones in the basal ganglia. Selective superficial cortical damage was seen in the cingulate gyri. Thalamic neurones were distorted due to transtentorial herniation, while the mid-brain and pons showed oedema, patchy infarction and Duret haemorrhage due to terminal brain stem herniation and compression. Other significant features included basal ganglia microcalcification and relative Purkinje cell preservation in the cerebellum.

Patient 3
A 47-year-old man with a 30-year history of poorly controlled insulin-dependent diabetes was found unconscious in hospital before breakfast, 7 days following an aortic valve replacement operation. Blood glucose was less than 1 mmol L⁻¹ and he was treated with intravenous dextrose. During this episode, the patient had a grand mal seizure which was complicated by aspiration of gastric contents and a respiratory arrest. He was intubated and ventilated and initial CT scan of the brain showed no intracerebral abnormality, although a repeat scan 2 days after the episode showed gross cerebral oedema. Despite treatment with mannitol and dexamethasone, his conscious level remained depressed and the patient died 33 days following the episode of severe hypoglycaemia. Permission was not obtained for an autopsy. Serial blood samples were obtained for S-100 level estimation only.

Results
The NSE and S-100 data for the 'hypo' and 'control' patients are presented in Figures 3 and 4. There was no significant effect of group (F=0.09, p>0.05) or time (F=4.09, p>0.05) on mean serum NSE concentrations. The group by time interaction was also not significant (F=0.75, p>0.05). Similarly, the main effects of group (F=1.09, p>0.05), time (F=2.12, p>0.05) and group by time (F=2.37, p>0.05) did not reach statistical significance for mean serum S-100 concentrations.

The NSE and S-100 concentrations of Patient 1 were comparable with the 'control' patients at both time points (Figures 3 and 4). In Patient 2, however, NSE and S-100 concentrations were markedly elevated, at approximately 8 and 130 times respectively, the levels noted in the 'control' subjects (Figures 3 and 4). In Patient 3 there was also a large rise in S-100 levels, which peaked at 24 hours after the episode of severe hypoglycaemia; S-100 concentrations fell thereafter, but were still above baseline levels at 17 days (Figure 5).

Figure 2. Section of the superficial cortex (layer 2) from Patient 2. There is neuronal acidophilia with shrinkage in the cingulate gyrus. (Haematoxylin and eosin x360)
Markers of Neuronal Damage After Severe Hypoglycaemia

Figure 3. Serum concentrations of NSE at T1 and T2 in the 'hypo' and 'control' subjects, and in Patients 1 and 2. There was no significant difference between 'hypo' and 'control' subjects in concentrations of NSE at either time point (p>0.05). Serum NSE concentrations did not rise in Patient 1, but were markedly elevated in Patient 2.

Figure 4. Serum concentrations of S-100 at T1 and T2 in the 'hypo' and 'control' subjects, and in Patients 1 and 2. There was no significant difference between 'hypo' and 'control' subjects in concentrations of S-100 at either time point (p>0.05). Serum S-100 concentrations did not rise in Patient 1, but were markedly elevated in Patient 2.

Figure 5. Time course of serum concentrations of S-100 in Patient 3 from the day of the episode of severe hypoglycaemia (Day 1) to 17 days afterwards. The S-100 concentrations peaked 24 hours after the detection of severe hypoglycaemia, but remained elevated up to Day 17.
Discussion

Acute hypoglycaemia in humans provokes neurological dysfunction with evidence of cognitive impairment and neurophysiological and EEG abnormalities. The possible clinical sequelae of an episode of severe hypoglycaemia vary widely, ranging from complete recovery to permanent neurological disability or, rarely, death [1–3]. Even when neurological recovery is apparently complete, recurrent exposure to severe hypoglycaemia may result in cumulative cognitive decrements [4–7], presumably because of chronic, low-level neuronal damage. When significant neuronal destruction does occur during acute hypoglycaemia, it may be very difficult in the early stages to predict the likely clinical outcome. The patient's condition may be affected by other intercurrent illness, pharmacologically induced sedation, or the need for artificial ventilation. CT scanning of the brain, while providing valuable information, may show no intracerebral abnormality initially (as in Patient 3), despite significant neuronal damage. Therefore, any measurable neurological index that could assist with prediction of the likely clinical outcome after an episode of severe hypoglycaemia would be of value in planning the optimal medical management for affected patients.

NSE and S-100 offer such a possibility, because serum concentrations of both proteins are elevated following neuronal damage from other causes and correlate with clinical outcome after stroke [23–25,38]. Acute changes in the serum levels of these markers have not been examined previously in diabetic patients exposed to severe hypoglycaemia.

The present pilot study has demonstrated that serum concentrations of NSE and S-100 did not alter significantly in diabetic patients who had made a complete neurological recovery following an episode of severe hypoglycaemia, either at 36 hours or 8.5 days after the event. Previous studies in patients with various cerebral disorders, particularly stroke, have shown consistently that serum concentrations of NSE and S-100 become elevated within 48 hours of the acute insult and remain so for several days, or even weeks, afterwards [21, 23–25,38] and indeed such a pattern of S-100 concentrations was seen in Patient 3. Therefore, in the present study, it is unlikely that a transient rise in serum NSE and S-100 had occurred in the hypoglycaemia patients at the times other than those of the blood sampling.

Fortunately, episodes of severe hypoglycaemia which result in permanent neurological abnormality are relatively rare in clinical practice, but we were able to measure the changes in serum concentrations of NSE and S-100 before death in three fatal cases. The serum concentrations of NSE and S-100 were low in the diabetic patient who died nearly two weeks following an episode of severe hypoglycaemia and whose neuronal damage was centred primarily on the hippocampus. The significant CA1 and end-folial hippocampal damage may at least partly have pre-dated the fatal illness, and would be consistent with damage from previous episodes of hypoglycaemia [41–43]. However, the acidophilia of the remaining neurones in the hippocampus implies that acute neuronal damage had also occurred at the time of the episode of severe hypoglycaemia that preceded death. The patient’s seizures were mild and were treated promptly, and while they may have contributed to the hippocampal damage, they are unlikely to have been the principal cause.

By contrast, serum concentrations of NSE and S-100 were markedly elevated in a young diabetic patient who died within 48 hours of the development of profound hypoglycaemia, after a probable deliberate insulin overdose. This patient suffered very extensive brain damage. The involvement of the dentate gyrus and the relative preservation of Purkinje cells and neurones of larger basal ganglia and deep cingulate cortex are consistent with the effects of hypoglycaemia [41–43], although there is overlap between the neuropathological effects of hypoglycaemia, hypoxia/ischaemia and seizures. In addition, serum concentrations of S-100 were significantly elevated in a middle-aged patient who had a prolonged episode of severe hypoglycaemia, seven days following cardiac surgery. Initial serum concentrations of S-100 were low and CT scan of the brain was normal. However, within 24 hours of the episode, S-100 levels had risen significantly and a subsequent CT scan confirmed gross cerebral oedema. Serum S-100 concentrations remained elevated, albeit at lower levels, for several days afterwards. This patient had a grand mal seizure and a respiratory arrest which were treated promptly, but both events may have contributed to the subsequent neurological damage and the rise in S-100 concentrations.

In both the above cases, the serum concentrations of the neurological markers were elevated to levels equivalent to those observed after a large ischaemic cerebral infarction [25,38]. Following acute stroke, elevations in serum concentrations of NSE and S-100 are related to the size of the cerebral infarction [25,38]. Thus, NSE and S-100 levels may not rise following small strokes where only modest degrees of acute neuronal injury have occurred. Therefore, the results from the patients who made a complete neurological recovery following the severe hypoglycaemic episode should not be interpreted as implying that no neuronal damage has taken place. It is possible that a degree of neuronal injury did occur, but at a level that was below the detection of the NSE and S-100 assays.

The fact that serum concentrations of the neuronal markers rose in two of the three patients who subsequently died following an episode of severe hypoglycaemia raises the prospect that NSE and S-100 may have a role in predicting both the severity of neuronal damage and the clinical outcome of patients who remain comatose following prolonged severe hypoglycaemia. The potential value of using such markers is that they are easy to measure, are relatively inexpensive and show a rapid response following acute
neurological insult which causes neuronal damage. Therefore prognostic information could be available to clinicians at an early stage of management, and possibly before any abnormality is detectable by neuroimaging techniques.

In conclusion, the present study suggests that serum concentrations of NSE and S-100 do not rise in patients who have experienced an individual episode of severe hypoglycaemia without persistent neurological impairment. However, measurement of the serum concentrations of these proteins could have prognostic value in patients who remain comatose following prolonged severe hypoglycaemia. This requires validation in a larger series of patients experiencing hypoglycaemic coma, with or without cerebral oedema, relating the increments in these serum markers to clinical outcome and neuropathological findings.

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Recovery of Cognitive Function and Mood After Severe Hypoglycemia in Adults With Insulin-Treated Diabetes

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OBJECTIVE — Acute hypoglycemia in humans impairs cognitive functions and alters mood states. The time required for cognitive functions and moods to return to normal after an acute episode of severe hypoglycemia is unknown.

RESEARCH DESIGN AND METHODS — Cognitive functions and moods were studied prospectively in 20 subjects with insulin-treated diabetes who had recently experienced a spontaneous episode of severe hypoglycemia ("hypo" subjects) and 20 matched control subjects with insulin-treated diabetes who had not experienced severe hypoglycemia during the preceding year. The hypo subjects had a history of a greater number of episodes of severe hypoglycemia (P = 0.001). Cognitive function tests and mood scales were administered at 1, 5, 9, and 30 days after the severe hypoglycemia and at similar intervals for the control subjects.

RESULTS — For most of the cognitive tests, no evidence of a "hangover" effect of the acute hypoglycemia on cognitive function was observed (P > 0.05). A trend was noted for levels of hedonic tone (P = 0.082) and energetic arousal (P = 0.033) to improve with time in the hypo subjects but not in the control subjects. However, the hypo subjects had chronically elevated levels of depression (P = 0.011) and anxiety (P = 0.049) and persistently performed more poorly in several cognitive tests, such as the Digit Symbol Test (P = 0.009) and the Stroop Task (P = 0.007).

CONCLUSIONS — These results suggest that, in general, recovery from any acute cognitive decrement after severe hypoglycemia was complete by 1.5 days. The cognitive decrements and altered mood states noted in the hypo subjects may be persistent and may be a consequence of previous exposure to recurrent episodes of severe hypoglycemia.

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Acute hypoglycemia induces cognitive dysfunction as a consequence of neuroglycopenia. Cognitive functions that deteriorate include simple and choice reaction times, speed of arithmetic calculation, verbal fluency, color identification, trail making and digit symbol test performance, digit span, and other short-term memory indices (1). Mood states are also affected by mild hypoglycemia, which causes a reduction in levels of energetic arousal and an increase in tense arousal (2).

Acute hypoglycemia can provoke longer-term effects, even after normoglycemia has been restored. In nondiabetic and diabetic subjects, the symptomatic and neuroendocrine responses to acute hypoglycemia are diminished after antecedent (episodic) hypoglycemia that has occurred within the previous 72 h (3–7). Indeed, the catecholamine response to hypoglycemia may take between 6 days and 4 weeks to return to a normal magnitude (8). The effects of acute mild hypoglycemia on cognitive functions appear to be less prolonged, in that cognitive function recovers within 1 h of the restoration of normoglycemia (9–13). The long-term effects of acute hypoglycemia on other mental states such as mood and general well-being have received less attention. Mood changes during acute hypoglycemia persist for at least 30 min after restoration of normoglycemia (2), whereas relatively brief nocturnal hypoglycemia is not associated with any cognitive impairment the following morning (14,15) but is associated with greater levels of fatigue in response to the next day (15).

Severe hypoglycemia, which is defined as an episode requiring external assistance for recovery, causes pronounced neuroglycopenia that results in a profound degree of cognitive dysfunction and (rarely) can cause permanent neurological impairment (16–18). Fortunately, most patients appear to make a complete and rapid recovery after normoglycemia is restored, although anecdotal observation suggests that some patients have cognitive impairment for more protracted periods. Exposure to repeated episodes of severe hypoglycemia may possibly result in persistent cognitive impairment in some patients (19–23), although this has been disputed (24,25).

No previous studies have examined the timing of recovery of cognitive function and mood after a single episode of severe hypoglycemia. Delayed recovery of cognitive functions after severe hypoglycemia could have potentially important effects on performance of activities such as work or driving.

The purpose of the present study was to examine the temporal changes in mood states and cognitive functions after a single spontaneous episode of severe hypoglycemia in people with insulin-treated diabetes.
Table 1—History of episodes of severe hypoglycemia

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Episodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypo</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>13</td>
</tr>
</tbody>
</table>

Data are n.

RESEARCH DESIGN AND METHODS — The study was approved by the local medical ethics advisory committee, and all subjects gave written informed consent after the nature of the study was explained. A total of 40 subjects with insulin-treated diabetes were studied, most of whom were attending the Diabetes Outpatient Department at the Royal Infirmary of Edinburgh for regular review. Of the subjects, 20 had experienced a recent episode of severe hypoglycemia (the “hypo” group) and 20 had not experienced severe hypoglycemia (the control group) during the preceding year. Most of the hypo patients were recruited after they received treatment in the Accident and Emergency Department of the hospital or after their hospital admission.

Severe hypoglycemia

Severe hypoglycemia was defined according to the criteria used in the Diabetes Control and Complications Trial as an episode that required external assistance to aid recovery (26). In addition, the patients’ blood glucose concentration had to be documented at ≤5.8 mmol/l, and/or the clinical manifestations had to have been reversed with oral carbohydrate, intramuscular glucagon, or intravenous glucose (26).

The hypo subjects received treatment for the severe hypoglycemia either in the community or in the hospital emergency department; none had experienced persistent neurological dysfunction after the restoration of normoglycemia. Of the subjects, 11 were treated with intramuscular glucagon, 2 were treated with intravenous dextrose, 2 were treated with a combination of intravenous dextrose and intramuscular glucagon, and 7 were treated with oral carbohydrate. All subjects who required parenteral glucagon or dextrose reported a transient loss of consciousness at the time of hypoglycemia. Where possible, the patient’s history of hypoglycemia was verified by scrutinizing contemporaneous notes made by the patients’ general practitioner (family physician) or the hospital case records and by obtaining a direct account from a witness. The latter involved completion of a short questionnaire documenting the clinical features demonstrated by the affected patient, the nature of the treatment administered, and the patient’s subsequent response. The history of previous severe hypoglycemia in the control group was confirmed by inter-viewing the individual patients, by scrutinizing hospital case records, and, where possible, by asking a relative or friend to complete a short questionnaire regarding previous episodes of severe hypoglycemia that the patient had experienced. Hypoglycemia history was graded on a four-point scale: 1 = no previous episodes of severe hypoglycemia, 2 = one or two previous episodes, 3 = three to five previous episodes; 4 = more than five previous episodes. The hypo subjects had a history of a significantly greater number of previous episodes of severe hypoglycemia than the control subjects (Table 1; P = 0.000). None of the hypo subjects experienced further exposure to severe hypoglycemia during the follow-up period of the study.

Patient characteristics

One subject in each group had insulin-treated type 2 diabetes, and all other subjects had type 1 diabetes. All patients had received routine annual assessment in a diabetes out-patient clinic within the previous year, and none had evidence of macrovascular disease. Few patients in either group had evidence of diabetic microangiopathy. Subjects were excluded if they had a history of cerebrovascular disease, previous head injury, epilepsy, serious systemic disease, or major psychiatric illness or if they were taking medication that could alter cognitive function, such as psychotropic agents or steroids. The corrected visual acuity of all subjects was 20/30 or better. Hypoglycemia awareness was assessed by using a visual analog scale from 1 to 7 (1 = always aware of the onset of hypoglycemia, 7 = never aware of the onset of hypoglycemia). When using this scale, a score of ≥4 has been associated with impairment of hypoglycemia awareness (27). HbA1c was measured at two time points (t1 and t2) using high-performance liquid chromatography (A. Menarini Diagnostic, Firenze, Italy) based on an ion-exchange reverse-phase partition method (Hi Auto A1c HA 8121). The nondiabetic range for HbA1c measured in our laboratory is 5.0-6.5%.

The clinical characteristics of the subjects are shown in Table 2. The subjects in the two groups were well matched regarding age, sex, BMI, and duration of diabetes. Mean HbA1c concentrations did not change significantly in either group throughout the duration of the study (data not shown), but the hypo subjects had a lower mean HbA1c level and a tendency toward a higher total insulin dose than the control subjects (Table 2). The mean hypoglycemia awareness scores were higher in the hypo group than in the control group (Table 2), and nine subjects in the hypo group had a score of ≥4 on the visual analog scale versus only one patient in the control group (P = 0.003).

Neuropsychological testing

Subjects underwent comprehensive neuropsychological testing at three time points (t1, t2, and t3) during a period of 1 month. To minimize any potential effect of sleep deprivation on the results of the cognitive tasks, all subjects had at least one night of undis-turbed sleep before testing. For the hypo subjects, this involved ensuring that they had obtained a full night of sleep after the episode of severe hypoglycemia, and therefore t1 took place a mean of 1.5 days after t2, t2 took place a mean of 8.9 days after, and t3 took place 30 days after severe hypoglycemia. The control subjects were tested at 306
comparable time intervals (P > 0.05). Blood glucose was measured on three occasions during the cognitive testing sessions (beginning, middle, and end). Subjects were allowed to consume a snack if necessary to ensure that blood glucose concentrations did not fall to <4.0 mmol/L during testing.

The neuropsychological test battery provided a comprehensive assessment of the main cognitive domains, including fluid intelligence, memory, attention and concentration, frontal lobe function, information processing, and mood state. Performance of many of the tests has been shown previously to be impaired during acute hypoglycemia in diabetic and non-diabetic subjects (1,28). The order of the tests remained constant for each session. Where appropriate, subjects underwent a brief practice session with each test before the recorded performance. The cognitive test battery included the following tests:

- Wechsler Adult Intelligence Scale-Revised (WAIS-R) performance intelligence quotient (IQ) subtests (29);
- National Adult Reading Test (NART) (30);
- Forward (FDS) and Backward (BDS) Digit Span (29);
- Trail-Making Test B (TMIB) (31);
- Logical Memory (LM) (immediate and delayed recall) (32);
- Figural Memory (FM) (32);
- Inspection Time (IT) (28);
- Visual Change Detection (VCD) (28);
- Borkowski Verbal Fluency Task (33);
- Paced Auditory Serial Addition Task (PASAT) (34);
- 1-, 2-, 4-, 8-Choice Reaction Time (RT) (35);
- Stroop Task (36);
- University of Wales Institute of Science and Technology (UWIST) Mood Adjective Checklist (37);
- Hospital Anxiety and Depression Scale (HAD) (38); and
- Cognitive Function Self-Appraisal Scale (CFSA) (39).

Many of the performance IQ subtests of the WAIS-R were subject to a practice effect (i.e., performance improved significantly after repeated administrations of the task). Therefore, performance IQ was measured solely at t1; at the subsequent sessions, only the Digit Symbol (DS) and Block Design (BD) tasks were used because these were subject to the least practice effect. The NART is resistant to the effects of organic brain damage and thus provides a measure of "best" global cognitive function ever attained regardless of time (30). The NART was measured during t1 only. An IQ decrement index was calculated by subtracting the present IQ (as measured by the WAIS-R performance IQ subtests) from the premorbid IQ (as estimated by the NART).

**Statistical analyses**

All data are presented as means ± SD. To allow comparison of the effects of severe hypoglycemia on the different cognitive tasks and mood scores, the individual test scores have been standardized in a Z score format such that the mean score for each test is 0 with an SD of 1. Baseline characteristics of subjects were compared using Student's t tests for unpaired samples, and the χ² test was used for categorical data. Differences in individual cognitive test scores between the hypo and control groups at the three time points were compared before standardization using a mixed model analysis of variance (ANOVA) with group (hypo or control) as a between-subjects factor and time (t1, t2, or t3) as a repeated measure. Simple effects (unpaired two-tailed t tests) at particular time points were examined only when significant group and/or interaction effects occurred.

The principal outcome statistic was any group-by-time interaction in the ANOVA. This statistic can indicate evidence of "catch-up" on behalf of the hypo group, which may be hypothesized to underperform at the first and perhaps the second time points. Significant main effects for group indicate the overall superiority of the hypo or control groups on the test. Significant effects of time are expected and indicate practice effects on the tasks.

Where significant simple effects were evident, cognitive and mood outcome scores were adjusted for individual differences in severe hypoglycemia history (with the exception of hypoglycemia scoring system) using univariate linear regression. The group effect on the standardized adjusted scores was subsequently examined using Student's t tests at the appropriate time points. This was an attempt to test the hypothesis that any cognitive impairment was secondary to exposure to previous episodes of severe hypoglycemia.

P < 0.05 was considered to be significant given the exploratory nature of this study. The risk of a type I statistical error could be reduced by applying the Bonferroni correction in which P = 0.05 was divided by the number of comparisons that would provide an overall significance of 0.004.

**RESULTS**

**Baseline cognitive function and IQ decrement index**

No significant difference in mean NART error scores was observed between the hypo subjects (20.9 ± 9.1) and the control subjects (19.9 ± 5.0) (P = 0.65), which indicates that the two groups were well matched regarding premorbid IQ. The actual performance IQ of the hypo subjects, which was measured at t1, was on average 6.8 ± 10.8 points lower than would have been predicted by the NART score (the IQ decrement index). By contrast, the actual performance IQ of the control subjects was 1.7 ± 10.2 points higher than predicted (P = 0.05). Thus, the mean difference in baseline performance IQ between the two groups was 8.5 points. The standard means ± SD of the scores of the remaining cognitive tests, mood scales, and memory questionnaire are shown in Tables 3 and 4.

**Group-by-time interactions on cognitive and mood tasks**

Significant group-by-time interactions were found with the BD (P = 0.044), PASAT 4 second (P = 0.020), and VCD (P = 0.028) tasks. In the cases of the BD and PASAT tasks, the interaction reflected significant differences between the two groups at t1 that were not present at t2 or t3. The interaction for the VCD task represented an apparently anomalous difference between the groups at t2 (with the hypo subjects actually attaining better scores) that was not statistically significant (P = 0.06) and that was not present at t1 or t3. The interactions for the Hedonic Tone (P = 0.012) and Energetic Arousal (P = 0.05) subscales of the UWIST Mood Adjective Checklist approached statistical significance, although none of the simple effects was significant. No other cognitive or mood tests demonstrated a statistically significant group-by-time interaction (P > 0.05).

**Main effects of group on cognitive tests**

The hypo group performed significantly poorer overall on the DS (P = 0.009), PASAT 4 seconds (P = 0.014), FDS (P = 0.014), and BDS (P = 0.021) tasks compared with the control subjects. In addition, the hypo subjects performed more poorly than control
## Table 3—Cognitive test results

<table>
<thead>
<tr>
<th>Test</th>
<th>Time point</th>
<th>Hypo vs control</th>
<th>Group-by-time interaction</th>
<th>Simple effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$t_1$</td>
<td>$t_2$</td>
<td>$t_3$</td>
<td>$t_1$</td>
</tr>
<tr>
<td><strong>DS</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Hypo</td>
<td>-0.75 (0.91)</td>
<td>-0.35 (1.06)</td>
<td>-0.05 (0.95)</td>
<td>0.009</td>
</tr>
<tr>
<td>Control</td>
<td>0.11 (0.80)</td>
<td>0.41 (0.86)</td>
<td>0.64 (0.82)</td>
<td></td>
</tr>
<tr>
<td><strong>BD</strong></td>
<td></td>
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</tr>
<tr>
<td>Hypo</td>
<td>-0.74 (0.85)</td>
<td>-0.03 (1.11)</td>
<td>-0.04 (1.07)</td>
<td>0.060</td>
</tr>
<tr>
<td>Control</td>
<td>0.09 (0.83)</td>
<td>0.22 (0.97)</td>
<td>0.50 (0.80)</td>
<td></td>
</tr>
<tr>
<td><strong>Stroop Task (Incongruent Colors)</strong></td>
<td>1.05 (1.43)</td>
<td>0.14 (0.89)</td>
<td>-0.15 (0.64)</td>
<td>0.007</td>
</tr>
<tr>
<td>Hypo</td>
<td>0.10 (0.60)</td>
<td>0.47 (0.66)</td>
<td>-0.66 (0.57)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>PASAT 4 second</strong></td>
<td></td>
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<tr>
<td>Hypo</td>
<td>-0.79 (1.46)</td>
<td>-0.06 (0.97)</td>
<td>-0.01 (0.87)</td>
<td>0.043</td>
</tr>
<tr>
<td>Control</td>
<td>0.13 (0.81)</td>
<td>0.36 (0.61)</td>
<td>0.36 (0.61)</td>
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</tr>
<tr>
<td><strong>PASAT 2 second</strong></td>
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</tr>
<tr>
<td>Hypo</td>
<td>-0.62 (0.91)</td>
<td>-0.25 (1.04)</td>
<td>0.10 (1.01)</td>
<td>0.073</td>
</tr>
<tr>
<td>Control</td>
<td>-0.15 (0.82)</td>
<td>0.29 (0.95)</td>
<td>0.63 (0.85)</td>
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</tr>
<tr>
<td><strong>TMS</strong></td>
<td></td>
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<tr>
<td>Hypo</td>
<td>-0.19 (0.90)</td>
<td>-0.41 (1.09)</td>
<td>-0.41 (1.04)</td>
<td>0.014</td>
</tr>
<tr>
<td>Control</td>
<td>0.30 (0.97)</td>
<td>0.38 (0.78)</td>
<td>0.33 (0.90)</td>
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<tr>
<td><strong>BOS</strong></td>
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<tr>
<td>Hypo</td>
<td>-0.35 (1.26)</td>
<td>-0.31 (0.79)</td>
<td>-0.28 (0.82)</td>
<td>0.021</td>
</tr>
<tr>
<td>Control</td>
<td>0.14 (0.89)</td>
<td>0.27 (0.90)</td>
<td>0.53 (1.02)</td>
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</tr>
<tr>
<td><strong>Borkowski Verbal Fluency (total)</strong></td>
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<tr>
<td>Hypo</td>
<td>-0.45 (0.92)</td>
<td>-0.25 (0.87)</td>
<td>0.13 (1.06)</td>
<td>0.188</td>
</tr>
<tr>
<td>Control</td>
<td>-0.12 (1.00)</td>
<td>0.11 (0.90)</td>
<td>0.56 (0.96)</td>
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<tr>
<td><strong>TMB</strong></td>
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</tr>
<tr>
<td>Hypo</td>
<td>0.59 (1.52)</td>
<td>0.08 (1.02)</td>
<td>0.09 (1.10)</td>
<td>0.109</td>
</tr>
<tr>
<td>Control</td>
<td>-0.03 (0.63)</td>
<td>-0.25 (0.55)</td>
<td>-0.44 (0.57)</td>
<td></td>
</tr>
<tr>
<td><strong>LM (immediate recall)</strong></td>
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</tr>
<tr>
<td>Hypo</td>
<td>-0.75 (0.86)</td>
<td>0.42 (1.13)</td>
<td>0.01 (0.78)</td>
<td>0.750</td>
</tr>
<tr>
<td>Control</td>
<td>-0.31 (0.88)</td>
<td>0.42 (0.93)</td>
<td>0.00 (1.09)</td>
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</tr>
<tr>
<td><strong>LM (delayed recall)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Hypo</td>
<td>-0.62 (0.96)</td>
<td>0.34 (1.13)</td>
<td>0.07 (0.65)</td>
<td>0.551</td>
</tr>
<tr>
<td>Control</td>
<td>-0.33 (1.19)</td>
<td>0.44 (1.11)</td>
<td>0.14 (0.84)</td>
<td></td>
</tr>
<tr>
<td><strong>FM</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Hypo</td>
<td>-0.03 (0.82)</td>
<td>0.01 (0.92)</td>
<td>0.20 (1.03)</td>
<td>0.627</td>
</tr>
<tr>
<td>Control</td>
<td>-0.35 (1.19)</td>
<td>-0.21 (1.15)</td>
<td>0.39 (0.76)</td>
<td></td>
</tr>
<tr>
<td><strong>IT</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Hypo</td>
<td>0.37 (1.02)</td>
<td>-0.19 (0.65)</td>
<td>-0.30 (0.54)</td>
<td>0.761</td>
</tr>
<tr>
<td>Control</td>
<td>0.15 (1.02)</td>
<td>0.05 (0.93)</td>
<td>-0.08 (1.51)</td>
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<tr>
<td><strong>VCD</strong></td>
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<td></td>
</tr>
<tr>
<td>Hypo</td>
<td>-0.05 (1.06)</td>
<td>0.36 (0.97)</td>
<td>0.13 (0.98)</td>
<td>0.298</td>
</tr>
<tr>
<td>Control</td>
<td>-0.28 (0.89)</td>
<td>-0.22 (1.03)</td>
<td>0.03 (1.04)</td>
<td></td>
</tr>
<tr>
<td><strong>8-Choice RT (Median Decision Time)</strong></td>
<td>0.35 (1.05)</td>
<td>0.04 (1.01)</td>
<td>-0.08 (0.99)</td>
<td>0.647</td>
</tr>
<tr>
<td>Hypo</td>
<td>0.08 (1.06)</td>
<td>-0.16 (0.90)</td>
<td>-0.22 (1.01)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>8-Choice RT (Median Movement Time)</strong></td>
<td>0.31 (1.04)</td>
<td>0.05 (1.3)</td>
<td>-0.01 (1.3)</td>
<td>0.417</td>
</tr>
<tr>
<td>Hypo</td>
<td>0.04 (0.71)</td>
<td>-0.19 (0.82)</td>
<td>-0.21 (0.68)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>CFSA</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Hypo</td>
<td>0.68 (1.03)</td>
<td>0.40 (1.08)</td>
<td>0.23 (1.20)</td>
<td>0.002</td>
</tr>
<tr>
<td>Control</td>
<td>-0.32 (0.99)</td>
<td>0.47 (0.63)</td>
<td>-0.52 (0.69)</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SD of standardized cognitive test scores [2 scores]. *Cognitive test where a lower score indicates better performance, in the remaining tasks, a higher score indicates better performance.
subjects on the Color-Associated Words (data not shown; \( P = 0.014 \)) and Incongruent Colors (\( P = 0.002 \)) subscales of the Stroop Task. Post hoc simple effects analysis demonstrated that performance on the DS and Stroop Task was poorer in the hypo subjects at all three time points (\( P < 0.05 \)), whereas performance on the FDS and BDS was significantly poorer in the hypo subjects only at \( t_1 \) and \( t_2 \) (\( P < 0.05 \)). The main effect of group on the PASAT 2 second (\( P = 0.073 \)) and BD (\( P = 0.066 \)) tasks just failed to reach conventional levels of statistical significance. No significant main effects of group were demonstrated on the information processing (IT, VCD, RT), memory (FM, LM), TMB, and Borkowski Verbal Fluency tasks (\( P > 0.05 \)).

**Main effects of group on mood scales**

Significant main effects of group (hypo vs. control subjects) were observed on the HAD Depression (\( P = 0.011 \)) and HAD Anxiety (\( P = 0.049 \)) scales. The hypo subjects had significantly higher HAD Depression scale scores at all three time points (\( P < 0.05 \)) and significantly higher HAD Anxiety scale scores at \( t_1 \) (\( P = 0.017 \)). No significant group effects were recorded with any of the UWIST mood scores (\( P > 0.05 \)).

**Main effects of group on self-rated cognitive ability**

A significant main effect of group (hypo vs. control subjects) was noted for the CFSA total score (\( P = 0.002 \)), with the hypo subjects having significantly lower levels of self-rated cognitive ability at all three time points (\( P < 0.05 \)). At \( t_1 \), three subjects in the hypo group gave a self-rating on the CFSA change score that suggested that a large deterioration in cognitive ability had occurred with time, eight subjects stated that cognitive ability had declined slightly with time, and nine subjects stated that they had experienced no change. In the control group, 18 subjects indicated that no change in their cognitive ability had occurred with time, and 2 subjects recorded a slight decline (\( P = 0.008 \)). These responses were not significantly different at \( t_1 \) and \( t_2 \) (\( P > 0.05 \)).

**Main effects of time**

All cognitive tasks, with the exception of the FDS (\( P = 0.743 \)) and BDS (\( P = 0.233 \)) tests, showed significant learning effects (as indicated by a statistically significant main effect of time) (\( P < 0.05 \)). The UWIST Energetic Arousal scale and the HAD Depression scale scores did not show a significant effect of time (\( P > 0.05 \)), but a time effect was seen with the UWIST Hedonic Tone scale (\( P = 0.014 \)) and Tense Arousal scale (\( P = 0.006 \)) scores and the HAD Anxiety scores (\( P = 0.001 \)). These scores do not affect the interpretability of the effect of the experimental variable (hypo vs. control subjects) because they apply to both groups.

**Effects of previous episodes of severe hypoglycemia**

After adjusting for severe hypoglycemia history differences in DS, BD, Stroop Task, FDS, BDS, PASAT 4 second, HAD Anxiety, HAD Depression, and CFSA scale scores (at time points where significant simple effects were obtained) became nonsignificant (\( P > 0.05 \)).

**CONCLUSIONS**

To our knowledge, the present study is the first to attempt to evaluate changes in moods and cognitive functions prospectively in patients with insulin-treated diabetes who have experienced a spontaneous episode of acute severe hypoglycemia. In the statistical analysis, we anticipated that a significant interaction between group and time effects may be indicative of a "hangover" effect of severe hypoglycemia on the cognitive parameter measured, with performance improving with time in the hypo subjects more than in the control subjects. Such an interaction was statistically significant in only two cognitive tasks (BD and PASAT 4 second). However, the results for the PASAT 4 second task can be interpreted as showing a ceiling effect, with the performance of the control subjects at \( t_1 \) being very close to maximum with little scope for scores to improve with time.

Therefore, only 1 (BD) of the 14 cognitive tasks showed convincing evidence of a hangover effect, which implies that, in general, any cognitive decrement resulting from the acute episode of severe hypoglycemia had recovered by the time of the initial testing session at 1.5 days. Evidence existed that some aspects of mood may have taken longer to recover because happiness and
energy, as measured by the UWIST scale, increased with time in the hypo subjects. However, although such mood alterations in response to severe hypoglycemia might be expected, the simple effects for the statistical interactions were not significant, and the interpretation that acute severe hypoglycemia has a hangover effect on mood must be made with caution.

The two study groups were well matched regarding standard demographic characteristics and premorbid IQ, but subjects in the hypo group had a previous history of significantly more episodes of severe hypoglycemia. Significant main effects of group (hypo vs. control subjects) were observed with several of the psychometric tests that measure fluid-type intelligence and attention and concentration skills. In addition, the hypo subjects had higher levels on the HAD Depression and HAD Anxiety scales and had higher self-rated levels of cognitive impairment (CFSA), but no significant group effects were observed with the memory or information processing tasks. Several previous cross-sectional studies have suggested that recurrent exposure to episodes of severe hypoglycemia is associated with cumulative cognitive impairment (19-23). In the present study, the differences in cognitive performance and mood between the two groups were abolished when we statistically adjusted for the number of previous episodes of severe hypoglycemia experienced by subjects. In addition, the difference in mean IQ decrement index scores (i.e., the difference in IQ scores as predicted by the NART scale and as measured by the WAIS-R scale) for the hypo and control subjects was 8.5 points. This is comparable with the mean difference in IQ decrement scores of 5.8 points observed by Langan et al. (20) when comparing diabetic subjects who had a history of severe hypoglycemia with those who had no history of severe hypoglycemia. Therefore, the difference in cognitive ability noted in the current study between the hypo and control subjects may possibly have been a consequence of the previous exposure of the former group to many more episodes of severe hypoglycemia. However, the study did not have power to investigate this issue specifically, and the statistical adjustment for the effects of exposure to previous severe hypoglycemia should be interpreted with caution because severe hypoglycemia was itself a significant variable between the study groups. The small sample size precluded a sufficiently powerful correlational analysis between cognitive performance and previous severe hypoglycemia history in the hypo subjects alone.

The elevated anxiety and depression scores recorded in the hypo subjects have been noted previously in diabetic patients exposed to recurrent severe hypoglycemia (40,41) and may be either a consequence or a cause of the previous severe hypoglycemia. Unfortunately, performing a surrogate retrospective assessment of mood in a similar manner to the use of the NART for intellectual ability is not possible. Gonder-Frederick and Cox (42) prospectively measured symptoms of depression in 20 subjects who experienced two or more episodes of severe hypoglycemia during a 6-month period and in 20 control subjects who experienced no severe hypoglycemia during that time. Depression scores (as assessed by the Beck Depression Inventory) were comparable between the groups at baseline but at 6 months were significantly higher in the subjects exposed to severe hypoglycemia (42).

Most of the cognitive tasks showed a practice effect, despite the fact that subjects underwent training in the individual cognitive tasks before the formal test performance. This highlights the importance of a parallel longitudinal control group in studies of this type. The UWIST Tense Arousal and Hedonic Tone and the HAD Anxiety scale scores also showed a significant main effect of time, with levels of tense arousal and anxiety decreasing with time and levels of hedonic tone or happiness increasing with time. This effect is likely to reflect the natural initial apprehension of subjects regarding participation in a clinical study.

This study has several limitations. These include the relatively small sample size, the absence of prehypoglycemia cognitive and mood data, and the need for multiple statistical testing. The small sample size means that a small hangover effect of severe hypoglycemia on cognitive function may not have been detected and precluded meaningful analysis to determine whether large individual differences existed regarding the effect of severe hypoglycemia. Major difficulty was encountered in recruiting insulin-treated diabetic patients after severe hypoglycemia, despite the high frequency of this problem in clinical practice. Most episodes of severe hypoglycemia are treated effectively in the community (43), and only a small selected group requires hospitalization (44). An attempt to improve recruitment by advising the patients attending our clinic and all local general practitioners in advance that the study was ongoing was unsuccessful. In addition, as observed previously in a local survey of acute severe hypoglycemia (44), recruitment was hampered by the fact that many of the diabetic patients attending the hospital for emergency treatment were elderly, had concurrent medical disorders, or were infrequent attendees at the diabetic clinic. Furthermore, many patients who were eligible for inclusion were unable to participate in the study because of the short notice or the time constraints necessitated by the study design. The collection of cognitive and mood data on the hypo subjects before the index episode of severe hypoglycemia would have necessitated testing a large number of diabetic subjects and a relatively long period of follow-up to identify subsequent hypoglycemic episodes. Future studies could reduce the amount of subjects who would undergo cognitive (pro)testing by studying subjects who are at high risk for severe hypoglycemia (e.g., patients who have had an episode of severe hypoglycemia in the preceding year or who have impaired hypoglycemia awareness). The large number of statistical tests that were used in this study increases the risk of type 1 statistical errors. At the outset of the study, the specific areas of cognitive function and mood that may exhibit a hangover effect were not clear, and clearly a balance had to be struck between testing a wide number of cognitive functions and making type 1 errors versus including a limited number of cognitive measures and potentially missing an effect in a cognitive domain not tested. Given the exploratory and preliminary nature of the present study, the former approach (using a conventional level of statistical significance) was adopted, and an illustrative Bonferroni correction has been included.

In conclusion, these results imply that acute severe hypoglycemia does not have a prolonged effect on cognitive functions, and patients with insulin-treated diabetes may be reassured that performance of various tasks, such as work or driving is not likely to be impaired 36 h after an episode. To determine more precisely at what time cognitive function recovers after severe hypoglycemia would require further studies within the period of 1.5 days. However, such investigations would pose considerable ethical and practical difficulties. The persistent impaired cognitive performance and mood disturbance demonstrated by the hypo sub-
jects may be a long-term consequence of repeated exposure to previous episodes of severe hypoglycemia.

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Cognition after severe hypoglycemia

Acute hypoglycemia impairs the functioning of the central but not peripheral nervous system

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Abstract

Acute hypoglycemia impairs functions of the central nervous system, but few controlled studies have assessed the impact of hypoglycemia on the function of the peripheral nervous system. Sixteen non-diabetic humans underwent two separate hyperinsulinemic glucose clamp procedures on different study days, in a counter-balanced fashion. On one occasion, euglycemia was maintained (blood glucose, 5.0 mmol l⁻¹), and on the other occasion, hypoglycemia (blood glucose, 2.6 mmol l⁻¹) was induced. During each condition, subjects performed a combined psychometric, cognitive-experimental and psychophysical test battery, and measures were made (in the dominant median and common peroneal nerves) of the motor nerve conduction velocities and the amplitudes of the motor action potentials. Hypoglycemia caused impaired performance of general cognitive and information processing tasks (P < .05), but nerve conduction velocities and the amplitudes of motor action potentials were unaffected. Conduction velocities of the common peroneal nerve decreased from baseline within each experimental condition, perhaps due to hyperinsulinemia. Overall, these results demonstrate that multiple levels of information processing in the brain may alter while peripheral nerve function remains intact, and imply that peripheral neurons do not have the same obligate requirement for glucose as a metabolic fuel as neurons of the central nervous system. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Hypoglycemia; Cognition; Nerve conduction; Information processing

1. Introduction

Acute hypoglycemia impairs function of the central nervous system in humans. Controlled studies, with experimental hypoglycemia induced in a laboratory setting, have demonstrated that a wide range of mental functions deteriorate when arterialized venous blood glucose concentrations decline to between 2.8 and 2.4 mmol l⁻¹ [1]. Cognitive functions that deteriorate include simple and choice reaction times, speed of arithmetical calculation, verbal fluency, color identification, trail-making and digit symbol test performance, digit span and other short-term memory indices [1]. Considerably less attention has focused on the effects of hypoglycemia on the peripheral nervous system, but limited evidence in humans supports the existence of a putative 'hypoglycemic neuropathy' [2]. Several studies in diabetic and non-diabetic rats have demonstrated that hypoglycemia (blood glucose below 2.5 mmol l⁻¹) of prolonged duration (at least 12 h) results in axonal degeneration of peripheral nerves with consequent reduction of nerve conduction velocities and evoked muscle action potentials [3–5]. In the spontaneously diabetic BB rat, prolonged hypoglycemia (blood glucose less than 3.0 mmol l⁻¹ for 6 days) is associated with the development of a motor peripheral neuropathy characterized by loss of anterior horn motor neurons, loss of large myelinated fibers and Wallerian degeneration [6]. In humans, peripheral neuropathy is a rare, but recognized, complication of insulinoma (a tumor associated with chronic hypoglycemia) [7–9]. The characteristic findings are those of a predominantly motor peripheral neuropathy, which is distal and symmetrical. Upper limb involvement is generally more frequent and severe, and neurophysiological studies suggest
that the axon is the primary site of damage [7]. Following resection of the insulinoma, weakness improves and sensory symptoms may resolve completely [7]. Earlier observations of psychiatric patients undergoing insulin coma therapy suggested that peripheral sensory disturbances and, in some instances, sensory loss followed prolonged insulin-induced hypoglycaemia [10—12]. In addition, anecdotal reports exist of peripheral neuropathy being precipitated by the onset of strict glycemic control in diabetic patients [13], in whom recurrent episodes of moderate hypoglycaemia are common.

Few data are available on the effects of more moderate degrees and durations of hypoglycaemia on peripheral nerve function. Tamburrano et al. [14] did not identify any change in median nerve sensory conduction velocity in six non-diabetic human subjects in whom mean blood glucose was maintained at 2.4 mmol l$^{-1}$ for 60 min using a glucose clamp technique. However, nerve conduction studies were not performed on nerves in the lower limb, and motor nerve conduction velocities were not measured. In a different study, acute hypoglycaemia (blood glucose, 2.4 mmol l$^{-1}$) had no effect on median sensory nerve conduction velocities in seven patients with insulinoma [15]. However, no information was provided on how, or if, hypoglycaemia was avoided in the period leading up to the experimental studies, the order of the study sessions (euglycaemia vs. hypoglycaemia) was not randomized and, in the hypoglycaemia studies, the neurophysiological readings were performed immediately after the blood glucose nadir was reached so that insufficient time may have elapsed for significant alterations in peripheral nerve function to have occurred.

Moderate hypoglycaemia is known to have significant impact on functions of the central nervous system, and any differential effect of hypoglycaemia on the peripheral nervous system may offer insights into the metabolic requirements of central and peripheral neurons. The primary aim of the present study was, therefore, to consider the impact of 1 h of hypoglycaemia (arterialized blood glucose, 2.6 mmol l$^{-1}$) on the motor function of the median and common peroneal nerves in non-diabetic humans.

A secondary aim of the investigation was, within a single study, to examine comprehensively the effects of controlled hypoglycaemia on different levels of human information processing. In a reductionist scheme, from the most complex to the simplest level, efficiency of information processing in humans can be assessed using psychometric tests (e.g. the processing speed test of the British Ability Scales), cognitive-experimental tasks (e.g. choice reaction time procedures), psychophysical tests (e.g. backward masking procedures such as visual inspection time (IT) [16]) and physiological tests (such as nerve conduction velocity [17]). Speed of processing at all the above levels of description is related in normal humans to mental ability as measured using psychometric (IQ type) tests [18]. By testing all such levels during hypoglycaemia in the same group of subjects, the lower level processes that may contribute to the decrements in higher-level mental performance can be investigated.

2. Methods

2.1. Subjects

Sixteen non-diabetic human subjects (eight males) were recruited from the staff at the Royal Infirmary of Edinburgh. None had any relevant previous medical history or family history of diabetes, and none were taking regular medication (other than the oral contraceptive pill). All subjects had a corrected visual acuity of 6/6 or better, as measured with a Snellen chart. The mean age (±S.D.) was 31.1 (±8.3) years, and the mean body mass index (±S.D.) was 23.1 (±2.2) kg m$^{-2}$. All of the subjects had above average intellectual ability as assessed by the National Adult Reading Test (NART) [19] and the Alice Heim 4 (AH4) Test [20]. The mean (±S.D.) NART error score for 15 of the 16 subjects was 15.7 (±5.6), which predicted a mean (±S.D.) intelligence quotient (IQ) of 111.2 (±6.9); the NART was not administered to one of the subjects whose first language was not English. The mean (±S.D.) AH4 score for all 16 subjects was 98.8 (±17.0). Written informed consent was obtained from all subjects and the study was approved by the local medical ethics advisory committee.

2.2. Experimental procedure

Subjects attended three study sessions, each separated by at least 2 weeks. The purpose of the initial visit was to familiarize subjects with the tests that would be used in the experimental sessions, so as to reduce the effects of practice on test results. The results from this session were discarded. In the following two study sessions, the participants underwent the experimental procedures during conditions of either hypoglycaemia or euglycaemia (Fig. 1). The subjects were not informed which condition was being studied at each visit. The subjects completed both experimental conditions in a counterbalanced fashion, i.e. half of the subjects underwent the euglycaemia study first followed by the hypoglycaemia study and the other half underwent the studies in the reverse order.

During the two experimental visits, subjects underwent a glucose clamp procedure. Following a light breakfast at 07:00 hours, subjects were asked to go to the department at midday. A Teflon cannula was inserted into an ante-cubital vein in the subject's non-dominant arm under local anaesthetic (1% lignocaine). This cannula was used to infuse human soluble insulin (Actrapid, Novo Nordisk Pharmaceuticals, Crawley, UK) and 10% dextrose. A second cannula was inserted in a retrograde direction into a vein on the dorsum of the same hand, which was then placed in a heated box (Plexiglas) at 60°C to arterialize the venous
blood. Arterialized blood samples were obtained throughout the study for the measurement of whole blood glucose at the bedside using a glucose oxidase method (Yellow Springs Instrument 2300 Stat, Yellow Springs, OH, USA). To maintain patency, both cannulae were flushed frequently with heparinized saline.

A modified hyperinsulinemic glucose clamp technique was used to maintain the blood glucose at predetermined levels [21]. After a brief priming regimen, insulin was infused at a steady rate (based on whole-body surface area) of 60 mU m⁻² min⁻¹ using an IMED Gemini PCI pump; 10% dextrose was infused, using an IVAC Site Saver pump, at a variable rate according to the blood glucose value. Arterialized blood glucose was initially measured every 3 min, until a stable level had been achieved, and then at 5-min intervals. Counter-regulatory hormone concentrations were not estimated.

At each laboratory session, the arterialized blood glucose concentration was initially stabilized at 5.0 mmol l⁻¹ for a period of 1 h during which baseline peripheral nerve conduction velocities were measured and the subject completed the mental test battery (baseline, Fig. 1). Following this, the blood glucose concentration was either maintained at 5.0 mmol l⁻¹ (euglycemia, Fig. 1) or lowered to 2.6 mmol l⁻¹ (hypoglycemia, Fig. 1) and maintained at this level for 1 h, during which peripheral nerve conduction velocities were measured every 30 min and the neuropsychological test battery was repeated. The subjects were not informed about their blood glucose concentration during any phase of the study. A period of 20 min was allowed to elapse between the baseline and the attainment of euglycemia or hypoglycemia to allow the blood glucose concentration to stabilize. The target glucose concentration was maintained for a further 10 min before the tests were administered.

2.3. Nerve conduction studies

Motor nerve conduction studies were performed by the same investigator on the dominant-side median and common peroneal nerves using a Medelec SapphireII 2ME electromyograph (Medelec, Old Woking, Surrey, UK). Skin surface electrodes were utilized and these remained in position during the course of each experimental study. Motor nerves were stimulated orthodromically with supra-maximal electrical signals; the sites for nerve stimulation were marked at the outset of each study session and remained constant thereafter. Limb temperature was kept constant at 34°C using a DISA thermostatically-regulated heater (DISA, Bristol, UK). Recordings were made of the median and common peroneal nerve motor conduction velocities (ms⁻¹) and of the amplitudes of the motor action potentials (mV). The ratio of the amplitudes of the motor action potentials after distal and proximal stimulations was also calculated as an index of acute conduction block. If acute conduction block occurred, then this would give a distal to proximal amplitude ratio of greater than 1.00. Measurements were made once during the baseline, at the beginning (T0) of the study session (hypoglycemia or euglycemia) and after 30 (T30) and 60 (T60) min.

2.4. Cognitive test battery

A combined psychometric, cognitive-experimental and psychophysical test battery was applied during the baseline and experimental phases of the euglycemia and hypoglycemia conditions. The order of the tests remained the same during each phase of the visit, and the battery was interrupted only to allow measurement of peripheral nerve conduction velocities at prerequisite time points. Symptoms
of hypoglycemia were also measured using the Edinburgh Hypoglycaemia Scale (EHS) [22].

2.4.1. Tests of general cognitive ability

Two complex psychometric tests were used to assess general, high-level cognitive function. In many studies, these have proved sensitive to moderate hypoglycemia:

2.4.1.1. Digit symbol task (DS). This is a subtest of the Wechsler Adult Intelligence Scale-revised (WAIS-R) [23]. In the task, nine digits were represented by nine different symbols, and subjects were required to write down the appropriate symbol for each in a given array of numbers for over 90 s. The number of correct responses was recorded.

2.4.1.2. Trail-making test B (TMB). This is a test from the Halstead Reitan Neuropsychological Battery [24]. The subject had to connect correctly an alternating series of numbers (1–13) and letters (A–L) in their respective orders as quickly as possible. The time taken (in seconds) to complete the task correctly was recorded.

2.4.2. Tests of information processing

Three tasks were employed to assess the efficiency of information processing at the psychometric, cognitive-experimental and psychophysical levels. Nerve conduction assessed a still more basic level.

2.4.2.1. Speed of information processing (SIP). This is a subtest of the British Ability Scales [25]. Subjects were presented with a card on which 25 rows of five numbers were printed. The complexity of the numbers increased from the top to the bottom of the page. Subjects were asked to circle the highest number on each row as quickly as possible. Two cards were presented to each subject: one containing ‘easy’ numbers, which were one to four digits in length, and the other containing ‘difficult’ numbers, which were three to six digits in length. For each card, ‘easy’ and ‘difficult’, the time taken to complete the task was recorded, as was the number of errors made.

2.4.2.2. Reaction time (RT). Information processing was measured at the cognitive-experimental level using a Hick-type reaction time device [26]. The device, by employing a ‘home’ button and two clocks, allows the separation of the decision and movement components of reaction times. Measures were made of four-choice reaction time. Forty trials were attempted and the median decision time (DT) and median movement time (MT) were recorded in milliseconds. DT represents the time interval between the stimulus light onset and the subject lifting his/her hand off the ‘home’ button. MT represents the time interval between the subject lifting his/her hand off the ‘home’ button and striking the stimulus light button.

2.4.2.3. Inspection time. This is a two-alternative, forced-choice discrimination task that utilizes an LED screen and assesses the efficiency of the early stages of visual information processing using psychophysical procedures. The format and theoretical basis of the test have been described in detail [16,27], but, in brief, subjects have to indicate which of two parallel vertical lines, of markedly different lengths, is longer. During the test, the experimenter varies the stimulus duration, with shorter durations being more difficult. Only correctness of response is recorded; response speed was not measured, and the subject is instructed to respond at leisure to achieve maximum accuracy. The IT test’s adaptive staircase algorithm measures the stimulus presentation time (in milliseconds) required by each subject to achieve 85% accuracy in responding (50% representing chance responding).

2.5. Edinburgh Hypoglycaemia Scale

This is a subjective self-rating system, using a Likert scale, [22] in which subjects are presented with a list of symptoms of hypoglycemia and are asked to score the severity of each symptom from 1 (not present) to 7 (very intense). Symptoms scores were summed and classified into three groups: autonomic (e.g. sweating, tremor, hunger), neuroglycopenic (e.g. confusion, drowsiness, inability to concentrate) and nonspecific (nausea and headache).

2.6. Statistical analysis

All data are presented as means±standard deviation (S.D.). Each measure of cognitive and peripheral nerve function was analyzed independently.

The effects of acute hypoglycemia on symptom scores and cognitive performance were assessed using a mixed-model analysis of variance (ANOVA). 'Order of session' was used as a between subjects factor with two levels

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Fig. 2. Mean blood glucose concentrations (±S.D.) during the baseline and study conditions of both the euglycemic (●) and hypoglycemic (■) glucose clamps. There was no difference in blood glucose concentrations during the baseline conditions. Blood glucose concentration was approximately 5.0 mmol l⁻¹ during the euglycemia experimental condition and 2.6 mmol l⁻¹ during the hypoglycemia experimental condition.
Table 1
Results of cognitive function tests and symptom scores during euglycemia and hypoglycemia

<table>
<thead>
<tr>
<th></th>
<th>Euglycemia</th>
<th>Hypoglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Study</td>
</tr>
<tr>
<td>Digit symbol (score)</td>
<td>72.5 ± 10.8</td>
<td>77.2 ± 10.2</td>
</tr>
<tr>
<td>Trail-making B (s)</td>
<td>26.5 ± 10.1</td>
<td>24.6 ± 8.0</td>
</tr>
</tbody>
</table>

**Speed of information processing**

| Easy numbers (s)     | 48.1 ± 8.2 | 49.1 ± 9.5   | 51.4 ± 14.8 | 57.6 ± 16.8 | 3.4   | .085          |
| Easy errors (no.)    | 1.0 ± 1.5  | 1.2 ± 1.3    | 0.9 ± 1.7   | 1.1 ± 1.2   | 0.3   | .873          |
| Difficult numbers (s)| 69.7 ± 12.3| 65.6 ± 13.0  | 72.5 ± 18.4 | 83.6 ± 20.8 | 13.1  | .003          |
| Difficult errors (no.)| 1.7 ± 1.8 | 2.1 ± 1.8    | 1.4 ± 1.3   | 1.9 ± 2.5   | 0.1   | .726          |

**Four-choice reaction time**

| DT (ms)               | 300.9 ± 22.1| 298.5 ± 20.8 | 304.4 ± 30.1| 328.9 ± 46.5| 7.0   | .019          |
| MT (ms)               | 115.1 ± 26.5| 109.2 ± 24.4 | 117.4 ± 24.5| 128.9 ± 34.3| 9.6   | .008          |
| IT (ms)               | 47.6 ± 19.1 | 47.3 ± 16.9  | 50.2 ± 20.3 | 63.4 ± 24.1 | 16.0  | .001          |

**Symptom scores**

| Autonomic            | 10.3 ± 2.0  | 10.4 ± 1.5   | 11.1 ± 2.5  | 21.1 ± 7.6  | 17.1  | .001          |
| Neuroglycopenic      | 10.4 ± 1.5  | 10.8 ± 3.3   | 10.4 ± 1.3  | 19.9 ± 12.0 | 8.0   | .013          |
| Malaise              | 2.0 ± 0.0   | 2.1 ± 0.3    | 2.4 ± 1.0   | 2.5 ± 1.1   | 0.0   | 1.000         |

Results are presented from the baseline and study conditions of the euglycemia and hypoglycemia study days (see Fig. 1). Values are means ± S.D. $^*$ and $P$ values refer to the interaction of "Time" vs. "Experimental condition" in ANOVA, i.e. they indicate, with reference to baseline scores, whether hypoglycemia leads to a deterioration in outcomes by comparison with euglycemia.

In the digit symbol task, a higher score represents better performance; in all other cognitive tasks, a lower score indicates better performance.

Higher symptom scores represent greater levels of hypoglycemia symptomatology.

A $P$ value of $<.05$ was considered to be significant. All analyses were performed using SPSS version 7.5.1 for Windows 95.

3. Results

Stable glycemic plateaus were achieved for every subject in each condition of the study (Fig. 2). The whole blood glucose (mean ± S.D.) during the baseline euglycemic clamps was 4.95 ± 0.15 mmol l$^{-1}$. During the experimental conditions of euglycemia and hypoglycemia, blood glucose values were 5.03 ± 0.07 and 2.63 ± 0.08 mmol l$^{-1}$, respec-

![Fig. 3. Mean motor nerve conduction velocities (± S.D.) in dominant-side median nerves at four time points — B (baseline), T0, T30, T60 (T = time; 0, 30 and 60 min). (●) Data from the euglycemia study day; (■) Data from the hypoglycemia study day. Moderate hypoglycemia had no significant effect on nerve conduction velocities.](image-url)
acute hypoglycemia (P < .05; Table 1), but the time taken to complete the 'easy' task failed to reach statistical significance (P = .09). There was no change in the number of errors made, which was low in each subtest. Moderate hypoglycemia also resulted in a deterioration in performance of the IT task and in the DT and MT components of the four choice reaction time (all P < .05; Table 1).

3.4. Nerve conduction studies

3.4.1. Effects of interaction of 'Experimental condition' and 'Time' on nerve conduction parameters

Acute hypoglycemia had no significant effect on the motor nerve conduction velocities of the median and common peroneal nerves (Figs. 3 and 4). Similarly, acute hypoglycemia did not alter the amplitudes of the evoked action potentials of either nerve after proximal or distal stimulation, nor did it affect the ratio of the distal to proximal amplitudes (Table 2).

3.4.2. Main effects of 'Experimental condition' on nerve conduction parameters

The amplitudes of median nerve evoked potentials after proximal stimulation were smaller during the hypoglycemia study day than the euglycemia study day (P = .02), and there was also a nonsignificant trend for the amplitudes after distal stimulation to be smaller during the hypoglycemia study day (P = .11). There were no significant main effects of 'Experimental condition' on median nerve conduction velocities or on any of the common peroneal nerve conduction parameters.

3.4.3. Main effects of 'Time' on nerve conduction parameters

There was a significant main effect of 'Time' on common peroneal nerve conduction velocities (P = .00), i.e. nerve conduction velocities in both experimental conditions declined with time within the experimental sessions. No

Table 2

Conduction amplitudes in median and common peroneal motor nerve after proximal and distal stimulation

<table>
<thead>
<tr>
<th></th>
<th>Euglycemia</th>
<th>Hypoglycemia</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>T0</td>
</tr>
<tr>
<td><strong>Median nerve amplitudes (mV)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>7.3±1.4</td>
<td>7.7±1.6</td>
</tr>
<tr>
<td>Distal</td>
<td>8.6±2.3</td>
<td>8.2±3.2</td>
</tr>
<tr>
<td>Ratio (D/P)</td>
<td>1.4±0.8</td>
<td>1.2±0.6</td>
</tr>
<tr>
<td><strong>Peroneal nerve amplitudes (mV)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>2.4±1.0</td>
<td>2.9±1.7</td>
</tr>
<tr>
<td>Distal</td>
<td>2.7±1.8</td>
<td>3.1±2.3</td>
</tr>
<tr>
<td>Ratio (D/P)</td>
<td>1.1±0.6</td>
<td>1.0±0.6</td>
</tr>
</tbody>
</table>

Results are presented from the baseline and study conditions of the euglycemia and hypoglycemia study days (see Fig. 1). Values are means ± S.D. The ratio of distal to proximal amplitudes is a measure of acute conduction block, which if present would be expected to result in a ratio of greater than 1.0.

* F and P values refer to the interaction of 'Time' vs. 'Experimental condition' in ANOVA, i.e. they indicate, with reference to baseline scores, whether hypoglycemia leads to a deterioration in outcomes by comparison with euglycemia.
main effect of 'Time' was observed on the common peroneal amplitude data, or on any of the median nerve neurophysiological parameters.

In summary, levels of information processing from psychometric, through cognitive to psychophysical were impaired during hypoglycemia, while nerve conduction velocities were unaffected.

4. Discussion

Neurons of the central nervous system are exquisitely sensitive to the prevailing blood glucose concentration, with even moderate hypoglycemia resulting in significant impairment of neuronal function, as evidenced by the development of cognitive dysfunction [1]. Experimental studies in animals [3–6] and anecdotal reports in humans [7–12] have also suggested that profound and protracted hypoglycemia adversely affects peripheral nerve function, and may precipitate the development of a peripheral neuropathy. The effects of more moderate degrees and durations of hypoglycemia on peripheral nerve function have not been definitively ascertained.

Previous studies demonstrated that acute moderate hypoglycemia had no effect on median nerve sensory function in both non-diabetic subjects [14] and in patients with insulinoma [15]. However, these studies had significant methodological limitations, which were described in the Introduction, and which included very small sample sizes. The present study has supported and extended these earlier observations by demonstrating that acute hypoglycemia had no effect on the motor conduction velocities of the dominant-side median and common peroneal nerves. The amplitudes of the action potentials were similarly unaffected, and there was no evidence of acute conduction block. The differences observed between the median nerve evoked potential amplitudes measured on the euglycemic and hypoglycemic study days (which included the baseline euglycemic measurements during both days) do not afford an obvious physiological explanation and might represent a chance occurrence (i.e. a Type 1 statistical error). The tendency for the ratio of median nerve distal to proximal amplitudes to be greater than 1.00 was predictable [28], since proximal stimulation may give rise to evoked potentials that are of slightly longer duration and lower amplitude than those observed following distal stimulation, because the impulses of slow-conducting fibers lag behind those of fast-conducting fibers over a long conduction path.

4.1. Methodological considerations

It is reasonable to speculate whether any methodological issues such as sample size, the depth of hypoglycemia attained, the nature of the nerves chosen for neurophysiological study or the nerve conduction studies themselves could have accounted for this null result.

The present study had ample power to detect differences in cognitive function during acute hypoglycemia. Moreover, because subjects acted as their own controls the sample size provided approximately 80% power to detect a 0.75 S.D. change in median nerve conduction velocities with a P value set at .05. Median and common peroneal nerve conduction velocities did appear to be consistently 1.0–1.5 m/s slower during hypoglycemia compared with euglycemia, but these differences were highly nonsignificant. It is just conceivable that these differences could have become statistically significant in a larger study, but the magnitude of such a difference would be of minimal clinical relevance.

An arterialized blood glucose concentration of 2.6 mmol 1 −1 was chosen for the hypoglycemia phase of the investigations as numerous previous studies have demonstrated that this blood glucose level is associated with significant impairment of cognitive functions [1]. Therefore, if peripheral nerves had a similar reliance on glucose as a metabolic fuel as central nervous system neurons, then this level of hypoglycemia should have been sufficient to demonstrate a decrement in function. Lower blood glucose concentrations are associated with increased physical discomfort for subjects, and it was not ethically justifiable to induce more profound hypoglycemia.

The common peroneal and median nerves were chosen for several reasons. Both nerves are readily accessible for performing neurophysiological studies, and their stimulation causes minimal discomfort to subjects. Peripheral neuropathies tend to affect longer nerves rather than shorter nerves, therefore, the common peroneal nerve was chosen on the theoretical basis that its long length may have made it more susceptible to the effects of moderate hypoglycemia. The median nerve was chosen since animal and human studies suggested that ‘hypoglycemic neuropathy’ affects predominantly upper limb nerves. Sensory neural function was not assessed in this study on the basis that ‘hypoglycemic neuropathy’ primarily affects motor nerves and two previous studies, albeit of limited methodological quality, had previously shown no susceptibility of peripheral sensory nerves to acute hypoglycemia [14,15]. In addition, the measurement of sensory nerve function causes considerably more discomfort to subjects and is open to greater technical errors because the amplitudes of the evoked potentials are considerably smaller (leading to a reduced signal-to-noise ratio) [28]. There was a limited amount of time available in the context of each clamp study to perform nerve conduction studies, and it was felt that this would best be utilized measuring motor nerve function.

In current clinical practice, nerve conduction studies, and, in particular, the measurement of nerve conduction velocities and the amplitudes of the evoked potentials, represent the most effective methodology for assessing the functions of peripheral nerves [28]. It is possible to measure other electrophysiological parameters, such as F
waves (which measure conduction time from the peripheral nerve to the spinal cord and back) and nerve refractory period (i.e. the shortest time interval between two sequential nerve stimuli that generate two sequential evoked potentials). It was not felt that the measurement of these parameters would have added considerably to the assessment of peripheral neural function. In particular, the measurement of refractory period is a time-consuming process and in a previous pilot study of four male Sprague-Dawley rats, acute hyperglycemia (arterial blood glucose nadir of 1.66 mmol L$^{-1}$ induced by insulin infusion) had no effect on the refractory period of the right femoral nerve [29].

The foregoing paragraphs, therefore, suggest that methodological issues do not account for the results of the present study and that the inability to detect any significant change in the neurophysiological parameters of peripheral nerves during hyperglycemia indicates resistance of peripheral neural function to neuroglycopenia.

4.2. Main effects of ‘Time’ on common peroneal nerve conduction velocities

The significant change in common peroneal nerve conduction velocities that occurred with time during the hyperglycemia and euglycemia studies was unexpected and was not observed in any of the other peripheral nerve neurophysiological measurements. Tamburrano et al. [14] did not report any impact of hyperinsulinemic euglycemia on median sensory nerve conduction velocities in five non-diabetic subjects, and in a later study on nerve conduction parameters in patients with insulinoma no time course data was provided [15]. While this observation could have resulted by chance, it should be noted that nerve conduction velocities are generally regarded as being highly reproducible within individual subjects (in contrast to amplitude measurements) [30]. It is, therefore, tempting to speculate that hyperinsulinemia per se affected the common peroneal nerve conduction velocities, although the reason for a lack of effect on median nerve conduction velocities would require explanation. One possibility would be that the greater length of the common peroneal nerve may have made it more susceptible to metabolic perturbation. The institution of intensive insulin therapy in diabetic patients with poor glycemic control can promote the development of a peripheral neuropathy [13], but, of course, in observational studies such as this, and others that have looked at ‘hyperglycemic neuropathy’, it is not possible to separate a potential direct toxic effect of insulin itself from its hyperglycemic actions. Greene et al. [31], however, reported no direct toxic effects of insulin on isolated endothelial preparations of rat peripheral nerve. Therefore, the observation that common peroneal motor nerve conduction velocities changed with time and the speculation that this may be related to hyperinsulinemia must be re-assessed in future appropriately controlled studies.

4.3. Symptoms of hypoglycemia

Acute hypoglycemia predictably caused an increase in autonomic and neuroglycopenic symptoms of hypoglycemia, but did not affect the ‘nonspecific’ symptoms of nausea and malaise. The insensitivity of the ‘nonspecific’ symptoms to hypoglycemia has been identified in other studies [27,32]. The three-factor model of hypoglycemic symptoms, which is utilized in the EHS, is based on a multifactor confirmatory factor analysis of symptoms of hypoglycemia that were retrospectively recalled by two large groups of diabetic subjects [33]. Therefore, the relative insensitivity of the ‘nonspecific’ symptoms, in the present and previous studies, suggests that this model may not be appropriate for symptoms of hypoglycemia induced during hyperinsulinemic clamp studies.

4.4. Implications for understanding human intelligence differences

By contrast to its effects on the peripheral nervous system, acute hyperglycemia caused significant disruption of central neural function as evidenced by impaired performance in the majority of tasks in the cognitive test battery. The time taken to complete the ‘easy’ subtest of the SIP task just failed to reach statistical significance, but moderate hypoglycemia had no significant effect on the number of errors made in either the ‘easy’ or the ‘difficult’ subtests of this task. These findings are consistent with previous studies suggesting that hypoglycemia causes greater impairment of complex cognitive tasks than simple tasks [1,27,32], and that accuracy of performance of a given task tends to be preserved at the expense of speed [34,35].

A significant correlation of low effect size ($r<.2$) exists between peripheral nerve conduction velocity and psychometric intelligence test scores [36]. It has been hypothesized that this association might explain some of the covariation found between mental ability differences and speed-of-information-processing indices such as reaction times [37] and IT [38], i.e. that more intelligent people have faster central neuronal functioning and that peripheral nerve conduction velocities may be a surrogate marker of this. A partial refutation of this hypothesis arises from studies that have found no reduction in correlations between psychometric intelligence and reaction times after statistically controlling for nerve conduction velocity differences (using partial correlation) [39,40]. The present study adds further evidence that may be interpreted as a refutation of the premise that differences in peripheral nerve conduction velocity form a partial basis for the association between psychometric intelligence and measures of information processing efficiency. Moderate hypoglycemia impaired psychometric ability test scores, reaction times and visual information processing, but peripheral nerve conduction velocity was unaffected. Of course, it remains perfectly feasible that speed of neural conduction within the central
nervous system could still relate to speed of information processing and intelligence, but further studies are clearly required to investigate this in more detail.

4.5. Explaining the differential sensitivity of the central and peripheral nervous systems to acute hypoglycemia

The marked disparity in the effects of acute hypoglycemia on peripheral and central neural system function implies that peripheral nerves do not have the same reliance on prevailing blood glucose concentrations as is demonstrated by neurons of the central nervous system. Neural components of peripheral nerves are separated from plasma and general extracellular fluid by endoneural capillaries and the perineural membrane, which form a ‘blood–nerve’ barrier that has similar properties to the blood–brain barrier in preventing the passive transfer of large molecules [41,42]. As with neurons in the central nervous system, glucose is the major substrate for energy production in peripheral neural tissue, when plasma concentrations of ketone bodies are not elevated [31]. Incubation of isolated nerve fascicles in a glucose-deficient medium produces a profound fall in glucose in nerve tissue, with reduced oxygen uptake and decrements in neural oxygen and ATP levels [31]. Glucose utilization in peripheral nerves does not appear to be regulated directly by insulin [31], which is also the case with neurons in the central nervous system. Peripheral neurons can, however, adapt to prolonged exposure to a low blood glucose. This was demonstrated by an elegant study of non-diabetic rats in which the anterograde fast component of axonal transport in peripheral neural tissue was reduced by 36% following the induction of acute hypoglycemia (blood glucose ~1.5 mmol l⁻¹ for 2 h) [43]. The reduction in axonal transport was prevented, however, by pretreatment of the animals for 3 days with insulin to induce prolonged moderate hypoglycemia [43]. The mechanisms behind this adaptive response to hypoglycemia are not clear, but it is also recognized that the brains of rats adapt gradually to chronic hypoglycemia over a period of days, by up-regulation of the GLUT 1 blood–brain barrier glucose transport protein [44]. This acts to increase extraction of glucose from the circulation during periods of hypoglycemia [45]. In human studies of non-diabetic and diabetic subjects, a similar adaptive mechanism has been demonstrated, preserving cerebral function at lower blood glucose concentrations than normal [46,47]. However, short-term adaptation of cerebral function to 1 h of hypoglycemia does not occur [48], indicating that this is a relatively slow process.

There are, therefore, several possible explanations whereby peripheral neural tissue may be unaffected by moderate hypoglycemia. Peripheral neurons have stores of glycogen, and these may provide sufficient energy to sustain neural metabolism during periods of short-lived, moderate hypoglycemia [31]. Alternatively, peripheral neurons may utilize other energy substrates, such as ketone bodies, fructose, lactate, amino acids and free fatty acids [31,49] more efficiently than the brain. It is also possible that the ‘blood–nerve’ barrier may have a constitutively greater ability at extracting glucose from the circulation, or that peripheral neurons have a lower overall glucose requirement.

5. Conclusions

In summary, acute hypoglycemia caused predictable disruption of cognitive functions as assessed by tasks of general cognitive ability and multiple levels of speed of information processing. However, acute hypoglycemia had no demonstrable effect on the function of peripheral nerves in healthy adult volunteers. This implies that neurons of the peripheral nervous system do not have the same obligate requirement for glucose as a metabolic fuel as neurons of the central nervous system. The data also partially refute the hypothesis that speed of conduction within the peripheral nervous system may be used as a marker of speed of nerve conduction within the central nervous system, and thus be a measure of speed of information processing and intelligence.

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