

**CEREBRAL EFFECTS OF HYPOGLYCAEMIA IN HUMANS**

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**Dissertation presented for the degree of Doctor of Medicine**

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*To my parents for their constant support and encouragement*

*"Quum multa legeris et cognoveris: ad unum semper oportet redire principium.  
Ego sum, qui doceo hominem scientiam et clariorem intelligentiam parvulis tribuo:  
quam ab homine possit doceri".*

Thomas à Kempis



## DECLARATION

- a) This thesis was composed by Ann E Gold.
- b) The studies presented were all performed, analysed and written by myself with the exception of *Study 4* (SPET scanning study); this study was performed with Dr Kenneth MacLeod of the Department of Diabetes, Royal Infirmary, Edinburgh. The contribution made by myself to this study included recruiting patients, executing the clamp procedure and performing the statistical analyses presented, with the exception of the correlational analyses.
- c) I hold the degree of MBChB (Edinburgh).
- d) This thesis has not been submitted for any other degree, diploma or professional qualification.

Ann E Gold

Date: 15/12/93.

UNIVERSITY OF EDINBURGH  
ABSTRACT OF THESIS (Regulation 3.5.10)

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Hypoglycaemia leads to cognitive impairment in humans. The literature relevant to the effects of acute hypoglycaemia and the effects of recurrent hypoglycaemia in normal human subjects and patients with diabetes is reviewed. The research described in thesis includes studies involving experimentally induced hypoglycaemia, and clinically based assessments of patients with insulin-dependent diabetes.

The effect of acute insulin-induced hypoglycaemia on cognitive function in humans was examined. The principal studies were performed: i) - to examine whether cerebral adaptation to acute neuroglycopenia occurs in normal subjects, ii) - to examine whether the degree of impairment of cognitive function during acute neuroglycopenia is related to the level of cognitive ability of the subject and iii) - to establish whether patients with insulin-treated diabetes, who have impaired awareness of hypoglycaemia exhibit a more severe degree of cognitive impairment during modest hypoglycaemia. In addition to cognitive function testing in patients with impaired awareness, studies of cerebral blood flow during hypoglycaemia were undertaken using Single Photon Emission Tomography (SPET). Mood changes during acute hypoglycaemia were also examined in non-diabetic subjects.

During 60 minutes of exposure to moderate hypoglycaemia there was no evidence of short term cerebral adaptation to neuroglycopenia in normal subjects. Cognitive ability did appear to have some influence on cognitive dysfunction during hypoglycaemia: those subjects of lower cognitive ability appeared to suffer a smaller degree of cognitive dysfunction during hypoglycaemia. During acute hypoglycaemia subjects were observed to have marked changes in mood: there was a decrease in hedonic tone, an increase in tense arousal and a decrease in energetic arousal.

Diabetic patients with impaired awareness of hypoglycaemia suffered greater and more prolonged cognitive dysfunction during hypoglycaemia than patients with normal awareness. Although hypoglycaemia did induce changes in cerebral blood flow during hypoglycaemia, there were no differences between the patients with impaired or normal awareness of hypoglycaemia.

A one year prospective study was undertaken to assess the frequency of severe hypoglycaemia in two groups of patients with insulin-dependent diabetes. Patients with impaired awareness of hypoglycaemia were shown to be at a sixfold increased risk of severe hypoglycaemia compared with the patients with normal awareness of hypoglycaemia.

A descriptive study examined the changes in personality, cognitive function and lifestyle in a small self-selected group of patients who have a long history of insulin-dependent diabetes and recurrent severe hypoglycaemia. These patients were observed to have become less extraverted and more neurotic, had problems with simple everyday activities and ability to interact socially had also declined.

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## CONTENTS

	Page no.
DECLARATION	1
ABSTRACT OF THESIS	2
ACKNOWLEDGEMENTS	3
CONTENTS	4-6
SUMMARIES OF INDIVIDUAL STUDIES	7-13
<b>PART I INTRODUCTION</b>	14
<u>Chapter 1</u> HYPOGLYCAEMIA	15
Incidence of hypoglycaemia	
Symptoms of hypoglycaemia	
Impaired awareness of hypoglycaemia	
<u>Chapter 2</u> HYPOGLYCAEMIA AND COGNITIVE FUNCTION	20
<b>Long Term Effects of Recurrent Acute Hypoglycaemia on Cognitive Function</b>	22
Recurrent hypoglycaemia and cognitive function in adults with diabetes	23
Recurrent hypoglycaemia and cognitive function in children with diabetes	26
Other factors affecting cognitive function in patients with diabetes	29
<b>Acute Hypoglycaemia and Cognitive Function</b>	30
Limitations of experimental models in the study of the effects of hypoglycaemia on cognitive function	31
Modalities of cognitive function affected by acute hypoglycaemia	33
Neurophysiological changes during acute hypoglycaemia	38
Studies combining cognitive tests and neurophysiological tests	39
Is there a "glycaemic threshold" for changes in cognitive function during acute hypoglycaemia ?	40
How are hypoglycaemic symptoms related to deterioration of cognitive function during acute hypoglycaemia ?	41
The effect of glycaemic control on cognitive during acute hypoglycaemia	44

<b>PART I</b>	(continued)	
<u>Chapter 2</u>	(continued)	
	Cerebral adaptation to acute hypoglycaemia	47
	<b>Clinical Relevance of Changes in Cognitive Function Caused by Hypoglycaemia</b>	50
<u>Chapter 3</u>	<b>HYPOTHESES FOR STUDIES</b>	52
<b>PART II</b>	<b>METHODS</b>	56
	Ethical Permission	57
	Subjects	57
	The Hyperinsulinaemic Glucose Clamp Technique	57
	Experimental Procedure for Clamp Studies	58
	Choice of Degree of Hypoglycaemia	59
	Catecholamine and Physiological Measurements	59
	Assessment of Symptoms of Hypoglycaemia	59
	Assessment of Mood Changes during Hypoglycaemia	60
	Cognitive Measurements	60
	- Paced Auditory Serial Addition Test (PASAT)	
	- Digit Symbol Substitution Test (DSST)	
	- Trail Making B (TMB)	
	- Rapid Visual Information Processing (RVIP)	
	- Four Choice Reaction Time (CRT)	
	Tests of Intelligence	63
	Statistical Methods	64
<b>PART III</b>	<b>EXPERIMENTAL INSULIN-INDUCED HYPOGLYCAEMIA STUDIES</b>	
<u>Chapter 1</u>		65
<i>Study 1 -</i>	Short term cerebral adaptation to acute insulin-induced hypoglycaemia in non-diabetic subjects	

<b>PART III (continued)</b>	
<u>Chapter 2</u>	88
<i>Study 2 -</i>	Effect of intelligence level on cognitive dysfunction during acute hypoglycaemia in non-diabetic subjects
<u>Chapter 3</u>	115
<i>Study 3 -</i>	Cognitive dysfunction during acute hypoglycaemia in patients with Type 1 diabetes and impaired awareness of hypoglycaemia.
<u>Chapter 4</u>	145
<i>Study 4 -</i>	Changes in cerebral blood flow during acute hypoglycaemia in patients Type 1 diabetes and impaired awareness of hypoglycaemia.
<u>Chapter 5</u>	159
<i>Study 5 -</i>	Mood changes during acute hypoglycaemia in non-diabetic subjects
<b>PART IV</b>	<b>CLINICAL STUDIES AND CASE REPORTS</b>
<u>Chapter 1</u>	173
<i>Study 6 -</i>	The frequency and morbidity of hypoglycaemia in patients with Type 1 diabetes who have impaired awareness of hypoglycaemia
<u>Chapter 2</u>	196
<i>Study 7 -</i>	Personality and behavioural changes in patients with long duration Type 1 diabetes and recurrent exposure to severe hypoglycaemia.
<b>PART V</b>	<b>CONCLUSIONS AND FUTURE RESEARCH</b>
	221
<b>APPENDIX</b>	226
QUESTIONNAIRES AND FORMS USED IN STUDIES	227
ABSTRACTS	243
<b>REFERENCES</b>	250

## **STUDY 1 SUMMARY**

**Aim of study:-** Acute hypoglycaemia causes cognitive dysfunction both in normal subjects and patients with diabetes, and it has been suggested that cerebral adaptation may occur in response to short-term hypoglycaemia. This was examined in the present study by measuring serial changes in cognitive function and in symptoms after 60 minutes of continuous hypoglycaemia.

**Methods:-** Hypoglycaemia was induced with a hyperinsulinaemic glucose clamp on two separate occasions in 24 non-diabetic human subjects. Cognitive function was assessed using the following cognitive test battery : Paced Auditory Serial Addition Test (PASAT), Rapid Visual Information Processing (RVIP), Trail-Making B (TMB), Digit Symbol Substitution Test (DSST) and Four Choice Reaction Time (CRT). In Study A the blood glucose was maintained at 4.5mmol/l throughout. On two separate occasions (Study B and Study C) the blood glucose was stabilised at 4.5mmol/l for 30 minutes, lowered to 2.5mmol/l for 60 minutes and restored to 4.5mmol/l for 30 minutes. In each study the cognitive test battery was performed immediately after stabilisation of blood glucose at 4.5 mmol/l and the subsequent battery was repeated at different time intervals: Study A - after a further 40 minutes of euglycaemia; Study B - after 5 minutes of hypoglycaemia; Study C - after 40 minutes of hypoglycaemia.

**Results:-** Acute hypoglycaemia induced a significant deterioration in cognitive function which was manifest in all tests except TMB ( $p < 0.05$ ), but performance ability did not differ between Studies B and C. Hypoglycaemic symptoms assessed by a scaled questionnaire, increased significantly during hypoglycaemia ( $p < 0.001$ ) but no differences were detected between the scores at 30 minutes and 60 minutes.

**Conclusion:-** In non-diabetic humans, no improvement appears to occur, either in cognitive function or in symptom scores after 40 to 60 minutes of hypoglycaemia (2.5 mmol/l), suggesting that cerebral adaptation does not occur during this period of time.

## STUDY 2 SUMMARY

**Aim of study:-** There is a large inter-individual variation in the cognitive dysfunction observed during hypoglycaemia, the reason for which is not apparent. The present study examines whether IQ exerts a differential effect on cognitive performance during acute hypoglycaemia.

**Methods:-** Hypoglycaemia was induced with a hyperinsulinaemic glucose clamp on two separate occasions in 24 non-diabetic subjects (as described in *study 1*). The subjects were divided into high and low IQ groups according to their results on the Alice Heim Test and National Adult Reading Test. Cognitive function was assessed as described in study 1.

**Results:-** Multivariate analysis of variance demonstrated a deterioration in cognitive performance irrespective of IQ group ( $p < 0.005$ ). Acute hypoglycaemia induced a significant deterioration in cognitive function in all tests except TMB ( $p < 0.05$ ). There was no overall effect of IQ on deterioration in cognitive performance; univariate analysis of variance revealed group differences in two of the tests: the lower IQ group deteriorated significantly less than the higher IQ group during hypoglycaemia in the 4 second PASAT ( $p = 0.03$ ) and tended to have higher false alarm rates in the RVIP ( $p = 0.06$ ). There were no differences in performance between Studies B and C, indicating that duration of hypoglycaemia does not have a differential effect on performance in subjects of lower intelligence. Symptoms assessed by a scaled symptom questionnaire changed significantly during hypoglycaemia ( $p < 0.001$ ), but there were no identifiable differences between the IQ groups.

**Conclusion:-** Intelligence level may play a minor role in the degree of cognitive dysfunction experienced during acute hypoglycaemia with patients with higher IQ performing more poorly.



### **STUDY 3 SUMMARY**

**Aim of study:-** Previous studies in patients with Type 1 diabetes and impaired awareness have suggested that cognitive function differs both during euglycaemia and hypoglycaemia compared with patients with normal awareness of hypoglycaemia. Changes in cognitive function were examined in 20 patients with Type 1 diabetes, 10 of whom had normal awareness of the onset of hypoglycaemia (Group A) while 10 had impaired awareness of hypoglycaemia (Group B).

**Methods:-** A hyperinsulinaemic glucose clamp was used to produce the following conditions: (a) euglycaemia (blood glucose concentration 4.5 mmol/l), (b) hypoglycaemia (2.5mmol/l) and (c) restoration of euglycaemia (4.5mmol/l). On a separate day a euglycaemic control study was performed (4.5mmol/l). Cognitive function was assessed using Rapid Visual Information Processing (RVIP), Trail Making B (TMB), Paced Auditory Serial Addition Test (PASAT) and Digit Symbol Substitution Test (DSST).

**Results:-** Multivariate analysis of variance demonstrated a significant difference in performance during the hypoglycaemic study compared with the euglycaemic control study ( $p < 0.01$ ). A trend was observed towards an overall effect of awareness on performance ( $p = 0.08$ ). Further analysis of the individual tests demonstrated a significant effect of awareness on RVIP correct responses across time ( $p = 0.05$ ), and an interaction of awareness by study by TMB ( $p = 0.03$ ). Analysis of the simple effects revealed that during hypoglycaemia, subjects in Group A were less accurate in their responses (RVIP misses  $p = 0.03$ ) and, on recovery from hypoglycaemia, cognitive function remained impaired in Group B compared to Group A (TMB  $p = 0.04$ , RVIP correct responses  $p = 0.02$ , RVIP misses  $p = 0.04$ ).

**Conclusion:-** Patients with impaired awareness of hypoglycaemia appeared to exhibit more profound cognitive dysfunction during acute hypoglycaemia in some tests and the dysfunction persisted for longer following restoration of euglycaemia.

## STUDY 4 SUMMARY

**Aim of study:-** Changes in regional cerebral blood flow (rCBF) during acute hypoglycaemia have been described previously. The significance of these changes with regard to cognitive function and awareness of hypoglycaemia is not known.

**Methods:-** Regional cerebral blood flow was measured in 20 patients with Type 1 diabetes, 10 of whom had normal awareness of hypoglycaemia (Group A) while 10 had impaired awareness of hypoglycaemia (Group B). Blood glucose concentrations were manipulated as described in *study 3* using the hyperinsulinaemic glucose clamp and rCBF was measured during i) euglycaemia (4.5mmol/l) and ii) hypoglycaemia (2.5 mmol/l). The distribution of the isotope  $^{99m}\text{Tc}$ -Exametazime was determined using a single slice multi-detector head scanner. 250MBq of isotope were injected during euglycaemia and a further 250 MBq was injected during hypoglycaemia. The rCBF was estimated in 30 regions of interest, derived from a standard neuroanatomical atlas, on two parallel slices at 40 and 60mm above the orbito-meatal line.

**Results:-** Analysis of variance did show a significant difference between the groups in right occipital blood flow, irrespective of the scan conditions. Both groups also exhibited increased rCBF to both superior pre-frontal cortices and the right thalamus with concomitant reduced flow to the right putamen compared with the baseline scans. When changes in cognitive function during hypoglycaemia were correlated with changes in rCBF, greater prefrontal increments in tracer uptake were associated with a smaller deterioration in performance in the Paced Auditory Serial Addition Test (PASAT).

**Conclusion :-** These results suggest that the rCBF may have a protective role in the prevention of cognitive dysfunction during acute hypoglycaemia.

## **STUDY 5 SUMMARY**

**Aim of study:-** Patients with diabetes frequently report changes in mood during acute hypoglycaemia and patients exposed to recurrent hypoglycaemia have been shown to be less 'happy' and more anxious. However few studies have addressed acute changes in mood occurring during a single episode of hypoglycaemia.

**Methods:-** Hypoglycaemia was induced on two occasions using a hyperinsulinaemic glucose clamp in 24 non-diabetic human subjects (see *study 1*); on a third occasion a euglycaemic 'placebo' control study was performed. Serial changes in mood were assessed using the UWIST Mood Adjective Checklist before, during and after 60 minutes of controlled hypoglycaemia (2.5 mmol/l).

**Results:-** Analysis of variance demonstrated that hypoglycaemia induced a significant reduction in hedonic tone (ie subjects became less happy;  $p=0.027$ ), a significant increase in tense arousal (ie subjects became more tense;  $p=0.001$ ) and a trend towards a decline in energetic arousal (ie became less energetic;  $p=0.056$ ) in comparison with the euglycaemia control study. The changes in tense arousal persisted for 15 minutes after restoration of euglycaemia and the changes in energetic arousal were still present after 30 minutes following the restoration of euglycaemia. In comparison hedonic tone returned to baseline scores within 15 minutes of restoration of euglycaemia.

**Conclusions:-** In non-diabetic subjects, during acute hypoglycaemia, profound changes in mood were observed, towards a state called 'tense-tiredness', which persisted for at least 30 minutes after restoration of euglycaemia. Laboratory induced hypoglycaemia may provide a means by which to investigate the biological basis of mood. In addition, these findings may be of clinical relevance in patients with diabetes who are exposed frequently to hypoglycaemia in daily life.

## **STUDY 6 SUMMARY**

**Aims of study:-** Diabetic patients with impaired awareness of hypoglycaemia are recognised to be at an increased risk of developing severe hypoglycaemia but the actual frequency is unknown.

**Methods:-** A prospective study was undertaken for 12 months in 60 patients with Type 1 diabetes: 29 had impaired awareness of hypoglycaemia and 31 had normal awareness of hypoglycaemia. The two groups of patients were matched for age, age of onset of diabetes, duration of diabetes and glycaemic control. Episodes of severe hypoglycaemia were recorded within 24 hours of the event and verified where possible by witnesses.

**Results:-** During the 12 months, 19 (66%) of the patients with impaired awareness had one or more episodes of severe hypoglycaemia in 12 months with an overall incidence of 2.8 episodes per patient per year. Only 8 (26%) of the patients with normal awareness experienced severe hypoglycaemia ( $p < 0.01$ ) with an annual incidence of 0.5 episodes per patient per year ( $p < 0.001$ ). Severe hypoglycaemia occurred at different times of the day in the two groups: patients with impaired awareness experienced a greater proportion of episodes during the evening ( $p = 0.03$ ) and patients with normal awareness experienced a greater proportion in the early morning ( $p = 0.05$ ). Fear of hypoglycaemia was also assessed; patients with impaired awareness of hypoglycaemia were observed to worry more about hypoglycaemia than patients with normal awareness ( $p = 0.008$ ), but this did not modify their behaviour.

**Conclusion:-** This prospective evaluation confirmed that impaired awareness of hypoglycaemia predisposes to an increased (sixfold) frequency of severe hypoglycaemia, much of which occurred at home during waking hours.

## **STUDY 7 SUMMARY**

**Aim of study:-** It has been proposed that recurrent severe hypoglycaemia may cause permanent cognitive impairment in insulin-treated diabetic patients. Changes in mood, personality and social function were examined in a group of five Type 1 diabetic patients, all of whom had a history of recurrent hypoglycaemia.

**Methods:-** Five patients with Type 1 diabetes were studied, aged 50 to 66 years, who had a duration of diabetes ranging from 24 to 47 years. Information on medical history and frequency of severe hypoglycaemia was obtained from their carers and hospital records. The patients' carers assessed the premorbid and present behaviour and personality of the patients using standard questionnaires.

**Results:-** All patients had experienced multiple episodes of severe hypoglycaemia and had impaired awareness of hypoglycaemia. Cerebral dysfunction pre-dated the development of identifiable diabetic complications which were mild in nature and had been apparent for between one and 17 years. Significant deteriorations were demonstrated in cognitive ( $p=0.04$ ) and social functions ( $p=0.04$ ), compared with assessment of premorbid function. Patients had tended to become more neurotic ( $p=0.08$ ) and less extravert ( $p=0.07$ ). All of the patients and three of the carers recorded scores suggestive of psychiatric morbidity on the General Health Questionnaire. The patients had experienced loss of employment and the carers described a reduction in the patients' social interactions.

**Conclusion:-** In the absence of other potential causes of cerebral dysfunction, these major changes in cognitive abilities, personality and social behaviour may be in part attributed to recurrent severe hypoglycaemia. The need for professional support for the carers of such patients should be recognised.

PART I

CHAPTER 1

**INTRODUCTION**



Patients with diabetes are exposed to a variety of metabolic insults including hyper- and hypoglycaemia, both of which affect cognitive function (Ryan 1988, Richardson 1990, McCall 1992). Although the pathogenetic role of hyperglycaemia in the development of various diabetic complications is well established, the adverse effects of acute and recurrent hypoglycaemia on the brain have received limited attention until recently, despite previous clinical observations that severe hypoglycaemia can cause cerebral damage and overt psychopathology (Moersch and Kernohan 1938, Sahs and Alexander 1939, Lawrence 1942, Murphy and Purtell 1943, Fischer and Dolger 1946, Jones 1947). The incidence and symptomatology of hypoglycaemia will be discussed before reviewing the effects of hypoglycaemia on cerebral function.

### **Incidence of Hypoglycaemia**

Since the advent of insulin therapy in 1922 for the treatment of patients with diabetes mellitus, hypoglycaemia has been recognised to be a common and potentially dangerous side-effect of such treatment (Paz-Guevera et al 1975, Deckert et al 1978, Hepburn et al 1989) and is one of the most feared complications of insulin treatment. The causes of acute hypoglycaemia are often multifactorial and may result from the unphysiological methods presently available for the administration of insulin in combination with varying degrees of patient error. Hypoglycaemia of varying severity is a common side effect of insulin therapy, although the frequency is difficult to estimate with accuracy unless confined to severe episodes (Pramming et al 1991). When severe hypoglycaemia is defined as the need for external assistance to resuscitate the patient, approximately 30% of insulin-treated diabetic patients experience severe hypoglycaemia at some time with an incidence of about 1.4-1.6 episodes<sup>-1</sup> patient<sup>-1</sup> year<sup>-1</sup>, and an unfortunate minority experience recurrent severe hypoglycaemia

(Goldstien et al 1981, Gale 1985, Pramming et al 1991). Comparisons of intensive and conventional insulin therapy have revealed that the frequency of severe hypoglycaemia is three times greater in patients exposed to intensive insulin therapy than in patients on conventional insulin regimens (DCCT Research Group 1991).

### **Symptoms of hypoglycaemia**

The symptoms which usually alert a patient to the onset of hypoglycaemia can be subdivided into three groups: those attributable to neuroglycopenia, which interferes with intellectual activity and impairs cognitive function, those resulting from activation of the autonomic (principally of the sympatho-adrenal) system and non-specific symptoms associated with malaise (Cooke 1934, Hepburn et al 1991a). Neuroglycopenic symptoms comprise confusion, drowsiness, odd behaviour, speech difficulty and incoordination. The 'malaise' symptoms include those such as headache and nausea. Stimulation of autonomic centres in the hypothalamus triggers an autonomic discharge (or 'reaction') which increases peripheral autonomic neural activity and is characterised by symptoms such as sweating, tremor, pounding heart and hunger. The simultaneous secretion of adrenaline probably augments some of these autonomic symptoms. Following the triggering of putative glucose-sensitive receptors located within the brain and also possibly in other peripheral organs (eg pancreatic / hepatic), various counter-regulatory hormones are released, the most important of which are glucagon and catecholamines in normal humans. In patients with Type 1 diabetes of over five years duration glucagon secretion may be diminished or absent and catecholamines are the principal counterregulatory hormones (Frier et al 1986). These hormones stimulate the restoration of blood glucose via metabolic actions on hepatic glycogenolysis and gluconeogenesis.



The symptoms of hypoglycaemia are idiosyncratic and may vary with circumstances and time, but usually comprise a combination of autonomic, neuroglycopenic and non-specific symptoms. A patient's ability to recognise the onset of hypoglycaemia may be affected by numerous factors including long duration of diabetes, strict glycaemic control, previous episodes of severe hypoglycaemia, sleep, alcohol and drugs such as hypnotics and non-selective beta-blockers. The interactions between hypoglycaemic warning symptoms, the integrity of glucose counterregulation and the effects of neuroglycopenia on cognitive function are important in determining the severity and morbidity of hypoglycaemia.

### **Impaired awareness of hypoglycaemia**

Impaired awareness of hypoglycaemia is the inability of the patient to perceive the onset of hypoglycaemia. It is important that a patient is able to detect hypoglycaemia in sufficient time to allow ingestion of carbohydrate to restore the blood glucose towards normal. However if there is a defect in warning symptoms the blood glucose may have already fallen too low, resulting in severe neuroglycopenia which will in turn impair the patient's ability to seek help. There are many early anecdotal reports of patients treated with insulin, in whom the perception of the onset of warning symptoms of hypoglycaemia has altered since the time of diagnosis (Maddock and Trimble 1928, Lawrence 1941, Balodimos and Root 1959). More recent studies have shown that approximately 50% of patients with Type 1 diabetes of over 20 years duration experience a change in their symptoms of hypoglycaemia, which usually results in a switch from predominantly autonomic symptoms to neuroglycopenic symptoms (Prasanna et al 1991). The term 'impaired awareness' is preferred to 'unawareness' of hypoglycaemia as the degree of perception of the episode will also be dependent on the situation eg a labourer will find it more difficult to perceive the onset of hypoglycaemia while

working outside on a hot day if his warning symptoms are predominantly those of sweating; in addition some patients can 're-educate' themselves to recognise new warning symptoms such as dizziness or yawning in place of sweating and trembling. However it has been shown that these patients with impaired awareness are at greater risk of experiencing recurrent severe hypoglycaemia (Hepburn et al 1990, Pramming et al 1991). In patients who have impaired awareness of hypoglycaemia, reliance on observers such as relatives or colleagues becomes paramount to detect the development of acute hypoglycaemia. The patient may be observed to be exhibiting manifestations of overt neuroglycopenia with impairment of cognitive function and increasing confusion which interferes with perception of the manifestations of severe hypoglycaemia.

Impaired awareness of hypoglycaemia appears to consist of two separate clinical entities at opposite ends of a spectrum, one of which is transient, reversible and related to strict glycaemic control and the other is chronic, irreversible and related to duration of diabetes (Amiel et al 1987, Hepburn and Frier 1991). Recurrent hypoglycaemia has been shown to result in 'hypoglycaemia-associated autonomic failure' (Cryer and Gerich 1990, Cryer 1992). This phenomenon comprises three clinical syndromes: defective glucose counterregulation, hypoglycaemia unawareness and elevated glycaemic threshold during effective insulin therapy (Cryer 1992). These three syndromes tend to cosegregate ie they occur in patients with a long duration of diabetes (Ryder et al 1990, Hepburn et al 1990) and appear to be perpetuated by recurrent hypoglycaemia. A single episode of hypoglycaemia results in elevated glycaemic thresholds for autonomic and symptomatic responses and impaired physiological defence to subsequent hypoglycaemia and may result in transient impaired awareness of hypoglycaemia. It is possible that the chronic form of impaired awareness of hypoglycaemia lies at the extreme end of the spectrum of 'hypoglycaemia associated autonomic failure', occurring in patients with longer duration diabetes and a history of recurrent

episodes of severe hypoglycaemia, and is irreversible, although various alternative causes have also been proposed (Heller and Cryer 1991, Clarke et al 1991, Hepburn and Frier 1991, Gerich et al 1991, Heller and Macdonald 1991). It is now clear that autonomic neuropathy *per se* does not cause impaired awareness of hypoglycaemia although it may also be found in many patients with a long duration of diabetes (Ryder et al 1990, Hepburn et al 1991b).

The pathophysiology of alterations in symptoms of hypoglycaemia is not known and may result from a defective activation of a glucose sensor which when exposed to hypoglycaemia triggers central neuronal activity which is eventually manifest as the autonomic discharge already described. Cerebral glucose metabolism has been reviewed previously (McCall 1992) and it appears that the brain may provide the location of a glucose sensor. In one study in dogs (Biggers et al 1989) peripheral hypoglycemia was maintained with concomitant cerebral euglycaemia (obtained by infusing glucose into cerebral arterial blood supply) and it was observed that the counterregulatory hormonal responses were significantly impaired compared with the responses which occurred when cerebral hypoglycaemia was allowed to occur. Although other studies have supported the role of this putative cerebral glucose sensor (Skov and Pryds 1992) a recent study again performed in dogs (Donovan et al 1993) proposed that hepatic glucose sensors mediate the counterregulatory responses. Until the glucose sensor in healthy subjects or patients with diabetes and normal awareness of hypoglycaemia is identified, it is difficult to hypothesise on the mechanism which is defective in patients who have impaired awareness of hypoglycaemia and defective counterregulation.

**PART I (continued)**

**CHAPTER 2**

**HYPOGLYCAEMIA AND COGNITIVE FUNCTION**

The evidence for the detrimental effects of hypoglycaemia on the brain has been documented for many years (Moersch and Kernohan 1938, Lawrence 1942, Fischer and Dolger 1946, Jones 1947) although the precise mechanisms involved and the resulting effects on behaviour, cognitive function and personality have only recently been the topic of scientific research. One of the reasons for this is that these areas of study are very difficult to quantify and were therefore previously neglected. Advances in statistical knowledge and psychometric analysis have now enabled the introduction of many standardised tests and questionnaires which allow quantification and comparison between subjects of variables such as cognitive function.

The results of such studies will be reviewed. Cognitive function will be considered with respect to :

- 1) permanent changes occurring as a result of the cumulative effects of recurrent exposure to severe hypoglycaemia.
- 2) transient changes in response to acute insulin-induced hypoglycaemia.

The methods required to study cognitive function during acute hypoglycaemia vary considerably from those necessary to assess the potential effects of recurrent severe hypoglycaemia.

## **LONG TERM EFFECTS OF RECURRENT ACUTE HYPOGLYCAEMIA ON COGNITIVE FUNCTION**

Anecdotal reports of diabetic patients who have experienced repeated severe hypoglycaemia have described the development of serious and often permanent deterioration in cognitive function, intellectual capacity and personality (Moersch and Kernohan 1938, Tyler and Ziskind 1940, Lawrence 1942, Murphy and Purtell 1943, Wilder 1943). 'Insulin shock' therapy was introduced in the 1930's as method of treatment for various psychoses; it was initially considered to be a relatively safe form of treatment although early published reports did acknowledge that this therapeutically induced hypoglycaemia was associated with a significant morbidity (Frostig 1938, Meduna and Fiedman 1939).

More recently the cumulative effects of recurrent episodes of hypoglycaemia on cognitive function have been studied in patients with Type 1 diabetes of various ages. The importance of these findings is that if recurrent severe hypoglycaemia is shown to impair cognitive function permanently, the therapeutic goals for glycaemic control which are currently recommended for patients with insulin-treated diabetes will need re-evaluation. It may be necessary to strive for suboptimal glycaemic control in individual patients who are at increased risk of severe hypoglycaemia, to avoid jeopardising intellectual function. Studies of adults and children should be differentiated, because recurrent severe hypoglycaemia is likely to impose different effects on the immature developing brain of a child compared with the adult who has attained maximum cognitive ability before being exposed to this metabolic insult.



## **Recurrent Hypoglycaemia and Cognitive Function in Adults with Diabetes**

A correlation between cognitive dysfunction and the estimated frequency of severe hypoglycaemia was demonstrated by Bale (1973) using a measure of global intelligence (the Walton Black New Word Learning Test) to examine cognitive performance in 100 Type 1 diabetic patients. In another study, cognitive function was measured with an extensive battery of cognitive tests, including the Wechsler Adult Intelligence Scale, and an association was reported between the number of episodes of severe hypoglycaemia and cognitive dysfunction in Type 1 diabetic patients (Skenazy and Bigler 1984). Patients with Type 1 diabetes who had a history of recurrent severe hypoglycaemia (Wredling et al 1990) were observed to exhibit impairment of motor ability, problem solving and visuo-spatial skills, and abnormalities of frontal lobe function (as assessed by finger-tapping, Necker cube reversal and maze tests). These patients were also observed to be more cautious at the expense of speed in deriving their answers, which is similar to observations of performance during acute hypoglycaemia (Holmes et al 1983).

Langan et al (1991) used the National Adult Reading Test (NART) (Nelson 1982) and Wechsler Adult Intelligence Scale - Revised (WAIS-R) (Wechsler 1981) to determine whether changes in IQ had occurred in 100 Type 1 diabetic patients who had no history or evidence of other organic brain disease. The NART (see Part II) is an assessment of the 'best' global cognitive function ever attained, irrespective of time, and is resistant to progressive brain disease. The comparison of pre-morbid assessment of IQ, using the NART, with measurements of the patient's present IQ (WAIS-R) can identify any significant deterioration which has occurred over a period of years. The diabetic patients who had previously experienced five or more episodes of severe hypoglycaemia had a definite deterioration in IQ and also had poorer reaction times. It was also possible to refute the suggestion that a lower premorbid IQ predisposes the patient to severe

hypoglycaemia, which could not be excluded in previous studies (Wredling et al 1990). Comparison of the same diabetic patients (Langan et al 1991) with a matched control group of 100 non-diabetic subjects demonstrated that the diabetic patients had lower WAIS-R performance and verbal IQ scores (Deary et al 1993a). However, after controlling for the frequency of severe hypoglycaemia, the performance IQ was similar both in the diabetic and the control groups although a significant difference in verbal IQ persisted. These findings suggest previous exposure to hypoglycaemia is associated with poorer performance IQ; factors such as the social impact of the diabetes may explain the lower verbal IQ which was apparent in the diabetic patients.

In order to determine which modalities of cognitive function were affected by previous exposure to severe hypoglycaemia the patients from Langan's study (1991) were also studied using Four Choice Reaction Time (CRT), Rapid Visual Information Processing (RVIP) and a memory task (Sternberg Memory Scanning)(Deary et al 1992). CRT measures the time taken for a subject to respond to one of a choice of lights, which is flashed on at random; both the time taken to decide an appropriate response (decision time) and the time taken to perform the response (movement time) are recorded. In the RVIP test the subject observes a screen, on which numbers are displayed individually, and is asked to press a button each time a sequence of three odd or three even numbers occurs; the number of correct responses, incorrect responses and the time taken to respond are obtained. In the diabetic patients the decision time for the CRT and the number of RVIP correct responses correlated significantly with the frequency of previous severe hypoglycaemia (Deary et al 1993a), and factor analysis suggested that decision and response initiation processes, were affected by a history of hypoglycaemia rather than processes such as encoding and storage.



These studies have all depended on retrospective estimation of the frequencies of severe hypoglycaemia which may be inaccurate. In a prospective study, the Stockholm Diabetes Intervention Study (SDIS), comparing intensive and conventional insulin regimens in a group of Type 1 diabetic patients (Reichard et al 1991), the frequency of severe hypoglycaemia increased during five years of strict glycaemic control, but no impairment of cognitive function was demonstrated over this period. However, a clear discrimination in the frequency of severe hypoglycaemia was not made between the intensively-treated and conventionally-treated patient groups, so diminishing the power of the study and increasing the susceptibility to a type II statistical error. In addition, five years may be too short a period for overt cognitive deterioration to emerge, and the limited battery of cognitive function tests which were used may not be of sufficient sensitivity to demonstrate subtle changes of cognitive ability (Deary and Frier 1992, Gold et al 1993).

The advent of sophisticated cerebral scanning techniques such as single photon emission tomography (SPET) have facilitated the demonstration that acute hypoglycaemia alters regional cerebral blood flow, in particular to the frontal lobes where blood flow was increased (Tallroth et al 1992). These changes were also present (and were more prominent) during normoglycaemia in diabetic patients who had a history of recurrent severe hypoglycaemia (MacLeod et al in press). Similarly changes in the frontal lobes have been identified in patients dying from hypoglycaemia (Terplan 1932).

## **Recurrent Hypoglycaemia and Cognitive Function in Children with Diabetes**

Premature infants are at risk of spontaneous hypoglycaemia and a prospective controlled study of non-diabetic children has demonstrated that infants exposed to hypoglycaemia subsequently had a lower IQ and had evidence of retarded development (Pildes et al 1974). In a study of children with Type 1 diabetes it was observed that IQ was significantly lower than that of age-matched, non-diabetic children with a similar educational background and the IQ deficit in the diabetic children was associated with the number of preceding episodes of severe hypoglycaemia (Ack et al 1961). Fallstrom (1974) showed that diabetic children had more perceptual disturbance, anxiety and behavioural problems compared with healthy non-diabetic children of the same age; the severity of these abnormalities correlated with the frequency of previous hypoglycaemic convulsions. Studies of children with Type 1 diabetes have also shown that up to 80% of children who had experienced five or more episodes of severe hypoglycaemia had permanent EEG abnormalities (Eeg-Olofsson and Petersen 1966, Eeg-Olofsson 1977, Haumont et al 1979, Soltész and Acsadi 1989). Visual evoked potential abnormalities have been shown to be more frequent in diabetic children compared with normal subjects (Cirillo et al 1984).

Adolescent diabetic patients have been demonstrated to have poorer cognitive function compared with normal controls and the poorest performance occurred in those diabetic children who had developed diabetes before the age of five years (Ryan et al 1984, 1985). Multiple regression analysis revealed that long duration of diabetes was associated with poorer verbal and learned skills, and that early age of onset principally appeared to affect the tests which measured the ability to process new information. Subsequent studies have attempted to correlate the frequency of hypoglycaemic episodes with changes in cognitive function (Rovet et al 1987, Golden et al 1989) and have shown that the diabetic children who

performed poorly on cognitive function testing had previously experienced more hypoglycaemic convulsions and had been exposed to frequent asymptomatic biochemical hypoglycaemia.

In a three year prospective study of 63 children with Type 1 diabetes no major deficits in cognitive function occurred during the study period (Rovet et al 1991). However those patients with onset of diabetes before five years of age had evidence of frequent biochemical (asymptomatic) hypoglycaemia and exhibited a poorer spatial ability, whereas those children with diabetes of later onset had more frequent symptomatic episodes of hypoglycaemia and had poorer verbal skills. One study has shown that relative youth may not necessarily indicate better cognitive and coping skills (Connis et al 1989). It has been suggested that diabetic children with early onset of diabetes should be considered for neuropsychological testing if they have a history of recurrent hypoglycaemia (Puczynski et al 1992).

The effects of recurrent severe hypoglycaemia on cognitive function are summarised in the Table 1.2.1.

TABLE 1.2.1

**EFFECTS OF RECURRENT SEVERE HYPOGLYCAEMIA  
ON COGNITIVE FUNCTION IN INSULIN-DEPENDENT  
DIABETIC PATIENTS**

- moderate reduction in IQ occur over a period of several years
- early onset of diabetes may affect ability to process new information
- accuracy of performance is maintained at the cost of speed
- decision but not encoding processes are affected by previous severe hypoglycaemia

## **Other factors affecting cognitive function in patients with diabetes**

Many other factors may potentially influence cognitive function which include age at onset of diabetes, duration of diabetes, quality of glycaemic control, the presence of other conditions which may potentially affect cognitive function and limited educational attainment through loss of schooling. The effect of recent poor metabolic control on cognitive function has been examined in patients with either Type 1 or Type 2 diabetes; while some studies have failed to demonstrate any such effect (Prescott et al 1990, Donald et al 1984, Lawson et al 1984) other studies have shown an association between poor metabolic control (predominantly hyperglycaemia) and impaired cognitive function (Holmes et al 1986, Reaven et al 1990). Holmes et al (1988) examined a group of Type 1 diabetic patients who had near-normal glycaemic control and observed that they appeared to perform more poorly both on auditory and visual reaction time tasks than a group of matched diabetic patients with moderate glycaemic control. In some studies, duration of diabetes and age at onset have been associated with poorer cognitive function (Holmes et al 1988, Reaven et al 1990) whereas others have been unable to demonstrate this relationship (Donald et al 1984, Prescott et al 1990). The problems of investigating changes in cognitive function after recurrent episodes of hypoglycaemia are exemplified by these conflicting and disparate results. Several other independent and confounding factors such as premature cerebrovascular disease, hypertension, alcoholism or previous head injury may interfere with cognitive function, and have to be excluded when investigating the putative effects of severe hypoglycaemia in diabetic patients.

## **EFFECT OF ACUTE HYPOGLYCAEMIA ON COGNITIVE FUNCTION**

Early anecdotal reports described the effects of neuroglycopenia resulting from hypoglycaemia either from the therapeutic administration of exogenous insulin or from the autonomous hyperinsulinaemia associated with insulin-secreting tumours (Dashiehl 1930, Wilder 1943, Fineberg and Altschul 1951). However, before the 1970's very few formal studies of the effects of acute hypoglycaemia on cognitive function had been reported. Subsequent studies have addressed the following questions and these will be reviewed:

- 1 Which modalities of cognitive function are impaired by hypoglycaemia ?
- 2 Is there a blood glucose 'threshold' at which cognitive impairment commences and how does that relate to the physiological and neuroendocrine responses to hypoglycaemia, including the perception of symptoms and the activation of counterregulatory mechanisms ?
- 3 In what way are the symptoms of hypoglycaemia related to cognitive impairment ?
- 4 Are there adaptive mechanisms in the brain which limit the degree of cognitive impairment ?



## **Limitations of experimental models in the study of the effects of acute hypoglycaemia on cognitive function**

Before it is possible to compare and contrast studies it is important to appreciate that there are many limitations in experimental models used in the study of acute hypoglycaemia on cognitive function. Analysis of the literature of such studies demonstrates many disparate results and it is important to consider certain methodological differences between studies before attempting to interpret and compare results.

### *Subjects*

Studies on diabetic subjects should not be compared with studies on non-diabetic subjects as the effect of diabetes *per se* and hyperglycaemia may confound results. In addition the type of diabetes must be considered ie Type 1 (insulin-dependent) or Type 2 (non-insulin-dependent), and the duration and age of onset of diabetes of the subjects differs between studies as well as the degree of glycaemic control.

### *Method of induction of hypoglycaemia*

Hypoglycaemia induced in the laboratory setting must be reproducible and safe and two principal methods of induction of hypoglycaemia have been utilised in the study of changes in cognitive function: 1) - insulin infusion, 2) - hyperinsulinaemic glucose clamp. In the former method insulin is infused at a steady rate until the threshold for the onset of hypoglycaemia is reached. The hyperinsulinaemic glucose clamp consists of a fixed rate insulin infusion and a variable rate glucose infusion which allows stabilisation of the blood glucose at predetermined concentrations. The merits of each of these techniques have been reviewed previously (Heine 1993), but in brief, while the insulin infusion technique is probably more physiological it does not allow prolonged cognitive testing at fixed

blood glucose concentrations or accurate determination of glycaemic thresholds for changes in symptoms, counterregulatory hormones or cognitive function. The hyperinsulinaemic glucose clamp is more reproducible and is the only method presently available to safely allow prolonged cognitive testing, although some responses to hypoglycaemia may be modified by hyperinsulinaemia. Comparison of studies using different methods of induction of hypoglycaemia are not therefore possible.

#### *Blood glucose sampling*

Blood glucose measurements vary depending on the site and method of sampling. Values have sometimes been cited as 'plasma' concentrations or as 'whole blood' concentrations and are not therefore directly comparable. Glucose concentrations also vary depending on whether sampling was 'venous' or 'arterialised'; in the fasted state there is little arterio-venous difference in glucose concentrations (Hampton et al 1986) but post-prandially or after an intravenous glucose load (as in the glucose clamp) this difference increases (Liu et al 1992).

#### *Cognitive test batteries*

Several different methods for assessment of cognitive function are available probably because there is no single test which can assess all aspects of cognitive function and in addition many studies have tried to analyse whether certain aspects of cognitive function are more affected by hypoglycaemia and therefore use more specific tests. The sensitivity of these tests to detecting subtle changes in cerebral function varies considerably. Improved performance in some tests arises as a result of repeated attempts at the tests and should be accounted for in the interpretation of results. These practice effects can be minimised by allowing subjects to have trial runs at the tests, although in many studies in the literature it is not made clear whether this has been done.



With these limitations in mind the results of the studies of acute hypoglycaemia on cognitive function will be reviewed.

### **Modalities of cognitive function affected by acute hypoglycaemia**

The initial studies of the effects of acute hypoglycaemia on cognitive function aimed to identify which modalities of cognitive function were affected. Frequently in these studies large cognitive test batteries were employed principally as an exploratory exercise. The main findings from these studies are shown in Table 1.2.2 and although it is not possible to describe all the studies performed in this area, a few of the principal results be described below, in chronological order.

Flenders and Lifshitz (1976) demonstrated impairment of fine motor coordination during acute hypoglycaemia in a group of Type 1 (insulin-dependent) adolescent diabetic patients. Holmes et al (1983), investigated insulin-treated diabetic patients using a hyperinsulinaemic glucose clamp method to induce controlled hypoglycaemia. Changes in memory tasks (using digit span and Rey Auditory Verbal Learning Test), general attention and psychomotor tasks (using matching tasks and visual reaction time), visuospatial-memory tasks (using Benton Visual Retention Tests) and academic ability tasks (using mathematical tests) were examined. They found that reaction time was slower during hypoglycaemia and fewer mathematical tasks could be performed in a given time although accuracy was maintained. Memory was not impaired, nor was visual perception affected. Herold et al (1985) similarly demonstrated slowing of reaction times during hypoglycaemia both in non-diabetic subjects and in insulin-treated diabetic adults. Considerable variability in response between subjects was noted which suggested differing degrees of individual susceptibility to acute hypoglycaemia. In a further study, Holmes et al (1984), observed that verbal fluency and Stroop test

performance were impaired in patients with Type 1 diabetes during acute hypoglycaemia.

Pramming et al (1986), used a variable rate insulin infusion to induce hypoglycaemia in a group of insulin-treated diabetic patients and showed changes in cognitive function using a battery of tests including subtraction tasks, trail-making tests, digit span, Bourdon-Story recall, categorisation and finger-tapping. Deterioration in the performance in all of these tests was observed, and differed from the results of Holmes et al (1983) by demonstrating that tests of immediate memory were also impaired. In an anecdotal case report, changes in the temporal lobe (which is involved in memory function) have been identified by magnetic resonance imaging in a diabetic patient who experienced severe but transient amnesia after hypoglycaemic coma (Chalmers et al 1991). However the degree of hypoglycaemia experienced by this patient was obviously far greater than that which is usually induced experimentally.

Hoffman et al (1989) described a deterioration in trail-making and pursuit-rotor tasks in patients with Type 1 diabetes, associated with prolongation of reaction times and also observed a marked variability between subjects as described previously (Herold et al 1985). Stevens et al (1989) demonstrated impairment of the digit-symbol substitution test (a subtest of the Wechsler Adult Intelligence test) in normal (non-diabetic) subjects but did not observe any effect on choice reaction times, although the degree of hypoglycaemia which was induced using a stepped hyperinsulinaemic glucose clamp technique was relatively modest (arterialised blood glucose 3.4 mmol/l). The variable results of these studies might be related to the differing techniques used to induce hypoglycaemia. In addition, variable degrees of severity and times of exposure to hypoglycaemia were induced before cognitive testing was applied and a wide range of cognitive test batteries of varying

sensitivity have been utilised by different workers.

The identity of the study population is important as previously mentioned and may provide variable results as patients with Type 1 diabetes have been shown to be more severely affected than normal subjects at arterialised blood glucose levels of 2.0mmol/l (Wirsen et al 1992).

In summarising the above studies it appears that there is a large inter-individual variation in performance but on the whole tests of complex cognitive function which require information to be perceived, processed and a response given appear to be most affected eg Stroop test, Trail-making tests, Digit Symbol Substitution and matching tasks. Driesen et al (1991) have also shown that during moderate hypoglycaemia (blood glucose 2.8-3.3 mmol/l) only choice reaction times were prolonged (which involve complex information processing) in patients with Type 1 diabetes, while at lower blood glucose concentrations (1.9-2.5 mmol/l) both simple and choice reaction times were impaired. This suggests that the degree of hypoglycaemia experienced is also important in determining which cognitive processes are impaired.

In general, during acute hypoglycaemia, skills which require more complex decision making and discrimination appear more likely to be impaired significantly than simple motor tasks (eg. simple reaction time). Trail-making B and pursuit rotor tests were impaired more significantly than trail-making A and visual reaction times (Hoffman et al 1989). This suggests that it is not primarily the motor component of these tasks which is compromised (Hoffman et al 1989), otherwise all of the tasks should be impaired to a similar degree. Further evidence in favour of this theory will be discussed later.

The results from the afore mentioned studies would lead one to expect that driving

skills would be impaired by hypoglycaemia as they involve complex decision making, but Hoffman et al (1989) did not observe abnormalities in insulin-treated diabetic patients using a driving simulator. However only a small number of subjects were tested and the type of simulator employed was thought unlikely to provide a very good comparison with actual driving conditions. The driving simulator may have awakened the subjects interest as an 'entertainment' rather than simulating the monotony of true driving conditions. However Cox et al (1991) have demonstrated significant impairment of driving skills during controlled hypoglycaemia using a more sophisticated driving simulator in a similar group of diabetic patients. In this study it was also observed that there was a compensatory slowing of driving in attempt to maintain accuracy of performance.

In conclusion, complex information processing is most profoundly affected by acute hypoglycaemia, although simple processes will be affected at more severe degrees of hypoglycaemia. There is also a marked variability between subjects, particularly between those with diabetes and those without diabetes.

TABLE 1.2.2 MODALITIES OF COGNITIVE FUNCTION IMPAIRED BY ACUTE HYPOGLYCAEMIA IN HUMANS

COGNITIVE FUNCTION TESTS IMPAIRED	TARGET BLOOD GLUCOSE (mmol/l)	METHOD OF INDUCTION OF HYPOGLYCAEMIA	SUBJECTS	REFERENCE
Visual reaction times Mathematical tasks	3.3 <sup>v</sup>	Clamp	IDDM	Holmes et al 1983
Visual reaction times	2.7 <sup>a</sup>	Clamp	IDDM Non-diabetic	Herold et al 1985
Verbal fluency Stroop test	3.3 <sup>v</sup>	Clamp	IDDM	Holmes et al 1984
Story recall Trail-making Digit span Finger-tapping	2.0 - 3.0 <sup>v</sup>	Variable insulin infusion	IDDM	Pramming et al 1986
Trail-making Pursuit rotor Driving skills	2.7 <sup>v</sup>	Biostator	IDDM	Hoffman et al 1989
Digit symbol substitution	3.4 <sup>a</sup>	Clamp	Non-diabetic	Stevens et al 1989
Decision and planning tasks	3.3 - 3.6 <sup>a</sup>	Clamp	Adolescent IDDM	Ryan et al 1991
Driving skills	2.6	Biostator	IDDM	Cox et al 1993
Choice reaction times Simple reaction times	2.8 - 3.3	Biostator	IDDM	Driesen et 1991

a arterialised blood glucose  
v venous blood glucose  
IDDM insulin-dependent diabetes mellitus

## **Neurophysiological changes during acute hypoglycaemia**

Neurophysiological measurements are not dependent on patient cooperation and may therefore add important additional information about cerebral function during acute hypoglycaemia, although the clinical significance of some of the tests may be difficult to interpret. Cerebral dysfunction during acute hypoglycaemia has been demonstrated during acute hypoglycaemia using neurophysiological tests. Electroencephalographic (EEG) changes occur during acute hypoglycaemia with decrements and slowing of alpha activity, and an increased occurrence of theta and delta waves, particularly in the frontal areas of the brain (Hoagland et al 1937, Harrad et al 1985, Pramming et al 1988, Tamburrano et al 1988, Bendtson et al 1991). Delays in visual evoked and brain stem potentials which record the cortical changes in electrical activity resulting from an aural or visual stimulus, have also been demonstrated during acute hypoglycaemia (Koh et al 1988, Jones et al 1990).

Another evoked potential, the p300 wave, which reflects the cortical activity of cognitive processes and so combines a measure of cognition and electrophysiology, is recorded by administering a varying visual or aural stimulus such as a combination of soft clicks and loud clicks. With the latter the subject is asked to count the number of soft clicks (which are less frequent than the loud clicks) while the cortical neurophysiological event (the p300 evoked potential) is recorded. This measurement appears to be associated with attention and short term memory but not with general intelligence and reasoning (Polich et al 1983). During acute hypoglycaemia, delayed transmission and reduction in amplitude of p300 evoked potentials have been documented (Jones et al 1990, Tallroth et al 1990). Changes in the p300 wave may be temperature-dependent (Durrant et al 1991), and as core body temperature may fall in response to acute hypoglycaemia



(Molnar and Read 1974), this variable should be measured when interpreting data.

Delays in brain-stem sensory evoked potentials have been observed during hypoglycaemia in Type 1 diabetic patients, with no concomitant alterations in the auditory nerve potentials; the changes observed could be rapidly reversed by the restoration of normoglycaemia (Ziegler et al 1991). This suggests that central rather than peripheral nerve function is most affected. In summary, these variations in EEG, brain-stem, visual evoked potentials and p300 evoked potentials suggest that neuroglycopenia causes widespread cerebral dysfunction during insulin-induced hypoglycaemia.

### **Studies combining cognitive function tests and neurophysiological tests**

The relevance of the alterations in neurophysiological parameters and how they relate to changes in cognitive processes observed during hypoglycaemia is unclear. In an attempt to correlate cognitive dysfunction with neurophysiological changes which occur during acute hypoglycaemia, p300 evoked potentials and reaction times have been studied simultaneously (Blackman et al 1990). Changes in p300 evoked potentials and reaction times occurred in parallel, but the p140 wave, which measures sensory processes, was not affected by hypoglycaemia. Accuracy of choice by the subjects was preserved but a marked variability was noted between subjects with respect to all of the neurophysiological and cognitive function tests. These results suggest that sensory processes are not affected by hypoglycaemia. The p300 wave and reaction time changes occurred in parallel, indicating that some common component of these tests must be affected to cause delayed transmission. As the p300 wave is not a measure of motor activity, presumably some other component of reaction time is affected such as the decision-making process and the central integration of information within the brain. Following the restoration of

blood glucose to normal, a lag phase of a minimum of 30 minutes, and possibly as much as 90 minutes, has been observed before cognitive function (measured by reaction times and p300 changes) returns to normal (Herold et al 1985, Wirsen et al 1992, Blackman et al 1992, Eckert et al 1992). This observation has obvious clinical relevance, in that diabetic patients should allow sufficient time for recovery of cognitive function after an acute episode of hypoglycaemia before attempting to resume tasks which involve decision-making and cognitive skills such as driving or operating machinery .

### **Is there a glycaemic 'threshold' for changes in cognitive function during acute hypoglycaemia ?**

The sequence of neurophysiological and cognitive events which occur during acute hypoglycaemia has been studied in normal subjects and in patients with insulin-dependent diabetes. Variable and disparate results have been obtained which may reflect the different techniques used to induce hypoglycaemia. The temporal sequence of responses which occurs as blood glucose declines is of obvious clinical importance as it may determine whether diabetic patients can detect the onset of hypoglycaemia, enabling appropriate action to be taken before their cognitive function becomes impaired by neuroglycopenia.

Holmes et al (1983, 1984) (using a hyperinsulinaemic glucose clamp), found that cognitive changes were evident in insulin-dependent diabetic patients at a venous blood glucose of 3.3 mmol/l while another study (Pramming et al 1986) (using an intravenous insulin infusion) showed that changes in cognitive functioning occurred when venous blood glucose had declined to between 3.0 and 2.0 mmol/l. By contrast, changes in p300 evoked potentials have been reported to occur at arterialised blood glucose concentrations of 4.0 mmol/l in non-diabetic volunteers, which most clinicians would consider to be normoglycaemia (De Feo et al 1988).

These findings have not been replicated by others who have demonstrated changes in p300 waves in healthy individuals at arterialised blood glucose concentrations of 3.0 (Jones et al 1990) and 2.6 mmol/l (Blackman et al 1990). Another study, using a comprehensive battery of cognitive function tests and a 'stepped' glucose clamp technique demonstrated that the initial changes of cognitive function occurred at an arterialised blood glucose threshold of 2.7 mmol/l (Mitrakou et al 1991). In a group of diabetic adolescents cognitive function was observed to deteriorate when arterialised blood glucose declined to between 3.3 and 3.6 mmol/l, which is above the glycaemic thresholds reported in many adult studies. This suggests that cognitive function in children with diabetes may be more sensitive to the effects of hypoglycaemia (Ryan et al 1990).

Thus it appears that glycaemic thresholds for cognitive dysfunction do occur but the blood glucose concentrations at which changes become apparent is highly variable depending on study techniques and the type and age of subjects studied.

### **How are hypoglycaemic symptoms related to deterioration of cognitive function during acute hypoglycaemia ?**

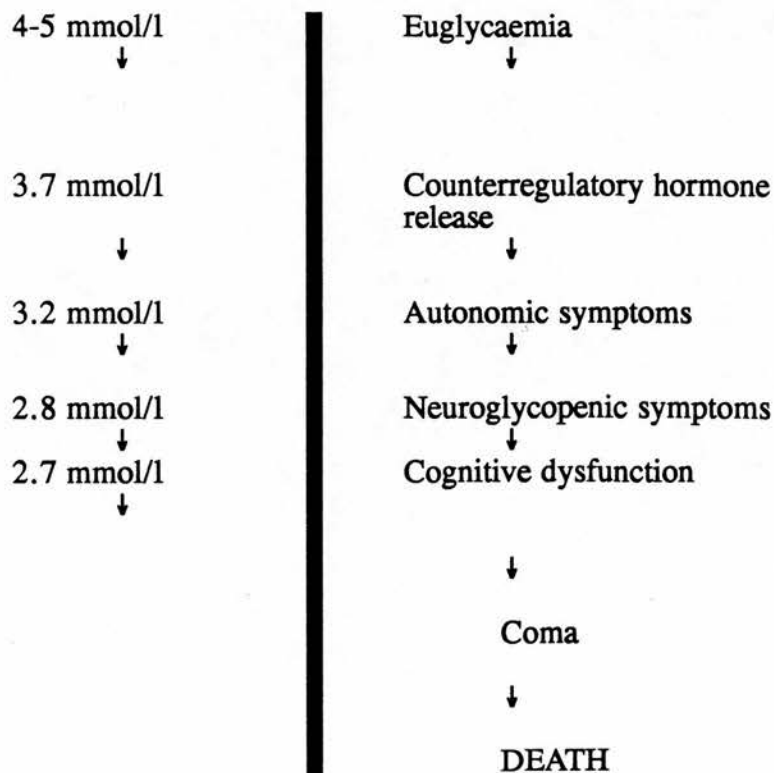
An important question is how the perception of the onset of hypoglycaemic symptoms relates to cognitive dysfunction and the neuroendocrine and neurophysiological responses. A hierarchy of responses has been described as blood glucose declines, commencing with counterregulatory hormonal release (Schwartz et al 1987), and followed by simultaneous changes in cognitive function and the perception of neuroglycopenic symptoms (Mitrakou et al 1991) (Fig. 1.2.1). Some studies in non-diabetic subjects have demonstrated a rise in plasma concentrations of counterregulatory hormones before any demonstrable changes in cognitive function (Ipp and Forster 1987, Stevens et al 1989). De Feo and his

colleagues (1988) reported the converse in non-diabetic subjects; they observed that the p300 evoked potential, as a correlate of cognitive function, developed an increased latency before counterregulatory hormonal secretion had commenced and before hypoglycaemic symptoms had appeared, suggesting that moderate neuroglycopenia precedes perception of symptoms. However the p300 event was the only measure of cognitive function which was examined and this conclusion has been challenged.

FIGURE 1.1.1

**HIERARCHY OF EVENTS DURING ACUTE HYPOGLYCAEMIA  
IN NON-DIABETIC PATIENTS**

(partially adapted from Mitrakou et al (1991))



This relation between perception of the onset of hypoglycaemia and the development of cognitive dysfunction has been investigated in other studies and some diabetic subjects were observed to be unable to perceive the development of hypoglycaemia before their cognitive function became impaired (Prasanna et al 1986). In Cox's study utilising a driving simulator it was observed that many of the subjects demonstrated a deterioration in driving skills at a blood glucose concentration of 2.6 mmol/l, before they were aware of the onset of hypoglycaemia (Cox et al 1991). Similar abnormalities have been demonstrated in studies of patients with insulin-dependent diabetes in whom subjective inability to detect the onset of symptoms of hypoglycaemia was evident during experimental hypoglycaemia so that the patients exhibited overt neuroglycopenia and cognitive impairment (Sussman et al 1963, Heller et al 1987, Hepburn et al 1991b).

The characterisation of diabetic patients with respect to their clinical history of impaired awareness of hypoglycaemia has varied considerably between studies, but definite evidence exists for an alteration in the glycaemic thresholds for autonomic (sympatho-adrenal) activation in affected patients. Diabetic patients with chronic impaired awareness of hypoglycaemia (unrelated to glycaemic control) were noted to have a poorer performance in trail making tests during normoglycaemia (Hepburn et al 1991b). This may reflect the effect of previous exposure to severe recurrent hypoglycaemia, the frequency of which is increased in patients with established loss of awareness of hypoglycaemia (Hepburn et al 1990). Only one patient with loss of awareness was able to complete the trail-making test at an arterialised blood glucose of 1.4 mmol/l while all of the patients with normal awareness of hypoglycaemia were able to complete the task (Hepburn et al 1991b). Patients with apparent impaired awareness of hypoglycaemia, during experimental hypoglycaemia also exhibit cognitive dysfunction (four choice reaction times) at a lower blood glucose than 'aware' control subjects with diabetes (Maran et al 1992), although this shift in glycaemic threshold may be mediated in part by strict



glycaemic control.

When cognitive function and hypoglycaemia symptom scores were assessed in a group of 24 diabetic children immediately after the resolution of symptomatic mild hypoglycaemia (capillary blood glucose less than 3.3mmol/l), cognitive function remained impaired even though the symptom score returned to normal (Puczynski et al 1990). This indicated a dissociation between the more rapid resolution of physical symptoms and the slower recovery of cognitive function following mild hypoglycaemia. In children with diabetes this may be of particular relevance to learning ability and to school examination performance following mild hypoglycaemia.

In summary, although there are few studies in this area it appears that the relation between the threshold for the onset of cognitive dysfunction and symptomatic awareness of the onset of hypoglycemia is variable and may depend on the patients previous history of impaired perception of hypoglycaemia.

### **The effect of glycaemic control on cognitive dysfunction during acute hypoglycaemia**

The results of studies which have examined non-diabetic human subjects (Mittrakou et al 1991) may not be extrapolated directly to patients with diabetes treated with insulin, in whom several factors can influence these putative glycaemic thresholds for the perception of symptoms, autonomic activation and the magnitude of the neuroendocrine responses. These include the quality of glycaemic control, the frequency of previous exposure to hypoglycaemia and the duration of diabetes (Severinghaus 1926, Maddock and Krall 1953, Simonson et al 1985, Lager et al 1986, Amiel et al 1987, Amiel et al 1988, Boyle et al 1988, Heller et al 1991,



Hepburn et al 1991b). In general it is more difficult to assess a change in the glycaemic threshold at which cognitive dysfunction commences as most cognitive tests take some time to administer, during which the blood glucose may have fallen further or cerebral adaptation may have occurred.

Widom and Simonson (1990) compared groups of Type 1 diabetic patients who had either good or poor glycaemic control. A hyperinsulinaemic 'stepped' glucose clamp was used to lower blood glucose and symptoms were subdivided into autonomic and neuroglycopenic groups. The glycaemic thresholds for neuroglycopenic symptoms and for impairment of cognitive function were unaffected by the antecedent glycaemic control, but more profound hypoglycaemia was required to activate counterregulatory hormonal release and autonomic symptoms in the diabetic patients with good glycaemic control. Similarly, in a mixed group of insulin-dependent diabetic patients with strict glycaemic control and non-diabetic patients who had chronic hypoglycaemia caused by an insulinoma, the thresholds for autonomic activation and counterregulatory hormonal secretion were higher ie a lower blood glucose was required for the initiation of these responses, while the threshold for neuroglycopenic symptoms was unchanged compared to a control group (Amiel et al 1991). EEG changes provoked by hypoglycaemia were most pronounced in the diabetic patients who had good glycaemic control, suggesting a greater cerebral vulnerability to neuroglycopenia and indicating a dissociation of the impairment of cognitive function from activation of the autonomic nervous system.

Blackman et al (1990) extended their studies in normal subjects to examine subjects with Type 1 diabetes with poor glycaemic control (Blackman et al 1992), and observed a wide range of thresholds of arterialised blood glucose (3.5-2.5 mmol/l) for increased p300 latency which did not differ significantly from those of a nondiabetic group. By contrast, Jones et al (1991), using a glucose clamp

technique, have demonstrated that the thresholds for p300 latency, the secretion of adrenaline and the onset of symptoms of hypoglycaemia all occurred at lower blood glucose concentrations in strictly controlled patients with Type 1 diabetes compared with non-diabetic control subjects, suggesting that susceptibility to cerebral dysfunction was diminished in the patients with diabetes. They postulated that patients with diabetes who are treated intensively with insulin develop a resistance to neuroglycopenia and do not release counterregulatory hormones or experience symptoms of hypoglycaemia until the blood glucose reaches a much lower level than in non-diabetic subjects.

The effect of glycaemic control on the latency and amplitude of p300 evoked potentials in patients with Type 1 diabetes has been examined during hypoglycaemia induced by a intravenous insulin infusion (Ziegler et al 1992). Those patients with strict glycaemic control developed cognitive dysfunction at significantly lower venous blood glucose values (1.6mmol/l) than the patients with poor glycaemic control (3.5mmol/l), ie the threshold for inducing cognitive impairment was higher. Although the authors acknowledge that the absolute thresholds cannot be determined using this technique the sequence of events demonstrated that the group with poor control often exhibited cognitive dysfunction before the counterregulatory hormonal responses had commenced and before they had reported subjective awareness of hypoglycaemia, suggesting that strict glycaemic control may induce a degree of cerebral 'adaptation' to modest neuroglycopenia. Similarly, patients with functional hypoglycaemia (as opposed to insulin-induced hypoglycaemia) have been shown to have a deterioration in cognitive function and onset of counterregulatory hormonal secretion at higher blood glucose concentrations than control subjects (Snorgaard et al 1991).

In conclusion the effect of glycaemic control on cognitive function remains

controversial, with some studies reporting a deterioration in cerebral function at the same blood glucose concentrations (Amiel et al 1991) while some report no effect on cerebral function (Widom and Simonson 1990, Blackman et al 1992) and yet others reporting a degree of cerebral adaptation to hypoglycaemia in patients with strict glycaemic control (Jones et al 1991, Ziegler et al 1992). These disparate results probably reflect the variable techniques and protocols for inducing hypoglycaemia and the tests used to measure cerebral function. In addition glycated haemoglobin which was used as measure of glycaemic control only gives information about average control two months prior to the study and does not necessarily reflect the frequency of episodes of mild or asymptomatic hypoglycaemia which may have occurred during that period.

### **Cerebral adaptation to acute hypoglycaemia**

The few studies which have examined how the symptoms of hypoglycaemia are modified in association with alterations in cognitive function suggest that some degree of cerebral adaptation to prolonged hypoglycaemia can occur. The total symptom score of hypoglycaemia has been found to parallel changes in reaction time in non-diabetic and in insulin-dependent diabetic patients. After exposure to constant mild hypoglycaemia for 40 minutes (arterialised blood glucose 3.0 mmol/l) induced by a glucose clamp, both the reaction times and the symptom scores improved in parallel (Kerr et al 1989, Kerr et al 1991a). This evidence may represent an important cerebral adaptive mechanism in insulin-dependent diabetic patients, which preserves and protects intellectual function even when exposed to moderate neuroglycopenia.

In rats subjected to chronic hypoglycaemia the uptake of glucose in the brain was increased (Cremer et al 1983, McCall et al 1986) as was the brain glucose transport capacity (Pelligrino et al 1990). During hypoglycaemia in non-diabetic

human subjects there appears to be facilitated transport of glucose from blood to brain during moderate hypoglycaemia (arterialised blood glucose 2.7mmol/l) which is accompanied by a reduction in brain glucose consumption (Blomqvist et al 1991). It is possible that further adaptation of glucose transporter mechanisms may occur in humans with insulin-dependent diabetes in a manner similar to that demonstrated in the animal studies.

While it is plausible that neuroglycopenic symptoms and cognitive function will change simultaneously and in parallel in response to acute hypoglycaemia, more investigation is required to confirm this premise. In addition, care must be taken with study design and with the methodology used to induce hypoglycaemia as high plasma insulin concentrations have been shown to modify the intensity of hypoglycaemic symptoms but do not appear to affect cognitive function (Kerr et al 1991b). It is not known whether neuroglycopenic symptoms and autonomic activation are dissociated during episodes of accidental insulin-induced hypoglycaemia in daily life or whether these changes are artefactual as a product of the artificial (and unphysiological) glucose clamp techniques used in the laboratory to induce hypoglycaemia.

The changes in cognitive function during acute hypoglycaemia are summarised in Table 1.2.3.

TABLE 1.2.3

**CHANGES IN COGNITIVE FUNCTION OBSERVED  
DURING ACUTE HYPOGLYCAEMIA**

- complex cognitive function tests such as choice reaction times, digit symbol substitution and trail-making B are affected
- accuracy is generally preserved at the expense of a slower response
- considerable inter-subject variability is often present
- diabetic patients may be more affected than normal controls
- effects on memory are less well defined but do not appear to be of major significance; in particular there are few demonstrable effects on immediate memory
- changes in neurophysiological function occur such as EEG changes and latency of evoked potentials occur
- recovery of cognitive function is delayed following hypoglycaemia (but not EEG changes).
- glycaemic thresholds at which cognitive dysfunction occurs vary depending on mode of investigation
- cognitive dysfunction may precede perception of symptoms and hormonal counterregulatory responses in some patients during acute hypoglycaemia, especially patients with hypoglycaemia unawareness or strict glycaemic control
- during prolonged acute hypoglycaemia initial cognitive dysfunction may resolve despite persisting hypoglycaemia, suggesting cerebral adaptation



## **CLINICAL RELEVANCE OF CHANGES IN COGNITIVE FUNCTION CAUSED BY HYPOGLYCAEMIA**

Changes in cognitive function of a variable degree may occur and in some cases may be sufficiently profound as to significantly affect a patient's ability to function in everyday life. In clinical practice it is uncommon for individual patients to develop significant cerebral damage or cognitive deficits after an isolated episode of severe hypoglycaemia, but more likely that recurrent severe hypoglycaemia has a cumulative effect producing a subtle and insidious decline in cognitive function and IQ over a number of years. As this occurs slowly, it may be erroneously ascribed to the degenerative changes associated with the progression of macrovascular complications, such as cerebrovascular disease, in patients with Type 1 diabetes of long duration.

The degree to which an average decline in IQ of 5.8 points (Langan et al 1991) will affect performance in daily tasks is not possible to predict, but it is likely that any decline in cognitive function is clearly of major importance to patients with Type 1 diabetes in occupations which are intellectually demanding and require the rapid handling of data and information. It is important to appreciate that the estimated decline in IQ of 5.8 points is an average measure, and that some patients exhibited a greater decrement, which may have a more significant effect on intellectual ability. Multiple episodes of severe hypoglycaemia may occur in a few unfortunate patients and they are therefore probably most at risk of a greater decline in IQ which might interfere with performance at work. Aspects of quality of life other than potential changes in cognitive function may also be affected by recurrent hypoglycaemia; patients who have experienced recurrent episodes of severe hypoglycaemia have been reported to be more anxious and to score lower in 'happiness' rating scales compared with diabetic patients without severe



hypoglycaemia (Wredling et al 1992).

The aetiology of cognitive dysfunction in diabetic patients is probably multifactorial, and may be associated with both peripheral and 'central' forms of neuropathy (Khardori et al 1986, Pozzessere et al 1988, Pozzessere et al 1991, Ryan et al 1992, Dejgaard et al 1991) resulting from a combination of chronic hyperglycaemia, and exposure to recurrent severe hypoglycaemia.

Not all patients exposed to hypoglycaemia develop problems with impaired awareness of hypoglycaemia and irreversible cognitive dysfunction which suggests that patients are not equally susceptible to the pathological effects of neuroglycopenia, and may not therefore develop cognitive dysfunction despite recurrent hypoglycaemia. Until vulnerable patients can be identified, one of the most important therapeutic dilemmas in the modern management of diabetes is whether diabetic patients should have to risk sacrificing intellectual capacity through an increased exposure to severe hypoglycaemia in order to prevent the complications of microangiopathy. This is of particular relevance to the management of diabetic children, especially those who are very young at the time of diagnosis. In these patients, recurrent severe hypoglycaemia may have such a profound effect on their cerebral and intellectual development as to seriously limit their potential achievement in adult life.



PART I (continued)

CHAPTER 3

**HYPOTHESES FOR STUDIES**

## **Acute Hypoglycaemia and Cognitive Function - Hypoglycaemic Clamp Studies**

There is a large inter-individual variability in the effects of acute hypoglycaemia and it is important to try and identify any factors that may make an individual more prone to developing cognitive dysfunction as a result of recurrent hypoglycaemia so that targets of glycaemic control can be modified in these patients. This would permit the majority of patients with Type 1 diabetes to aim for optimal glycaemic control to avoid or delay the development of the long term microvascular complications of diabetes. In addition little is known about the cerebral response to protracted acute hypoglycaemia ie the phenomenon of 'cerebral adaptation' remains poorly defined. Kerr and colleagues (Kerr et al 1989, 1991a) suggested that during hypoglycaemia of 40- 60 minutes the brain adapts to the effects of neuroglycopenia and the cognitive dysfunction which initially occurs, becomes less profound as the duration of hypoglycaemia increases. Further studies in this area are required to try and identify the presence of such adaptation and to define the possible mechanisms operating. The hypothesis that 'cerebral adaptation to hypoglycaemia occurs' was therefore postulated and a Study 1 was designed to investigate this further.

In view of the large inter-individual variation in the degree of cerebral dysfunction experienced during hypoglycaemia, two important factors were investigated as possible causes of this variability; firstly the effect of IQ on cognitive dysfunction during hypoglycaemia and secondly the effect of subjective awareness of hypoglycaemia on cognitive dysfunction during acute hypoglycaemia. The effect of other cerebral insults, such as alcohol, on cognitive function have been investigated previously and it was shown that subjects of greater IQ were more susceptible to cognitive dysfunction after alcohol consumption than those of a lower IQ (Maylor et al 1990). Using hypoglycaemia as the metabolic insult further information on cognitive processes as well as the nature of the effect of

hypoglycaemia could be investigated. Study 2 was therefore designed to assess the effect of IQ on the cognitive response to acute hypoglycaemia.

A previous study suggested that patients with impaired awareness of hypoglycaemia appeared to be more susceptible to the effects of acute hypoglycaemia (Hepburn et al 1991b). However this study had not been designed to address this question and the patient groups were not matched. Study 3 was designed to establish whether patients with insulin-treated diabetes, who have acquired impaired awareness of hypoglycaemia exhibit a more severe degree of cognitive impairment during moderate hypoglycaemia. The mechanism for altered perception of the symptoms of hypoglycaemia and the delayed initiation of counterregulatory responses may result from a defective glucose sensor. However no discrete glucose sensor has been located and if it was present within the brain it might be expected that the alteration in its function may result from defective delivery of blood glucose to the receptor ie an alteration in regional blood flow. Therefore as an adjunct to study 3, regional cerebral blood flow was measured during hypoglycaemia in patients with impaired awareness of hypoglycaemia (study 4).

Although patients frequently describe mood changes during and after episodes of hypoglycaemia occurring in daily life there have been no formal assessments of this. The subjects in studies 1 and 2 were therefore questioned about mood changes during acute hypoglycaemia, using a standardised questionnaire (study 5).

## **Clinical Studies of Patients with Type 1 Diabetes**

The actual frequency of severe hypoglycaemia in patients with impaired awareness has only been documented retrospectively and as this potentially fatal complication is more common in such patients it is important to know its true prevalence. A year long prospective study (study 6) was designed to assess and compare the frequency and morbidity of severe hypoglycaemia in patients with Type 1 diabetes with and without normal awareness of hypoglycaemia.

Although there are now reports of the long term effects of hypoglycaemia on cognitive function as tested by formal psychological tests already described, there are few reports of the actual impact of recurrent severe hypoglycaemia on personality, social function or everyday life. A carers' group identified five patients with a long duration of diabetes, all of whom had suffered problems with work, home life and relationships. A predominantly descriptive account (study 7) is provided of these problems and possible causes identified.

PART II

**METHODS FOR HYPOGLYCAEMIC CLAMP STUDIES**



### **Ethical Permission**

Permission was granted for each of the studies by the Lothian Health Board Ethics of Medical Research Subcommittee for Medicine and Clinical Oncology (reference numbers: MCO/71/91, MCO/134/91, MCO/78/91, MCO/77/91, MCO/76/91). Written consent was obtained from all subjects following detailed explanation of the nature of the studies (see Appendix for 'Subject Explanation Sheets').

### **Subjects**

The details of criteria for selection of subjects for each study are explained in the individual study chapters.

### **The Hyperinsulinaemic Glucose Clamp Technique**

All of the hypoglycaemia studies were performed using a modified manual hyperinsulinaemic glucose clamp technique to manipulate blood glucose (DeFronzo et al 1979). In this method insulin was infused at a fixed rate, and a variable rate of infusion of 20% dextrose was used to manipulate the blood glucose concentrations. Insulin was initially infused at a rate of 150 mU/m<sup>2</sup>/min and reduced to and then maintained 60 mU/m<sup>2</sup>/min over a period of 10 minutes (see Appendix). This loading dose saturated the insulin receptors to facilitate subsequent manipulation of the blood glucose. The 20% dextrose infusion was commenced after about 5 minutes in the non-diabetic subjects, initially at a rate of about 1 ml/kg/hour. In the diabetic subjects the dextrose infusion was only commenced after the blood glucose concentration had reached less than 5 mmol/l. The subsequent infusion rate of the dextrose was determined by the arterialised blood glucose concentrations measured at the bedside. In order to induce hypoglycaemia the dextrose infusion was stopped for about five minutes and then recommenced at about half of the rate used during stable normoglycaemia.

Although the glucose clamp technique is unphysiological, it is the only method available for reliably maintaining the blood glucose at predetermined concentrations for prolonged periods of time, sufficient to allow administration of the cognitive test batteries.

### **Experimental Procedure for Clamp Studies**

Subjects were fasted for at least four hours prior to study. A teflon cannula was inserted into an antecubital vein in the non-dominant arm under local anaesthesia (lignocaine 1%) for the infusion of human soluble insulin (Actrapid; Novo Nordisk Laboratories, Copenhagen, Denmark) and the variable rate infusion of 20% dextrose. Arterialised venous blood samples were obtained at three minute intervals from a retrograde cannula in a dorsal hand vein of the same arm; the hand was warmed in a heated box (55°C) and the cannulae were kept patent with 0.9% sodium chloride. Dextrose was infused using an IVAC Site Saver pump and insulin was infused (60mU/m<sup>2</sup>/min) using an IMED Gemini PC1 pump. The rate of dextrose infusion was adjusted every 3 minutes according to the blood glucose concentration measured at the bedside using a glucose oxidase method (Yellow Springs Instrument 2300 Stat, Yellow Springs, Ohio, USA). Warming the hand allows shunting of blood from the arterial to venous system and "arterialised" venous blood glucose concentrations have been shown to approximate very closely with true arterial blood glucose concentrations and are necessary as venous blood glucose concentrations are highly variable, particularly in combination with the glucose clamp technique (Liu et al 1992).

The subjects were not informed of their blood glucose concentrations during the experiments. The blood glucose concentration was initially stabilised and maintained at 4.5 mmol/l in all the studies and the subsequent manipulations of the concentrations are explained in the individual study chapters.

### **Choice of Degree of Hypoglycaemia**

In all of the studies, the degree of hypoglycaemia used was 2.5 mmol/l. The reason for the choice of this value was that although there is considerable variation in the glycaemic thresholds for changes in cognitive function, most of the recent studies have documented a change at blood glucose concentrations above this level (as discussed in Part I, Chapter 2). In order to be sure that the glycaemic threshold for cognitive dysfunction had been reached and in an attempt to produce as greater change in cognitive function as possible, the lowest glucose concentration which could be tolerated safely by the subjects, for the duration of at least one hour, was used.

### **Catecholamine and Physiological Measurements**

Blood was withdrawn at certain time points (indicated in the relevant chapters), before being separated and stored at -40°C. Plasma catecholamines were assayed using an HPLC method with an electrochemical detector (Goldstein et al 1981). Continuous heart-rate measurements were recorded from precordial electrodes using an ECG monitor, and blood pressure was recorded with a Copal sphygmomanometer at the times indicated in the individual studies.

### **Assessment of Symptoms of Hypoglycaemia**

A standard symptom questionnaire was devised (see Appendix) on the basis of previous studies assessing symptoms of hypoglycaemia (Hepburn et al 1991a, Deary et al 1993b). Each subject was required to complete the questionnaire at appropriate time points described in each study. As far as possible the subjects were allowed to complete the questionnaire in their own time without the interference of the experimenters in order to obtain accurate answers. The symptoms were divided into three groups according to the classification established in a previous study (Hepburn et al 1991a, Deary et al 1993b) a) - *autonomic* (anxiety, pounding heart, sweating, tremor, hunger, warmness and shivering), b) -

*neuroglycopenic* (difficulty speaking, confusion, drowsiness, dizziness, inability to concentrate, double vision and blurred vision) and c) - *non-specific* (tingling lips, nausea, headache, weakness). Each symptom was graded on a 1 to 7 scale, 1 indicating that the symptom was 'not present at all' and 7 indicating that the symptom was present 'a great deal'; the minimum total score was therefore 18 and the maximum possible score was 126.

### **Assessment of Changes in Mood during Hypoglycaemia**

The UWIST Mood Adjective Checklist (UMACL) (Matthews et al 1990) was used to assess mood at the same time points that symptoms were assessed (see Appendix). The UWIST questionnaire has previously been validated and measures the three factors of mood about which there is most agreement (Matthews et al 1990). The subjects completed the questionnaire on their own to avoid attribution bias. Mood-relevant adjectives were graded by intensity on a scale of 1 to 4 and were classified into three groups : hedonic tone, tense arousal and energetic arousal. Higher scores indicated a high 'happiness' rating for hedonic tone, a high 'anxiety' rating for tense arousal and a high 'energy' rating for energetic arousal. The maximum score which could be attained for each of the three mood components was 32 and the minimum score was four.

### **Cognitive Measurements**

Tests of complex cognitive processes were utilised as they have been shown to be more profoundly affected by acute hypoglycaemia than tests of simple processes. All of the tests have been well validated and many have also been used in other studies of cognitive function in patients with diabetes. In addition two of the tests, the Trail-Making B (TMB) and Digit Symbol Substitution Test are the most frequently used subtests (in psychopharmacological and neuropsychological studies) from the two best known neuropsychological batteries: the Holstead-Reitan and



Wechsler Adult Intelligence Scale respectively. Both of these subtests are known to be sensitive to mild cerebral insults and it was important to include such tests as these as deterioration in these tests provided confirmation that the degree of cerebral dysfunction induced was satisfactory. The other three tests the Paced Auditory Serial Addition Test (PASAT), Rapid Visual Information Processing (RVIP) and Four Choice Reaction Time (CRT) are also known to be sensitive to mild cerebral insults and were used because they are tests of complex information processing. In each of the studies a combination of these five tests, detailed below, was utilised, and the details of the order and duration of each test battery are given in the relevant study chapters. In each of the studies the tests were administered in a fixed order on each occasion.

1 - Paced Auditory Serial Addition Test (PASAT) (Deary et al 1991a)(see Appendix): the subject listens to a fixed random sequence of the numbers 1 to 9 read aloud at 4 second intervals and is required to add the first number to the second number and give the answer, the second number to the third number and give the answer and so on for a total of 61 numbers. The process is then repeated with the numbers being read at 2 second intervals. The score is the total number of correct answers on each run (ie maximum score of 60). The PASAT is said to be a measure of attention and concentration (Gronwall and Wrightson 1977, Deary et al 1991a); the 4 second PASAT has been shown to be associated with short term memory and whereas the 2 second test is more closely related to perceptual processing speed (Deary et al 1991a).

2 - Digit Symbol Substitution test (DSST) (Wechsler 1981)(see Appendix): in this subtest of the Wechsler Adult Intelligence Scale - Revised, a symbol must be substituted for each digit (using a key provided) and the score is the total number of correct symbols drawn in 90 seconds. This test provides a test of discretionary and decision-making skills.

3 - Trail-Making B (TMB) (Reitan et al 1974)(see Appendix): this is a subtest of the Halstead-Reitan Neuropsychological Test Battery. Nine alternative versions were produced and validated for equal degrees of difficulty to avoid potential practice effects which might have occurred if the same trail had been used repeatedly. They were administered in random order to each of the subjects in each study so that no subject performed the same trail more than once and all the tests were utilised equally and in different orders. The subject joins numbers to letters in sequence (eg 1 to A, A to 2, 2 to B, B to 3, 3 to C etc.) and the time required to complete the task is measured. This test provides a measure of visual searching, mental set shifting and psychomotor speed.

4 - Rapid Visual Information Processing (RVIP) (Wesnes and Warburton 1984, Deary et al 1992): a random sequence of digits (1-9) is shown individually on a monitor over a period of 5 minutes and the subject is asked to press a button each time a series of 3 consecutive even or odd numbers occurs. Three measures are taken from the test a) the total number of correct answers ie targets detected ('hits'), an estimate of ability to encode, store and classify digits, and make comparisons at speed; b) the number of false alarms ie responses to non-target sequences ('misses'), an indication of the response threshold or caution of the subject and c) the time taken to respond to each sequence ('reaction time' - RT) is recorded, an indication of the motor aspects of the decision making process. The subjects are not instructed to respond quickly in this test and so the significance of the 'reaction time' is doubtful. This test was run on a BBC microcomputer. The total number of hits over five minutes, the total number of misses and the mean reaction time were used in analyses.

5 - Four Choice Reaction Time (CRT) (Hick 1952, Jensen 1987, Deary et al 1992): a device is used which measures both decision time and movement time;



this consists of a black box with a sloped top on which lies a 'home' button and four equidistant response buttons. The subject is asked to press the home button with the preferred finger of the dominant hand; when one of the response buttons is lit, a timer begins and is stopped when the subject's finger leaves the 'home' button, recording the decision time (DT). When the finger is lifted from the home button this also starts a second timer which is stopped when the appropriate response button is pressed by the subject, using the same finger, giving a measure of movement time (MT). The subject completed 30 responses in three batches of ten, separated by a few seconds rest. The test was controlled by a BBC Master microcomputer. The mean scores for DT and MT for all 30 responses were used for analysis.

Before commencing the study each subject was required to perform all of the cognitive tests to minimise any practice effect during the experimental procedures. Similar instructions were given to each subject but as the patients were of different cognitive abilities, some required longer practice times in order to become familiar with the tests.

### **Tests of Intelligence**

Prior to enrolment into the studies, the level of cognitive ability in each subject was assessed using a combination of the Alice Heim 4 test (AH4, Heim 1968) and the National Adult Reading Test (NART, Nelson 1982). In the AH4 test, which is divided into two parts, the subject completes as many questions as possible in ten minutes in each part. A choice of answers is provided for each question; the first part of the test provides an assessment of verbal and numerical skills while the second part provides an assessment of visuo-spatial skills. The test does not provide an actual IQ but merely an indication of global cognitive ability. The maximum score in each part is 65. The NART assesses premorbid IQ, that is the peak level of mental ability attained by a subject prior to any cognitive

deterioration; the subject is asked to pronounce 50 irregular English words (eg ache, depot) and the number pronounced correctly is the score obtained. The NART has been validated against the Wechsler Adult Intelligence Scale and has been shown to correlate closely with this test in subjects without reason for cognitive decline (Crawford 1989).

### **Statistical Methods**

The individual tests utilised are detailed in the relevant study chapters. All statistical procedures were performed using an IBM computer with software package SPSS-PC or SPSS-X. Cognitive function scores in the general population are normally distributed and in each of the studies the scores were also demonstrated to approximate to a normal distribution and therefore the use of parametric statistical analysis was justified.

PART III

CHAPTER 1

*Study 1*

**SHORT TERM CEREBRAL ADAPTATION TO ACUTE INSULIN-INDUCED  
HYPOGLYCAEMIA IN NON-DIABETIC SUBJECTS**

## INTRODUCTION

The capacity of the brain to adapt to neuroglycopenia would represent an important adaptive mechanism to reduce the cognitive dysfunction which occurs during insulin-induced hypoglycaemia. Studies in rats have suggested that chronic hypoglycaemia results in adaptation of glucose transporters in order to increase brain glucose uptake (Cremer et al 1983, McCall et al 1986, Pelligrino et al 1990). In humans subjects a degree of cerebral adaptation has been demonstrated after prolonged hypoglycaemia of 56 hours duration (Nagy et al 1993). Previous studies in normal humans and in patients with Type 1 diabetes have suggested that tolerance to asymptomatic hypoglycaemia may develop within 60 minutes of hypoglycaemia, as a result of rapid cerebral adaptation to neuroglycopenia (Kerr et al 1989, Kerr et al 1991a). However in these studies only a single measure of cognitive function was used to assess cerebral changes during neuroglycopenia, and the method used to estimate symptom scores was primitive and of limited value. The aim of the present study was to use an extensive battery of cognitive tests and a validated symptom scoring system to assess the nature and degree of deterioration in cognitive function during hypoglycaemia and to examine whether any improvement in cognitive function and symptom scores could be detected after a period of continuous exposure to moderate hypoglycaemia, so indicating the development of cerebral adaptation.

## **METHODS**

### **Subjects**

24 healthy non-diabetic subjects were recruited (13 male, 11 female) with a mean age of  $29.5 \pm 4.3$  years ( $\pm$ SD) and a mean body mass index of  $23.9 \pm 2.7$  kg m<sup>-2</sup> ( $\pm$ SD). Eleven of the subjects were medical or paramedical staff and the remaining subjects had a variety of different educational backgrounds. Seventeen of the subjects were right handed and seven were left handed. None of the subjects had any relevant medical history of note and none was taking any regular medication other than the oral contraceptive pill. None of the subjects had a family history of diabetes.

### **Experimental Procedure**

All subjects were studied on three separate occasions, each at least two weeks apart. After a light breakfast the subjects fasted until conclusion of the study. The blood glucose concentrations were controlled using a hyperinsulinaemic glucose clamp as described in Part II.

The study design is shown in figure 3.1.1. On one occasion (Study A) the blood glucose concentration was stabilised and maintained at 4.5 mmol/l while the first battery of cognitive tests (T1) was administered; after completion of the tests the blood glucose was stabilised at 4.5mmol/l for a further 40 minutes, following which a second cognitive test battery was performed (T2). Twenty minutes after the second test battery was completed a further abbreviated test battery was presented (T3) while the blood glucose was maintained at 4.5 mmol/l, and this was repeated after a further 15 minutes (T4). This euglycaemic study (A) served as a control study for Studies B and C, both of which involved the induction of hypoglycaemia (2.5mmol/l). Induction of hypoglycaemia took about 10 minutes, after completion of the first cognitive test battery (T1). In Study B the second

cognitive test battery (T2) was administered immediately after induction of hypoglycaemia (2.5 mmol/l). In Study C the blood glucose concentration was maintained at 2.5 mmol/l for 40 minutes before the administration of the second cognitive test battery (T2), and was then returned to euglycaemia (4.5 mmol/l). In Studies B and C the blood glucose was maintained at 2.5 mmol/l for a total of 60 minutes before returning to euglycaemia (4.5 mmol/l). Abbreviated test batteries (T3 and T4) were administered 15 and 30 minutes after stable euglycaemia had been re-established in Studies B and C, corresponding to T3 and T4 in Study A (figure 3.1.1). As the order of the studies could be arranged in six possible sequences, four subjects were randomly allocated to each sequence to counterbalance the order of the study conditions. The subjects were not informed of their blood glucose concentrations during the experiments.

At each of the seven time points indicated in figure 3.1.1 by (S), the symptom questionnaire described in Part II was completed by each subject. In Studies B and C the symptom scores were given at identical times irrespective of difference in timing of the cognitive test batteries. Continuous heart-rate measurements were recorded at the times indicated (figure 3.1.1).

### **Cognitive Test Battery**

The cognitive test battery utilised in this study comprised five tests (described in Part II) and took approximately 20 minutes to administer in the following order:

- 1 - Paced Auditory Serial Addition Test (PASAT) (Deary et al 1991a)
- 2 - Four Choice Reaction Time (CRT) (Hick 1952, Deary et al 1992)
- 3 - Digit Symbol Substitution test (DSST) (Wechsler 1981)
- 4 - Trail-Making B (TMB) (Reitan 1974)
- 5 - Rapid Visual Information Processing (RVIP) (Deary et al 1992)



These five cognitive tests were administered at each of the times T1 and T2 in each of the studies. A shortened test battery comprising the Four Choice Reaction Time and the Digit Symbol Substitution Test, and lasting approximately 6 minutes, was administered at times T3 and T4 in order to assess recovery of cognitive function after hypoglycaemia.

On a separate day before commencing the study, each subject was required to perform all of the cognitive tests in the above order, to minimise practice effects during the experimental procedures.

### **Statistical Analysis**

Multivariate analysis of variance (MANOVA) was used to assess the effects of hypoglycaemia on cognitive function. The main independent variable was *Study*, a within subjects factor with three levels, "euglycaemia" (Study A), "early hypoglycaemia" (Study B) and "late hypoglycaemia" (Study C). The effect of *Study*, therefore, incorporates any effect of hypoglycaemia on performance, and differences between the two hypoglycaemia conditions.

There was a large inter-individual difference in baseline cognitive test scores for the subjects, as there were subjects of relatively low and relatively high IQ levels (see Part III Chapter 2). For this reason therefore the absolute *change* from the baseline cognitive score (T1) to the score obtained in the experimental condition (T2) was the measure used for each cognitive test. In all studies the first test period represents a baseline score assessed during euglycaemia (T1). The second test period (T2) represents the score during early hypoglycaemia in Study B, late hypoglycaemia in Study C, and a second test at euglycaemia in Study A. The dependent variables, comprising the within subjects factor *Test*, were the nine scores derived from the cognitive function tests : four and two second PASAT,

DSST, TMB, RVIP hits, RVIP misses RVIP reaction times, Four Choice decision time (DT) and movement time (MT).

The overall significance in the multivariate tests was calculated from the Wilks' Lamda test statistic. Separate univariate analyses of variance (ANOVA) were used to assess the effects of the differential study conditions on the individual cognitive tests, symptoms, blood glucose, blood pressure and heart rate. Significant results were further analysed using t-tests to determine specific differences between the study conditions. In order to minimise the Type 1 error rate, the following planned comparisons were made in the assessment of cognitive function scores:

i) Study B scores vs Study C scores - this test compares the effect of immediate hypoglycaemia (Study B) with prolonged hypoglycaemia (Study C).

ii) Study A scores vs mean of (Study B + Study C) scores - this test compares the effect of euglycaemia with hypoglycaemia *per se*.

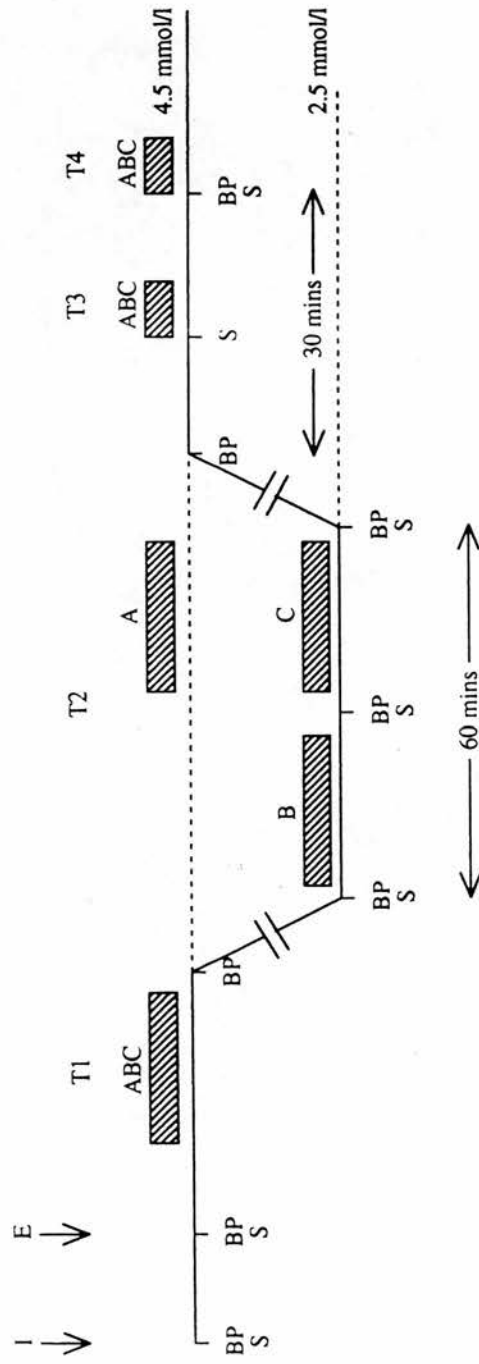


FIGURE 3.1.1  
**STUDY DESIGN.** I - time of commencing insulin infusion; E - euglycaemia; BP - time of measurement of blood pressure; S - time of symptom assessment; hatched area indicates time of cognitive testing T1 to T4 in each of studies A, B and C.

## **RESULTS**

### **Blood glucose**

The blood glucose profiles of all three studies are shown in figure 3.1.2. In the hypoglycaemia Studies (B and C) the profiles appeared very similar throughout which was confirmed by testing with analysis of variance. At the times of cognitive testing during hypoglycaemia, ie between 5 and 25 minutes of hypoglycaemia in Study B and between 40 to 60 minutes of hypoglycaemia in Study C, no differences in blood glucose concentrations could be discerned between the Studies B and C.

### **Blood pressure and heart rate**

The blood pressure and heart rate profiles are shown in figures 3.1.3a, b and c. The systolic blood pressure did not change substantially in any of the studies, although the expected fall in diastolic blood pressure and rise in heart rate were observed during both hypoglycaemia studies. Analysis of variance confirmed a significant difference among the studies across time (Study by Time interaction  $p=0.03$ ) for the diastolic blood pressure ie that the diastolic blood pressure fell significantly during the hypoglycaemia studies compared with the euglycaemia control study.

### **Cognitive function**

The results of the cognitive function testing are shown in figure 3.1.4. and the raw scores are shown in table 3.1.1. For all test variables a positive deflection indicates an improvement in function, whereas a negative deflection indicates a deterioration in function. At baseline, no significant differences were present among the three studies for any cognitive test variable.

### *Multivariate analysis*

Multivariate analysis of variance revealed a significant effect of *Study* on overall cognitive performance ( $p=0.01$ ), and also a significant effect of *Test* ( $p=0.01$ ), with a significant interaction between *Study* and *Test* ( $p=0.001$ ). This indicates that there were significantly different effects on cognitive performance among the three study conditions, and also that hypoglycaemia had a differential effect on the individual tests, necessitating further analysis of each test separately.

### *Univariate analysis*

The results of univariate analysis of variance for each test are shown in table 3.1.2. For the Four Choice reaction time and the DSST there is an additional "time" factor, because each of these tests was also performed twice during recovery from hypoglycaemia (T3 and T4).

PASAT - (Figure 3.1.4a). In Study A there was an improvement from baseline score, whereas in both Studies B and C there was a significant deterioration during hypoglycaemia. This applies to both the 4 second test and the 2 second test (table 1: Study effect). Further analysis with t-tests showed that although there was a significant effect of hypoglycaemia *per se* on performance (A v mean [B+C],  $p=0.006$  for 4 second and  $p<0.001$  for 2 second), no significant difference in performance occurred between Studies B and C, ie PASAT performance showed no improvement with persisting hypoglycaemia.

Four Choice Reaction Time - (Figures 3.1.4b and c). Performance remained stable during the euglycaemia control Study A, whereas slowing was observed both in DT and MT during hypoglycaemia (Studies B and C). DT and MT in Studies B and C returned towards baseline values during the recovery period. Analysis with t-tests confirmed a significant deleterious effect of hypoglycaemia on performance (A v mean [B+C],  $p=0.05$  for DT and  $p=0.002$

for MT). The time of testing after onset of hypoglycaemia (Study B vs Study C) did not affect DT performance. After prolonged hypoglycaemia (Study C), the MT was significantly slower than in Study B ( $p=0.018$ ), ie movement time *deteriorated* increasingly with prolonged hypoglycaemia. Performance during recovery did not differ significantly from baseline.

DSST - (Figure 3.1.4d). In Study A performance improved throughout the study, but in Studies B and C performance deteriorated during hypoglycaemia, returning towards normal in the recovery periods. Further analysis with t-tests confirmed the effect of hypoglycaemia on performance (A v mean [B+C],  $p=0.004$ ), and the absence of a significant difference between Studies B and C indicated that no adaptation had occurred. During the recovery periods the subjects' performances were not significantly different from the euglycaemia study (A vs [B+C]).

TMB - (Figure 3.1.4e). A trend towards an improvement in performance in Study A was observed with deteriorations in performance in Studies B and C. Analysis with t-testing confirmed a significant effect of hypoglycaemia (A v mean [B+C]  $p=0.007$ ). No difference in performance was observed between Studies B and C indicating that there was no adaptation in TMB performance as hypoglycaemia persisted.

RVIP hits - (Figure 3.1.4f). Performance did not change during Study A and deteriorated during hypoglycaemia (Studies B and C). Further analysis with t-tests confirmed the effect of hypoglycaemia (A v Mean [B+C]  $p=0.001$ ) but revealed no difference in performance between the hypoglycaemia Studies B and C, suggesting that RVIP hits performance did not adapt after persisting hypoglycaemia.



RVIP false alarms (misses) - (Figure 3.1.4g). No significant difference in RVIP misses was observed among any of the studies, suggesting that hypoglycaemia did not have an effect on performance on this variable.

RVIP reaction time - (Figure 3.1.4h). In Study A there appeared to be a slight improvement in performance during the study, with a slight deterioration in performance in Study C and no change in Study B. However, testing with ANOVA did not identify a significant difference among the studies, and t-tests did not reveal any effect of hypoglycaemia or any difference between Studies B and C.

### **Symptom Scores**

Results of the symptom scores at the 7 time points are shown in figures 3.1.5. The baseline for the y axis on each graph is taken as the minimum score possible. The symptom profiles across time were similar for autonomic and neuroglycopenic scores. During Study A total, autonomic or neuroglycopenic scores did not change across time. However, in both hypoglycaemia studies (symptom questionnaires were completed at similar times during the studies, irrespective of the times of cognitive testing) significant increments in symptoms were recorded (ANOVA Study effect  $p < 0.001$ , Study by Time interaction  $p = 0.004$ ) ie during hypoglycaemia symptoms scores increased compared with baseline and compared with the euglycaemia study indicating that the change in symptoms was attributable to the effect of hypoglycaemia and not simply the effect of time. There was no evidence to suggest that symptoms became less intense as hypoglycaemia persisted. The scores actually increased as hypoglycaemia progressed from 30 to 60 minutes, although the difference did not achieve significance.

TABLE 3.1.1

## SCORES FROM COGNITIVE FUNCTION TESTS IN CLAMPS A, B AND C

		Clamp A	Clamp B	Clamp C
<b>Cognitive Test</b>				
<b>PASAT - 4-second</b>	T1	56.8±5.9	56.8±4.1	57.1±3.5
	T2	58.3±2.9	56.3±4.1	55.9±3.5
<b>PASAT - 2-second</b>	T1	45.0±9.8	45.4±10.5	45.8±10.9
	T2	50.1±7.1	41.6±12.2	38.7±12.2
<b>CRT - DT / ms</b>	T1	325±44	332±45	334±47
	T2	329±46	352±46	351±53
	T3	325±45	332±48	339±44
	T4	320±45	328±45	326±37
<b>CRT - RT / ms</b>	T1	162±44	168±41	171±48
	T2	164±46	207±73	188±57
	T3	159±40	178±53	175±43
	T4	158±41	168±46	170±42
<b>TMB / s</b>	T1	31.5±11.3	32.5±11.1	29.1±10.4
	T2	28.6±9.3	35.0±11.9	31.1±14.1
<b>DSST</b>	T1	71.6±11.7	70.7±12.6	69.8±11.4
	T2	74.9±12.1	67.4±11.8	68.5±11.6
	T3	75.9±12.7	72.3±13.3	73.4±13.5
	T4	78.7±13.8	76.5±17.1	76.1±15.9
<b>RVIP 'hits'</b>	T1	25.4±8.5	26.1±7.8	26.5±7.5
	T2	26.4±8.7	22.1±6.4	22.7±7.1
<b>RVIP 'misses'</b>	T1	4.0±8.3	3.3±7.8	4.7±14.8
	T2	3.7±9.5	4.4±12.5	5.7±15.5
<b>RVIP 'reaction time' / ms</b>	T1	470±74	461±53	473±79
	T2	456±52	480±61	471±58

mean ± SD

TABLE 3.1.2

**UNIVARIATE ANALYSIS OF VARIANCE OF THE  
INDIVIDUAL COGNITIVE TESTS**

Cognitive Test	Study effect	SIGNIFICANCE	
		Time	Study by Time
PASAT 4 second	0.04	-	-
PASAT 2 second	0.001	-	-
Four Choice Reaction -			
decision time	NS	0.001	0.06
movement time	0.018	0.001	0.016
DSST	0.06	<0.001	0.039
TMB	0.08	-	-
RVIP -			
hits	0.001	-	-
misses	NS	-	-
reaction time	0.15	-	-

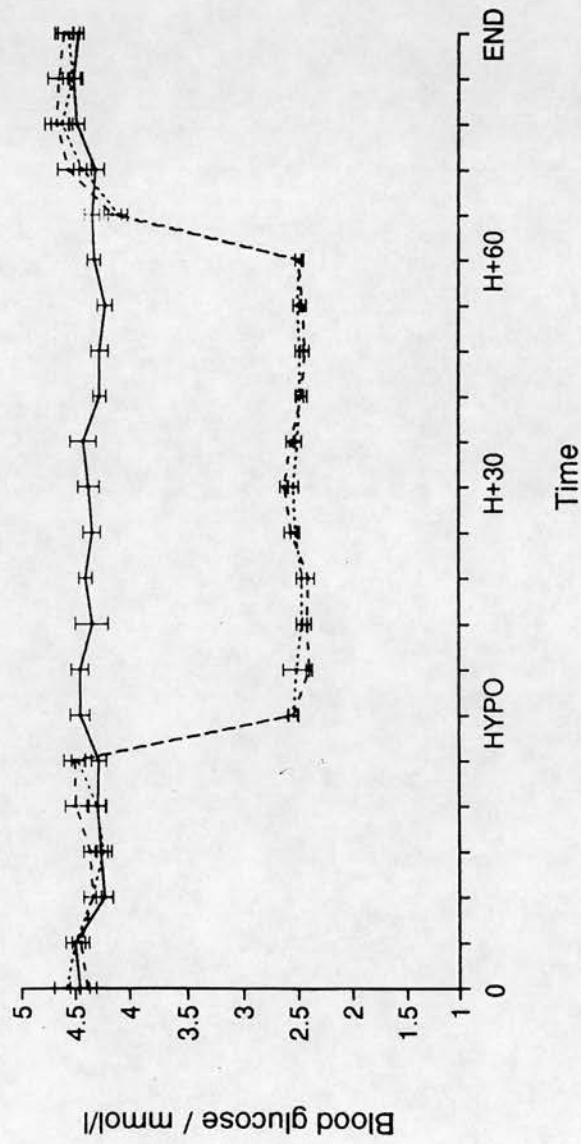


FIGURE 3.1.2  
 BLOOD GLUCOSE PROFILES DURING STUDIES A, B and C.  
 Solid line - Study A; broken line - Study B; dotted line - Study C.

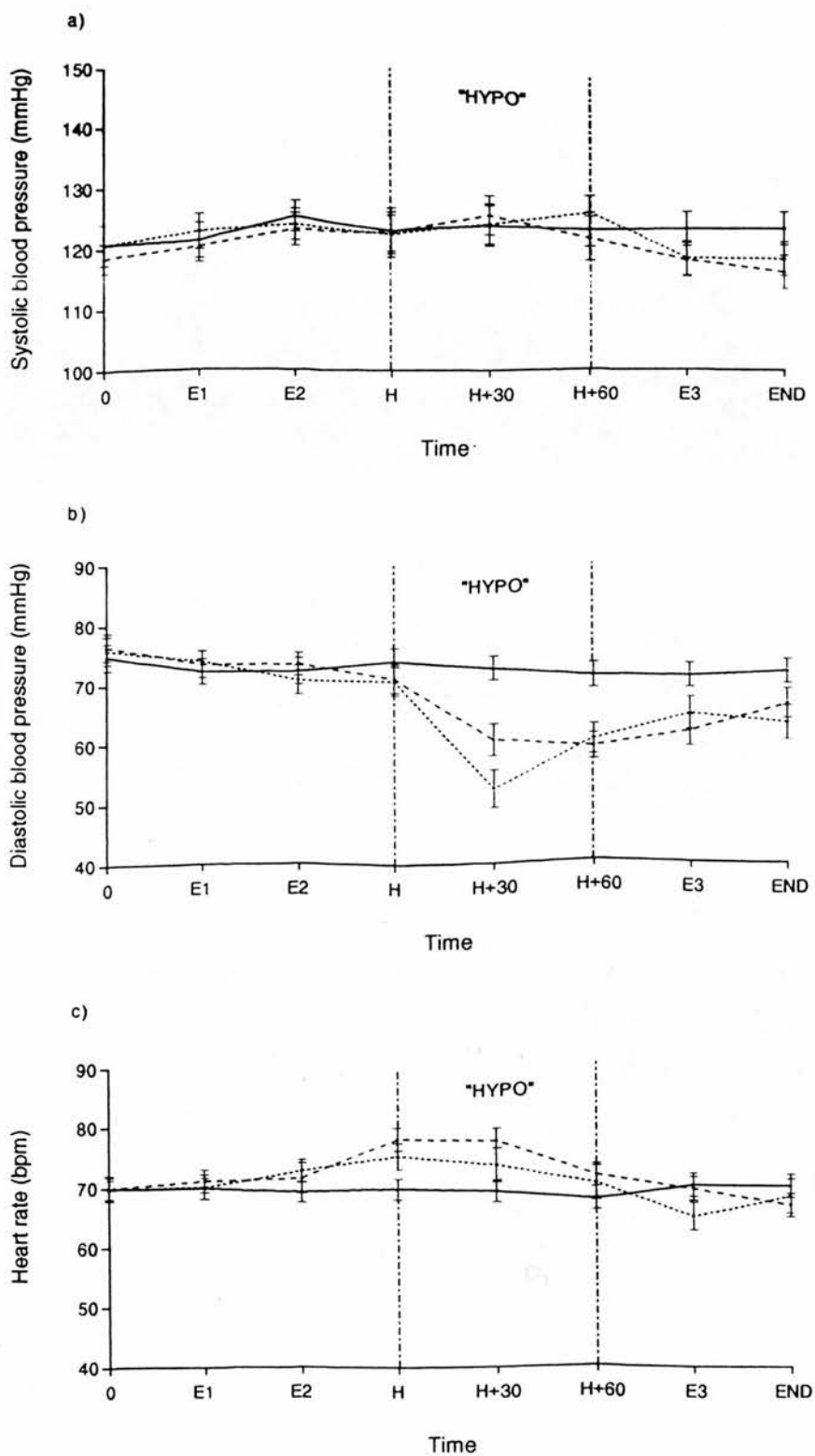


FIGURE 3.1.3  
 a - SYSTOLIC BLOOD PRESSURE DURING STUDIES A, B and C.  
 b - DIASTOLIC BLOOD PRESSURE DURING STUDIES A, B and C.  
 c - HEART RATE DURING STUDIES A, B and C.  
 Solid line - Study A; broken line - Study B; dotted line - Study C.

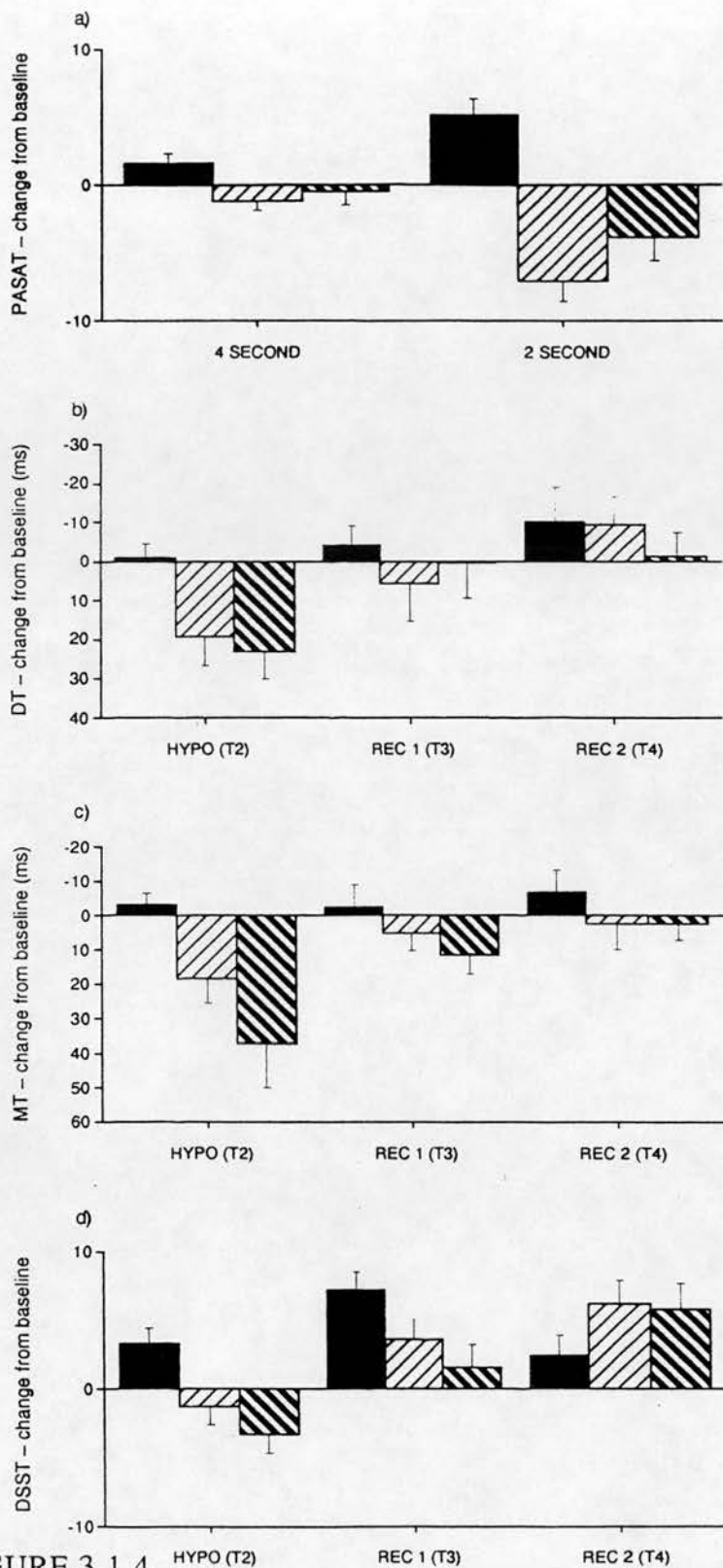


FIGURE 3.1.4 HYPO (T2) REC 1 (T3) REC 2 (T4)  
**COGNITIVE FUNCTION TEST RESULTS DURING STUDIES A, B and C, MEASURED AS CHANGES FROM BASELINE SCORES DURING INITIAL EUGLYCAEMIA. Solid area - Study A; close hatched area - Study B; wide hatched area - Study C.**

- a - PASAT 4 and 2 second scores
- b - 4 Choice reaction time decision time (DT)
- c - 4 Choice reaction time movement time (MT)
- d - DSST



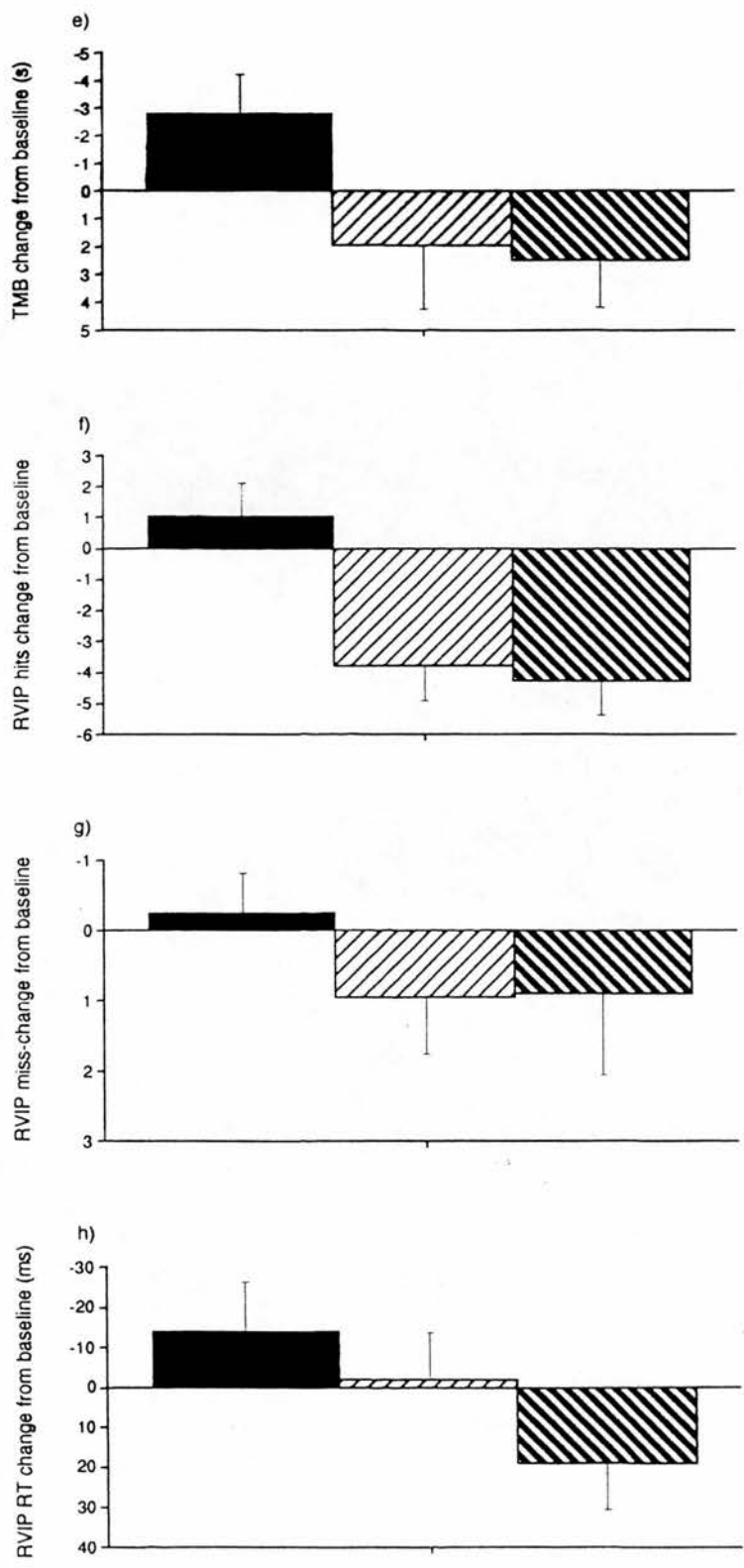
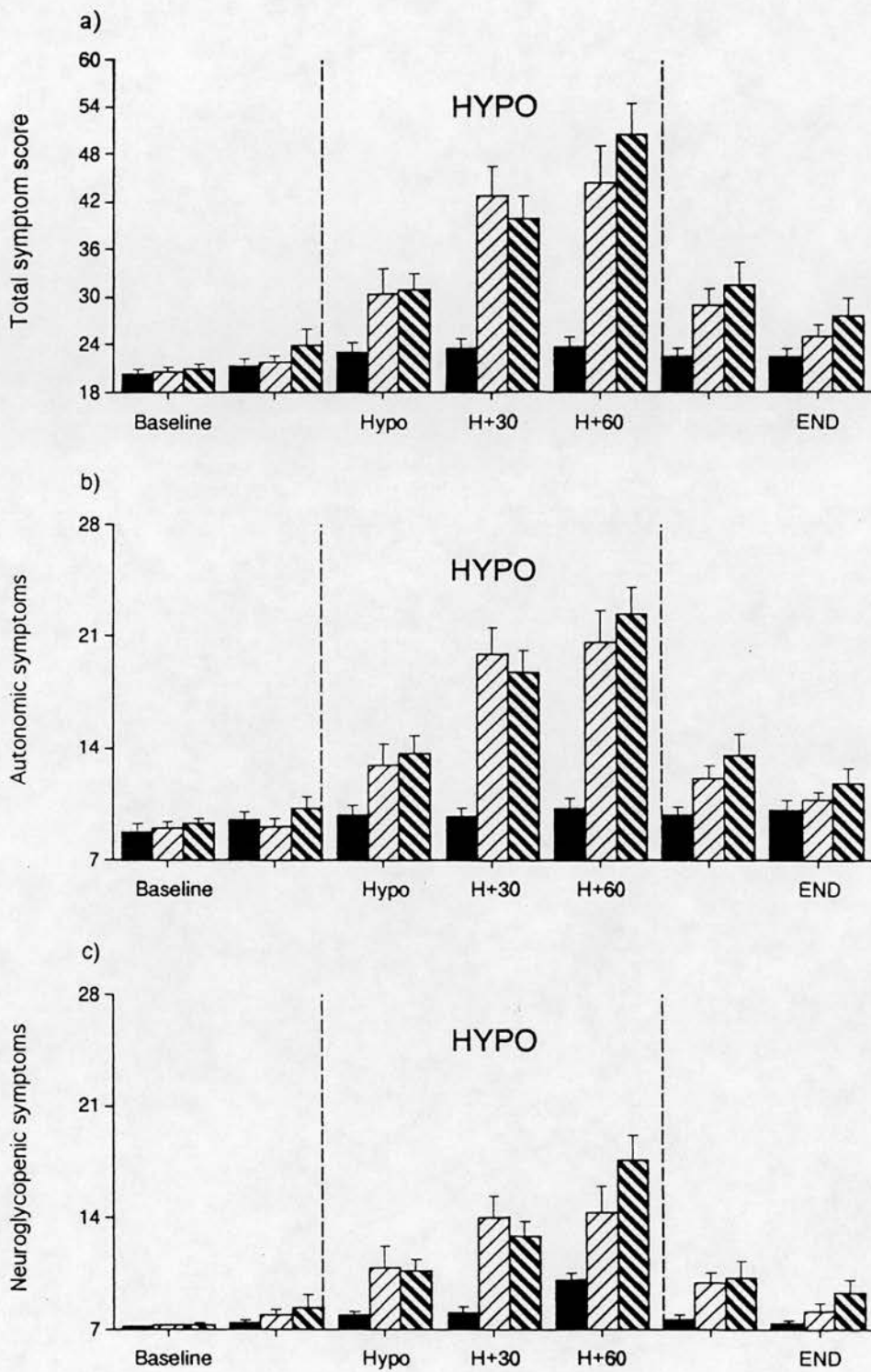


FIGURE 3.1.4 (CONTINUED)

- e - TMB
- f - RVIP hits
- g - RVIP misses
- h - RVIP reaction time



**FIGURE 3.1.5**  
**SYMPTOMS EXPERIENCED DURING HYPOGLYCAEMIA.** Solid area - Study A; close hatched area - Study B; wide hatched area - Study C.  
 a - Total symptom score (autonomic + neuroglycopenic + non-specific)  
 b - Autonomic symptoms  
 c - Neuroglycopenic symptoms

## DISCUSSION

The cognitive function test battery which was employed in the present study examined complex cognitive processes such as have typically been shown to be more affected by acute hypoglycaemia (Holmes et al 1983, Herold et al 1985, Pramming et al 1986, Hoffman et al 1989, Stevens et al 1989). In the present study, in which a relatively large number of subjects was examined, the multivariate analysis confirmed that the overall performance in the test battery deteriorated significantly during hypoglycaemia. Further analyses determined which tests were most affected by hypoglycaemia, and the extent of any differences between early and more prolonged exposure to hypoglycaemia.

All of the tests in the present study were significantly affected by hypoglycaemia with the exception of the TMB test, although the latter has previously been shown to be affected by hypoglycaemia in patients with Type 1 diabetes (Hoffman et al 1989, see also Part III Chapter 3). In a study which compared healthy non-diabetic subjects with patients who had Type 1 diabetes, the Trail Making B was used as part of the cognitive test battery and the patients with diabetes were observed to perform more poorly during hypoglycaemia (Wirsen et al 1992). This observation may explain why the performance in TMB in the non-diabetic subjects in the present study did not deteriorate significantly during hypoglycaemia.

The RVIP task is a signal detection task and not all of the variables derived from this task were affected by hypoglycaemia. The RVIP hits variable assesses the efficiency of signal detection and was affected by hypoglycaemia. The RVIP false alarms (misses) variable is a measure of response threshold or caution and *did not* deteriorate significantly during hypoglycaemia in the present study which is in agreement with the results of previous studies which have demonstrated that, during this stress, accuracy tends to be preserved at the expense of speed of performance (Holmes et al 1983, Herold et al 1985, Cox et al 1993a). The RVIP

reaction time is not as accurate a measure of reaction time as the Four Choice Reaction Time : in the former test the subjects are not instructed to complete the task as quickly as possible.

In the present study no relative improvement in cognitive performance was demonstrated after 40 to 60 minutes of hypoglycaemia when compared with performance after 5 to 25 minutes of hypoglycaemia. One test, the movement time (MT) of the Four Choice Reaction Time, was significantly *worse* after prolonged hypoglycaemia, but this test reflects predominantly motor function rather than decision-making processes and might indicate the development of fatigue associated with prolonged exposure to the prolonged hypoglycaemia. A previous study of healthy non-diabetic subjects (Kerr et al 1989) demonstrated that simple reaction times initially deteriorated when the arterialised blood glucose was lowered to 3.0 mmol/l, but subsequently improved after 60 minutes at the same blood glucose concentration, although they remained slower than the original baseline values. In that study (Kerr et al 1989), the data from any changes in the euglycaemia study were not presented, and it is not possible to ascertain whether this improvement could be attributed to a learning effect. In the present study the method of analysis corrects for any potential learning effect which may be occurring. The same authors repeated a similar study in patients who had Type 1 diabetes (Kerr et al 1991a); on that occasion the arterialised blood glucose was lowered to 2.8 mmol/l, at which the simple reaction times were noted to deteriorate. However, after 90 minutes of persisting hypoglycaemia, the reaction time was returning towards normal, although it still remained prolonged. Simple reaction time is not a good measure of changes in central cognitive processes during hypoglycaemia because it involves little cognitive processing, and motor function is emphasised. A recent study has confirmed that pure "cognitive " tasks are more sensitive to neuroglycopenia than motor tasks (Cox et al 1993b). These

studies (Kerr et al 1989, Kerr et al 1991a) cannot therefore be used to conclude that any changes observed were the result of "cerebral adaptation" to neuroglycopenia.

The degree of hypoglycaemia induced in the present study was more profound than that used by Kerr et al (1989). If cerebral adaptation to neuroglycopenia does occur it is possible that this process depends upon the degree of hypoglycaemia, and it may take longer than 60 minutes of exposure to a low blood glucose. However, a period of 60 minutes of continuous hypoglycaemia was considered to be similar to some episodes of biochemical hypoglycaemia (which are often asymptomatic) which occur in clinical practice when the diabetic patient is awake. More protracted hypoglycaemia may occur in diabetic patients during sleep and may promote cerebral adaptation, as no cognitive impairment has been demonstrated on the morning after severe nocturnal hypoglycaemia (Bendtsen et al 1992). A recent study in non-diabetic subjects (Nagy et al 1993), demonstrated that cognitive function (measured with Stroop test and finger-tapping) initially deteriorated at an arterialised blood glucose of 3.6mmol/l but, after chronic hypoglycaemia (2.8 mmol/l) had been maintained for 56 hours, cognitive function did not deteriorate until the blood glucose was lowered to 2.5 mmol/l. The results of this study suggest that, although a degree of adaptation to prolonged hypoglycaemia appeared to have occurred, the threshold for cognitive dysfunction was shifted to a more profound level of hypoglycaemia. One potentially confounding factor in this study (Nagy et al 1993) was that there was no control limb of the study to assess the effect of hyperinsulinaemia *per se* on cerebral function, which was required to maintain that degree of hypoglycaemia (Kerr et al 1991b). A more recent study of non-diabetic patients with insulinomas, using a hyperinsulinaemic glucose clamp technique, has suggested that when those patients were exposed to recurrent episodes of hypoglycaemia, pre-operatively, they appeared to be protected from cognitive dysfunction during acute hypoglycaemia; although following operative removal of the insulinoma they became as susceptible



to cognitive dysfunction as a control group (Mitrakou et al 1993). However it is important to remember that these patients did not have diabetes and therefore may be susceptible to different mechanisms of possible adaptation. Previous studies in rats have shown that, during chronic hypoglycaemia, uptake of glucose by the brain and the brain glucose transport capacity are increased (Cremer et al 1983, McCall et al 1986, Pelligrino et al 1990) and it is possible that a similar mechanism operates in human subjects exposed to chronic hypoglycaemia such as those with an insulinoma.

Symptoms of hypoglycaemia increased to a maximum after 60 minutes of hypoglycaemia in the present study. In a study of non-diabetic subjects reported by Kerr and colleagues (1989), symptoms of hypoglycaemia were beginning to resolve after 60 minutes at a blood glucose of 3.0 mmol/l. However, in the present study a more comprehensive symptom scoring system was used, and the greater degree of hypoglycaemia used in the present study compared with that used by Kerr et al 1989, may have influenced any potential cerebral adaptation to hypoglycaemia. In the study by Kerr et al (1989), although a reduction in symptom scores and improvement in reaction time were demonstrated, the rise in plasma catecholamine concentrations continued, suggesting that some dissociation was occurring between the hormonal responses, cerebral function and awareness of hypoglycaemia. The cognitive test battery used in the present study was more stressful, and previous studies have shown that such tests *per se* may promote a rise in catecholamines (Frankenhaeuser, 1971). However, in the present study, the symptom profiles did not differ between Study B and Study C despite the tests of cognitive function being administered at different times; if anxiety induced by the tests was a major contributing factor to the total autonomic symptom scores then the symptom profiles would have been expected to differ between Studies B and C.



Many previous studies have observed a delay in recovery of cognitive function following hypoglycaemia (Ryan et al 1990, Eckert et al 1992, Blackman et al 1992). This was not a significant finding of the present study, and may be a function of the different cognitive tests which were used and the different levels of hypoglycaemia induced even though the present study examined a larger number of subjects and utilised a very comprehensive cognitive test battery.

The aetiology of cognitive dysfunction during hypoglycaemia is probably multifactorial, resulting from a direct cerebral effect of neuroglycopenia combined with non-specific influences such as fatigue and headache. However, in clinical practice the important issue is the degree of dysfunction experienced by the diabetic patient treated with insulin. In the present study neither an improvement in cognitive function nor a diminution of symptoms of hypoglycaemia could be observed after 60 minutes of moderate hypoglycaemia, which suggests that cerebral adaptation does not occur at this degree and duration of hypoglycaemia.

In summary:-

Normal subjects do not exhibit short term cerebral adaptation to hypoglycaemia after being maintained at a blood glucose concentration of 2.5 mmol/l for 40 to 60 minutes.

PART III

CHAPTER 2

*Study 2*

**THE EFFECT OF INTELLIGENCE LEVEL ON  
COGNITIVE DYSFUNCTION DURING ACUTE HYPOGLYCAEMIA  
IN NON-DIABETIC SUBJECTS**

## INTRODUCTION

In humans intelligence is a manifestation of cerebral functioning which can be quantitated in individuals. The brain is uniquely dependent upon and very sensitive to the availability of glucose, fluctuations in which will induce changes in cerebral function and may therefore be used as a tool to investigate cognitive ability. The development of acute hypoglycaemia in the daily life of patients with diabetes, as a result of insulin treatment is not reproducible and varying degrees of hypoglycaemia are unsuitable for accurate evaluation of cognitive function. The hyperinsulinaemic glucose clamp provides the facility for accurate manipulations of blood glucose and can be used experimentally to study the effects of changes in blood glucose on various physiological processes.

Studies examining the effect of acute hypoglycaemia on cognitive function have revealed that there is a large inter-individual variation in the cognitive dysfunction observed during acute hypoglycaemia (Herold et al 1985, Hoffman et al 1989); the reason for these differences is not apparent. It has been suggested that patients with diabetes perform more poorly than non-diabetic control subjects during hypoglycaemia (Wirsen et al 1991), although in many previous studies, the non-diabetic control groups have often comprised doctors and other professional groups in whom IQ would be expected to be much higher than the general population. These control groups would therefore be atypical and might confound interpretation of the results.

The notion that high IQ might offer some protection against a metabolic form of cerebral 'insult' is often discussed but has seldom been submitted to experimental evaluation. The most common area of study is 'aging', but there is no clear evidence to support the supposition that high IQ individuals have greater 'reserve capacity' and thereby deteriorate less as they grow older (Raykov 1989, Baltes et al 1986, Christensen and Henderson 1991). Age is a notoriously difficult variable to

study, and much of the difficulty in deciding on the reserve capacity issue arises from the problems inherent in cross-sectional and longitudinal studies of aging.

A more convenient test of whether individuals with a high IQ have a greater reserve capacity is to subject high and low IQ groups to an acute cerebral insult in a controlled experiment. This was attempted by Maylor et al (1990) who stated that, "we might expect that bright subjects, having greater processing resources or reserve capacity, would be less adversely affected by alcohol than dull subjects". Maylor et al examined the effect of alcohol intoxication on performance in a recall test in high and low IQ groups (identified using the Alice Heim 4 test). Their results indicated that alcohol had a greater effect on the *high IQ* group, though they could not rule out 'floor' effects for the low IQ group. In other words the low IQ group were already performing so poorly that the tests may not have been sufficiently sensitive to identify any further deterioration.

The aim of the present study was to try and identify whether subjects with a high IQ suffer more or less cognitive deterioration than those subjects with a lower IQ during acute hypoglycaemia. Hypoglycaemia was utilised as a cerebral insult (because it is directly associated with brain metabolism and more highly controllable than other insults such as alcohol). In addition the present study examines whether IQ may play a role in any putative cerebral adaptation to hypoglycaemia (see Part III Chapter 1).

## **METHOD**

This study was performed in conjunction with *Study 1* ( see Part III Chapter 1) where the methods of study have already been described in detail, and will not be repeated here. After the initial analysis of the data from the 72 hyperinsulinaemic glucose clamps examining the presence of short term adaptation to hypoglycaemia, the data was reanalysed to examine the effect of IQ on deterioration of cognitive function during hypoglycaemia.

### **Subjects**

The same 24 healthy subjects studied in Part III Chapter 1 divided into two groups: one with higher cognitive ability (Group 1) and one with lower cognitive ability (Group 2) (Table 3.2.1). Cognitive ability was assessed using Alice Heim 4 test (AH4, Heim 1968) and the National Adult Reading Test (NART, Nelson 1982).

### **Experimental Procedure and Study Design**

The study design was identical to that described in Part III Chapter 1. Figure 3.1.1.

### **Symptom scores and Physiological Measurements**

These have been described previously in Part III Chapter 1.

### **Cognitive Test Battery**

The five cognitive tests comprising the principal cognitive test battery have been described previously (Part II):

- 1 - Paced Auditory Serial Addition Test (PASAT) (Deary et al 1991a)
- 2 - Four Choice Reaction Time (CRT) (Hick 1952, Deary et al 1992)
- 3 - Digit Symbol Substitution test (DSST) (Wechsler 1981)
- 4 - Trail-Making B (TMB) (Reitan 1974)
- 5 - Rapid Visual Information Processing (RVIP) (Deary et al 1992)

A shortened test battery comprising the Four Choice Reaction Time and the Digit Symbol Substitution Test was administered at times T3 and T4 in order to assess recovery of cognitive function after hypoglycaemia (Figure 3.1.1).

### **Statistical Analysis**

Multivariate analysis of variance (MANOVA) was used to assess the effects of hypoglycaemia on cognitive function. There were two independent variables:

- i) IQ with two levels, "high" (Group 1) and "low" (Group 2) ;
- ii) Study , with three levels, "euglycaemic placebo" (Study A), "immediate hypoglycaemia" (Study B) and "delayed hypoglycaemia" (Study C).

The key variable in all the analyses is IQ, and the most important statistical results are the interactions representing the effect of IQ on performance under different study studies (IQ by Study interaction). The effect of Study incorporates any effect of hypoglycaemia on performance, *and* differences between the two hypoglycaemia studies.

The absolute *change* from the baseline cognitive score (T1) to the score obtained in the experimental study (T2) was the measure used for each cognitive test. Percentage change from baseline was also assessed and the results obtained were very similar to the present method of analysis and so only the absolute change from baseline is presented. In all the studies the first test period represents a baseline score assessed during euglycaemia (T1); the second test period (T2) represents the score during early hypoglycaemia in Study B, late hypoglycaemia in Study C, and a second test at euglycaemia in Study A. The dependent variables comprising the within subjects factor "Test", were derived from the nine indices extracted from cognitive function tests : four and two second PASAT, DSST, TMB, RVIP hits, RVIP misses RVIP reaction times, Four Choice decision time (DT) and Four



Choice movement time (MT).

Separate univariate analyses of variance (ANOVA) were used to assess the effects of IQ and hypoglycaemia on the individual cognitive tests, symptoms, blood glucose, blood pressure and heart-rate.

The overall significance in the multivariate tests was calculated from the Wilks' Lamda test statistic. Significant results were further analysed using t-tests to determine specific differences between the study studies. The assumptions for parametric tests were satisfied for all variables unless otherwise stated. In order to minimise the possibility of a Type 1 error, the following *planned* comparisons were performed to assess the cognitive scores:

i) the mean of [Study B scores + Study C scores] in Group 1 vs Group 2 to reveal any effect of IQ on performance during hypoglycaemia.

ii) Study B scores v Study C scores - to compare the effect of immediate hypoglycaemia (Study B) with prolonged hypoglycaemia (Study C) in each group.

iii) Study A scores v mean of [Study B scores + Study C scores) - to compare the effect of euglycaemia with hypoglycaemia *per se* in each group.

It would be anticipated that with the addition of the 'IQ' factor into the analyses of variance, the actual cells comprising the analysis would differ from the analyses of variance described in Part III Chapter 1. This may result in small differences in the degree of significance of the main effects eg 'Study', 'Test'.

TABLE 3.2.1

## DEMOGRAPHIC DETAILS OF NON-DIABETIC SUBJECTS

	Group 1 'High IQ'	Group 2 'Low IQ'	
Age (years)	30.1±4.1	28.9±4.6	NS
Male / Female	5 / 7	8 / 4	NS
Right / Left handed	9 / 3	8 / 4	NS
NART errors	6.6±1.7	13.7±4.1	p<0.001
AH4 total score	106.1±7.7	79.3±11.1	p<0.001

## **RESULTS**

### **Blood glucose**

The blood glucose profiles of Studies A, B and C are shown in figure 3.2.1. There was little variation in blood glucose concentrations during each phase of the studies ie the standard errors during the euglycaemic and hypoglycaemic periods were all small. In the hypoglycaemia studies (B and C) the profiles appear very similar in both groups throughout which was confirmed by testing with analysis of variance. At the times of cognitive testing during hypoglycaemia, ie between 5 and 25 minutes of hypoglycaemia in Study B and between 35 to 55 minutes of hypoglycaemia in Study C, no differences in blood glucose concentrations between the Studies B and C or between groups could be discerned. In other words both groups were exposed to virtually identical degrees of hypoglycaemia and euglycaemia throughout all the studies.

### **Blood pressure and heart rate**

There were no significant differences in resting blood pressure or heart rate between the groups. Analysis of variance across time revealed no significant effect of hypoglycaemia on systolic blood pressure or heart rate in either group (IQ, Study, Study by Time, IQ by Study, and IQ by Study by Time were all non-significant). There was, however a significant fall in diastolic blood pressure in both groups during the hypoglycaemia study (Study by Time  $p=0.043$ ), but there was no difference between the groups.

### **Cognitive function**

The raw scores for the cognitive data are shown in Table 3.2.2. In all the tests, with the exception of the Four Choice reaction decision and movement times, significant baseline differences between the groups were observed: as expected Group 1 (high IQ) performed better than Group 2 (low IQ) (ie mean scores at T1 [Study A + Study B + Study C]; RVIP hits  $p=0.005$ , 4 second PASAT  $p=0.03$ ,

2 second PASAT  $p=0.03$ , TMB  $p=0.008$ , DSST  $p=0.036$ ). The change from baseline scores during hypoglycaemia is shown in figures 3.2.2 to 3.2.6. For all test variables a positive (upward) deflection indicates an improvement in function, whereas a negative (downward) deflection indicates a deterioration in function. In the 4 second PASAT (fig 3.3.2a) there appeared to be a deterioration in the high IQ group during hypoglycaemia (Studies B and C) although the low IQ group improved considerably in performance in the euglycaemia placebo study (A) and did not deteriorate during hypoglycaemia. In the 2 second PASAT (fig 3.2.2b) both groups performed more poorly during hypoglycaemia and also appeared to improve in the euglycaemia control study. Performance in the Four Choice Reaction Time (fig 3.2.3a and 3.2.3d) and DSST (fig 3.2.4a) during hypoglycaemia (Studies B and C), deteriorated during hypoglycaemia in both groups. In the DSST, there was also an improvement in scores during the euglycaemia study A. In the high IQ group performance deteriorated in the TMB test during hypoglycaemia but did not appear to change greatly in the low IQ group. During the recovery periods the performance in most subjects had returned to baseline in the Four Choice Reaction Time and had improved in the DSST to better than baseline scores in both groups.

### *Multivariate analysis*

Multivariate analysis of variance of changes from baseline scores during each of the studies revealed a significant effect of study conditions on performance ( $p=0.048$ ) and also a significant effect of Test ( $p=0.01$ ) with a significant interaction between Study and Test ( $p=0.005$ ). This indicates that there were significantly different effects of the study conditions on cognitive performance ie that hypoglycaemia generally lowered cognitive scores, and also that hypoglycaemia had a differential effect on the individual tests, necessitating further analysis of each test separately. There was no overall significant effect of IQ on

performance (the IQ, the effect of IQ by Study interaction and the IQ by Test by Study interactions were non significant), although IQ tended to have a differential effect on performance in the different tests irrespective of the study conditions (IQ by Test interaction  $p=0.068$ ).

#### *Univariate analysis*

The results of univariate analyses of variance for each test are shown in table 3.2.3. For the Four Choice reaction time and the DSST there is an additional "time" factor as each of these tests was also performed twice during recovery from hypoglycaemia (T3 and T4). It is possible that Type 1 errors might have occurred but it was considered that the univariate analyses were necessary because there are few other detailed studies of this type and the planned comparisons were used to minimise these errors. In addition a significant Study by Test interaction was noted which required further investigation.

*PASAT* - Figure 3.2.2a and b. In the 4 second PASAT, in Study A an improvement from baseline score was observed in both groups, particularly in those with a lower IQ. In both Studies B and C a significant difference between the groups emerged : in Group 1 (high IQ) there was a significant deterioration during hypoglycaemia which did not occur in Group 2 (Low IQ) (ANOVA effect of Study  $p=0.033$ ). Further analysis by comparing the scores from Study A at T2 with the mean of the scores from Studies B and C at T2, revealed that although a significant effect of hypoglycaemia compared with euglycaemia could be observed on the performance of Group 1 (high IQ;  $p=0.037$ ) this was not found in Group 2 (low IQ;  $p=ns$ ). Group comparisons then revealed that the low IQ group had significantly better scores during hypoglycaemia in comparison with the baseline scores than did the high IQ group ( $p=0.04$ ). In the 2 second PASAT a significant effect of hypoglycaemia on performance in both groups (Study effect  $p<0.001$ ) was found but no differences between the groups could be distinguished. No



significant difference within groups was demonstrated in the performance between Study B and C, ie PASAT performance showed no adaptation to hypoglycaemia.

It can be seen from table 3.2.2 that the standard deviations for the 4-second PASAT were considerably larger in the low IQ group than the high IQ group. For this reason, a non-parametric comparison between the groups (using Mann-Whitney U test) of the average change from baseline during hypoglycaemia for Studies B and C was performed, which still showed a trend towards a difference in performance between the groups ( $p=0.06$ ).

*Four Choice Reaction Time* - Figure 3.2.3a-f. Performance did not change during the euglycaemia placebo Study A in either group, but both DT and MT deteriorated in both groups during hypoglycaemia (Studies B and C). However this only reached significance for MT (ANOVA study by Time  $p=0.02$ ), and returned towards baseline values during the recovery period. IQ did not have an effect on performance. Analysis with t-tests confirmed a significant deleterious effect of hypoglycaemia on performance on MT in both groups (A v mean [B+C],  $p=0.009$  for Group 1 and  $p=0.05$  for Group 2). The time of testing after onset of hypoglycaemia (study B vs study C) did not affect performance in either group. Performance during recovery was not significantly different from baseline in either group.

*DSST* - Figure 3.2.4a, b, c. Changes in performance were similar in *both* groups: in Study A there was an improvement in performance throughout, but in Studies B and C performance deteriorated during hypoglycaemia (Study by Time  $p=0.043$ ), returning towards normal in the recovery periods. Further analysis with t-tests confirmed the effect of hypoglycaemia on performance in Group 1 (High IQ)(A v mean [B+C],  $p=0.01$ ), although this failed to reach significance



for Group 2. However no significant differences in performance were observed during hypoglycaemia between the groups (Mean [B+C]); in addition there was no significant difference between Studies B and C in either group, indicating the absence of a significant adaptation effect.

*TMB* - Figure 3.2.5. There was a trend towards an improvement in performance in Study A in both groups, with deteriorations in performance in Study B in both groups and Study C in Group 1 but not Group 2. However analysis of variance did not reveal a significant difference between the groups or a significant effect of hypoglycaemia overall.

*RVIP hits* - Figure 3.2.6a. Changes in scores were similar in *both* groups: performance did not change during Study A but deteriorated during hypoglycaemia (ANOVA Study effect  $p=0.002$ ). Further analysis with t-tests confirmed the effect of hypoglycaemia (A v Mean [B+C]  $p=0.02$  for Group 1 and  $p=0.03$  for group 2) but revealed no difference in performance between the groups in either Studies B or C.

*RVIP misses* - Figure 3.2.6b. IQ tended to have an effect on performance during hypoglycaemia (ANOVA, IQ effect  $p=0.06$ ): subjects in Group 2 made significantly more false positive responses during hypoglycaemia in both studies (A v mean [B+C]  $p=0.03$ ) unlike group 1 ( $p=NS$ ); comparison of the groups demonstrated a trend towards an increased number of false alarms in the low IQ group compared with the high IQ group (mean [B+C] of Group 1 v Group 2  $p=0.07$ ).

From table 3.2.2 it can be seen that the standard deviations for the RVIP misses were considerably larger in the low IQ group compared with the high IQ group. Therefore the average change from baseline during hypoglycaemia was compared

between the groups using a non-parametric analysis (Mann-Whitney U test); this confirmed a significant difference in performance between the groups during hypoglycaemia ( $p=0.025$ ).

*RVIP reaction time* - Figure 3.2.6c. Testing with ANOVA showed no significant effect of hypoglycaemia on performance and no difference between the groups.

### **Symptom Scores**

The symptom profiles did not differ between the groups for autonomic, neuroglycopenic and non-specific scores (see Part III chapter 1).

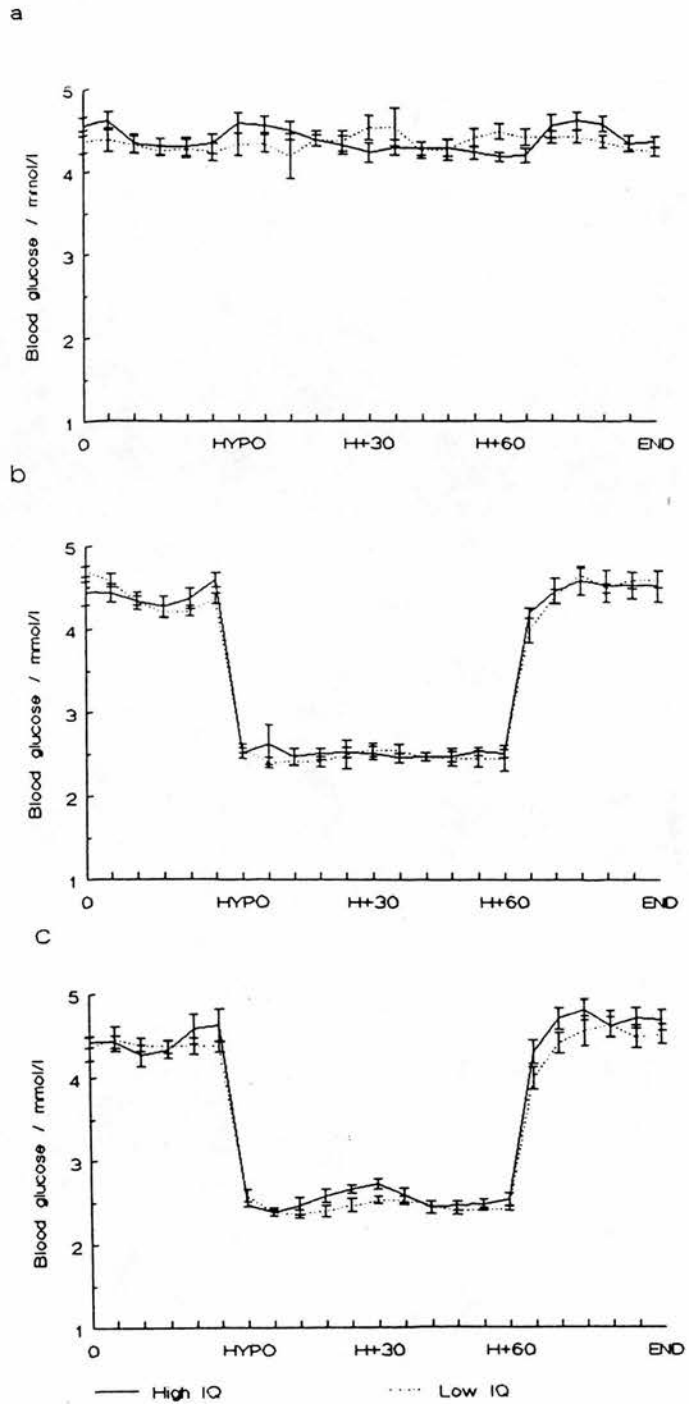
TABLE 3.2.2  
 SCORES FROM COGNITIVE TESTS IN BOTH HIGH AND LOW IQ GROUPS AT ALL TIME POINTS OF STUDY (score $\pm$ SD)

Cognitive test		Clamp A		Clamp B		Clamp C	
		High IQ	Low IQ	High IQ	Low IQ	High IQ	Low IQ
PASAT - 4-second	T1	58.6 $\pm$ 2.5	54.9 $\pm$ 7.6	58.3 $\pm$ 1.8	55.3 $\pm$ 5.2	58.8 $\pm$ 1.3	55.4 $\pm$ 4.2
	T2	59.3 $\pm$ 0.7	57.5 $\pm$ 3.9	56.5 $\pm$ 4.5	56.2 $\pm$ 3.8	56.2 $\pm$ 4.0	55.7 $\pm$ 3.1
PASAT - 2-second	T1	47.8 $\pm$ 8.6	42.2 $\pm$ 10.5	49.1 $\pm$ 7.3	41.7 $\pm$ 12.1	50.4 $\pm$ 7.2	41.2 $\pm$ 12.3
	T2	53.3 $\pm$ 5.9	46.9 $\pm$ 6.9	44.4 $\pm$ 11.7	38.8 $\pm$ 12.4	43.8 $\pm$ 10.6	33.7 $\pm$ 11.9
CRT - DT / ms	T1	317 $\pm$ 30	334 $\pm$ 53	329 $\pm$ 35	336 $\pm$ 55	331 $\pm$ 40	338 $\pm$ 56
	T2	322 $\pm$ 39	335 $\pm$ 52	346 $\pm$ 30	359 $\pm$ 58	347 $\pm$ 43	354 $\pm$ 63
	T3	318 $\pm$ 39	333 $\pm$ 51	324 $\pm$ 51	340 $\pm$ 47	337 $\pm$ 45	342 $\pm$ 45
	T4	309 $\pm$ 46	330 $\pm$ 44	327 $\pm$ 40	331 $\pm$ 51	322 $\pm$ 31	330 $\pm$ 43
CRT - RT / ms	T1	154 $\pm$ 42	171 $\pm$ 47	166 $\pm$ 41	169 $\pm$ 43	166 $\pm$ 38	177 $\pm$ 57
	T2	155 $\pm$ 41	173 $\pm$ 50	199 $\pm$ 71	214 $\pm$ 77	185 $\pm$ 50	192 $\pm$ 65
	T3	155 $\pm$ 36	163 $\pm$ 45	180 $\pm$ 59	177 $\pm$ 47	169 $\pm$ 37	181 $\pm$ 49
	T4	149 $\pm$ 34	167 $\pm$ 47	169 $\pm$ 46	167 $\pm$ 48	163 $\pm$ 39	178 $\pm$ 45
TMB / s	T1	26.3 $\pm$ 3.7	36.6 $\pm$ 13.9	28.0 $\pm$ 9.9	37.1 $\pm$ 10.7	22.8 $\pm$ 4.7	35.4 $\pm$ 10.7
	T2	24.4 $\pm$ 7.1	32.8 $\pm$ 9.5	30.8 $\pm$ 8.1	39.3 $\pm$ 13.8	27.6 $\pm$ 15.8	34.6 $\pm$ 11.8
DSST	T1	75.8 $\pm$ 7.8	67.3 $\pm$ 13.6	74.6 $\pm$ 10.7	66.8 $\pm$ 13.6	75.3 $\pm$ 8.9	64.4 $\pm$ 11.4
	T2	80.1 $\pm$ 6.8	69.7 $\pm$ 14.1	71.4 $\pm$ 11.4	63.3 $\pm$ 11.3	73.6 $\pm$ 8.3	63.5 $\pm$ 12.5
	T3	81.3 $\pm$ 9.0	70.5 $\pm$ 13.8	76.8 $\pm$ 11.8	67.8 $\pm$ 13.7	79.4 $\pm$ 12.2	67.4 $\pm$ 12.2
	T4	85.4 $\pm$ 9.8	72.1 $\pm$ 14.2	82.8 $\pm$ 16.0	70.3 $\pm$ 16.6	81.7 $\pm$ 13.0	70.5 $\pm$ 17.1
RVIP 'hits'	T1	30.6 $\pm$ 6.4	20.2 $\pm$ 7.2	29.5 $\pm$ 7.9	23.1 $\pm$ 6.6	30.5 $\pm$ 6.2	22.6 $\pm$ 6.6
	T2	30.4 $\pm$ 8.6	22.4 $\pm$ 6.9	24.7 $\pm$ 6.1	19.5 $\pm$ 5.7	25.4 $\pm$ 7.1	20.0 $\pm$ 6.4
RVIP 'misses'	T1	2.3 $\pm$ 2.1	5.8 $\pm$ 11.6	2.0 $\pm$ 2.2	4.4 $\pm$ 10.7	1.8 $\pm$ 1.3	7.6 $\pm$ 20.1
	T2	1.8 $\pm$ 2.4	5.7 $\pm$ 13.2	0.8 $\pm$ 1.2	7.3 $\pm$ 17.4	1.5 $\pm$ 0.9	9.8 $\pm$ 21.6
RVIP reaction time / ms	T1	478 $\pm$ 73	460 $\pm$ 77	482 $\pm$ 37	441 $\pm$ 58	483 $\pm$ 61	462 $\pm$ 95
	T2	460 $\pm$ 37	451 $\pm$ 65	384 $\pm$ 48	451 $\pm$ 65	495 $\pm$ 51	446 $\pm$ 56

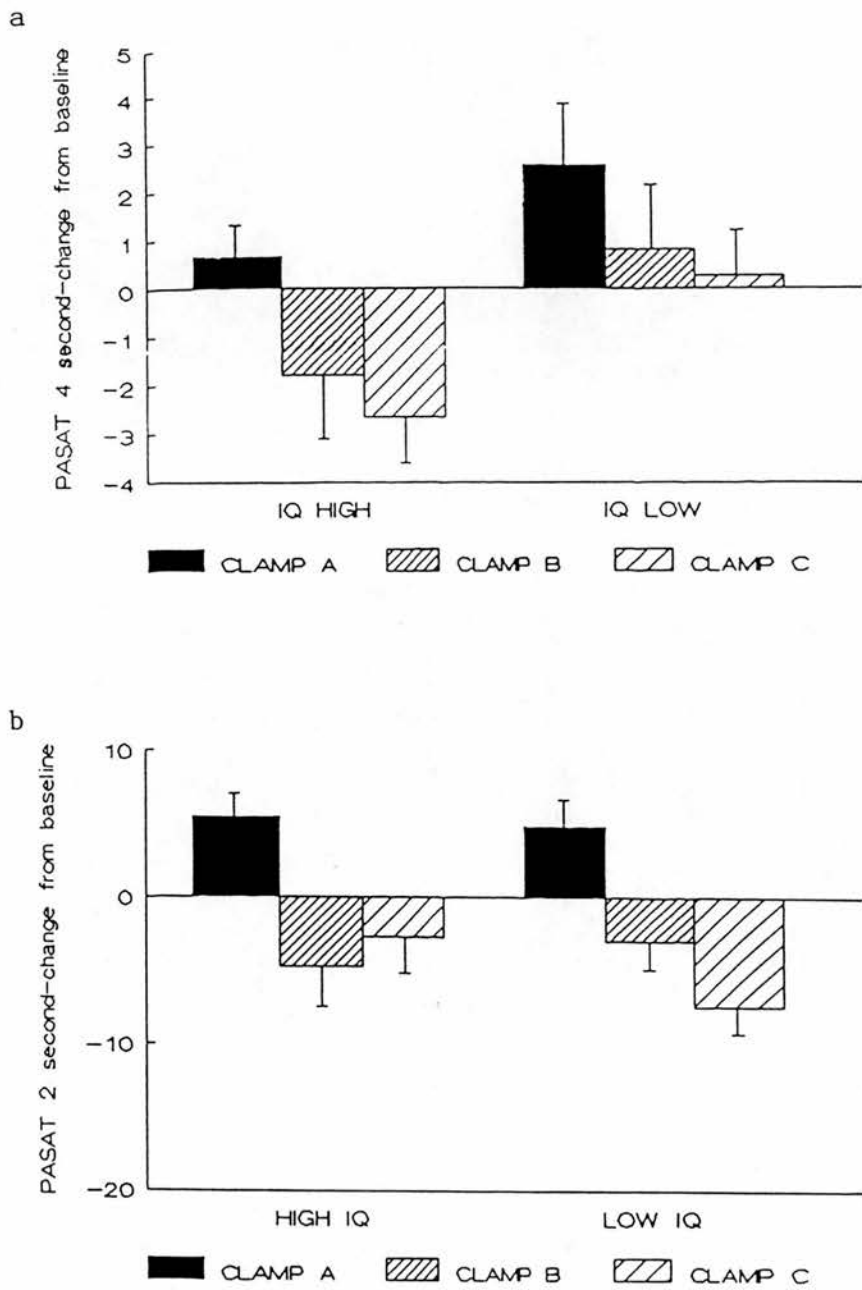
TABLE 3.2.3

UNIVARIATE ANALYSIS OF VARIANCE OF THE INDIVIDUAL COGNITIVE TESTS

Test	SIGNIFICANCE OF EFFECT					
	'IQ'	'Study'	'Time'	'Study by Time'	'IQ by Time'	'IQ by Study by Time'
PASAT 4 second	0.033	0.012	-	NS	-	-
PASAT 2 second	NS	<0.001	-	NS	-	-
4 Choice reaction DT	NS	NS	0.001	NS	0.07	NS
4 Choice reaction MT	NS	0.02	0.001	NS	0.02	NS
DSST	NS	0.07	<0.001	NS	0.043	NS
TMB	NS	0.1	-	NS	-	-
RVIP hits	NS	0.002	-	NS	-	-
RVIP false alarms	0.06	NS	-	NS	-	-
RVIP reaction time	NS	NS	-	NS	-	-

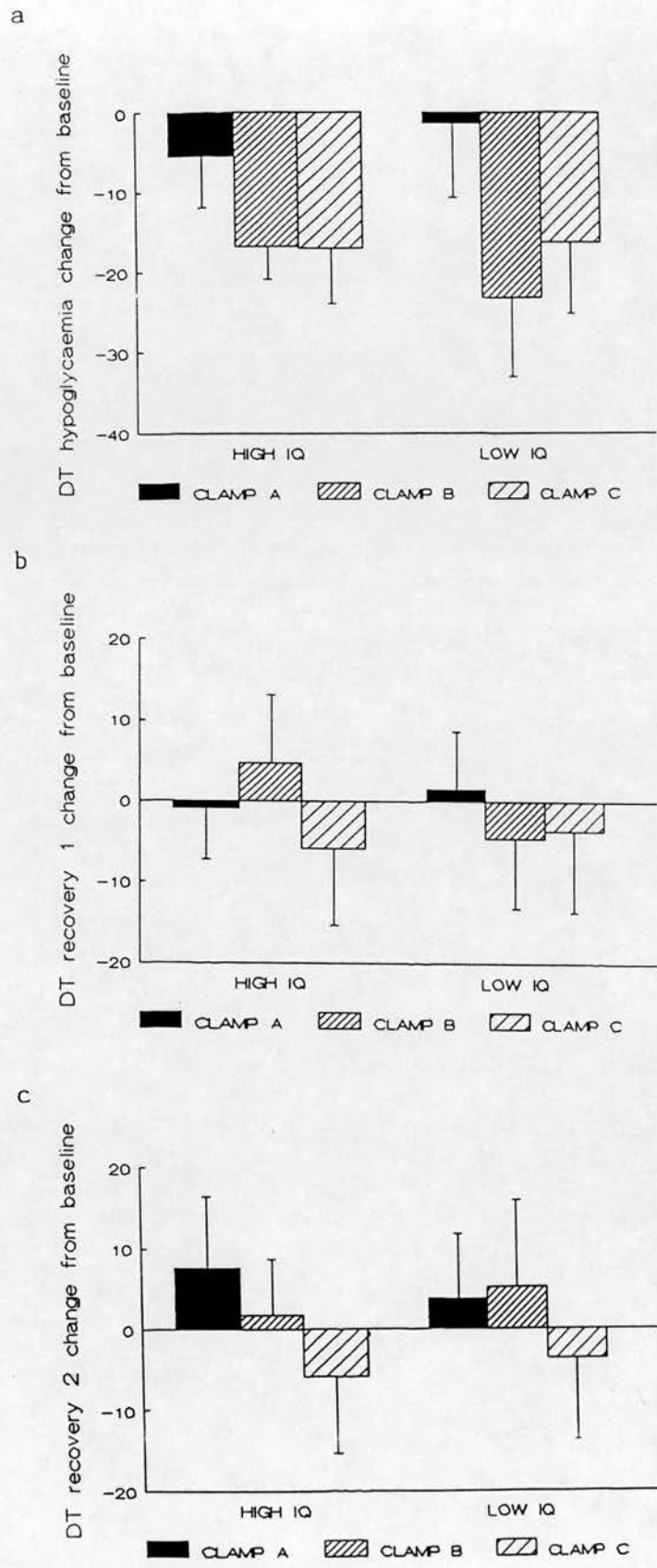


**FIGURE 3.2.1**  
**BLOOD GLUCOSE PROFILES DURING a) - STUDY A, b) STUDY B and**  
**c) STUDY C.**

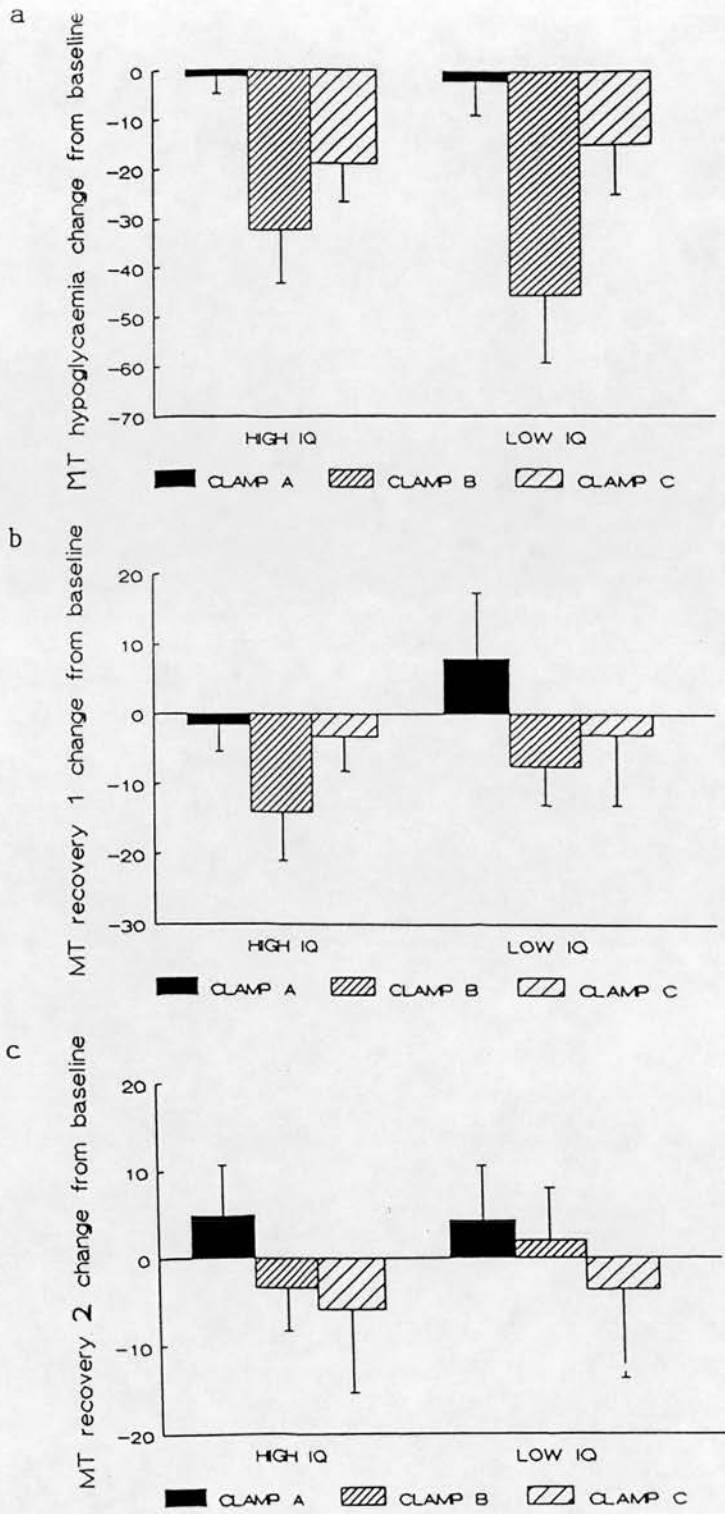


**FIGURE 3.2.2**  
**a) - PASAT 4 SECOND and b) PASAT 2 SECOND SCORES DEPICTED AS ABSOLUTE CHANGE FROM BASELINE SCORES.**

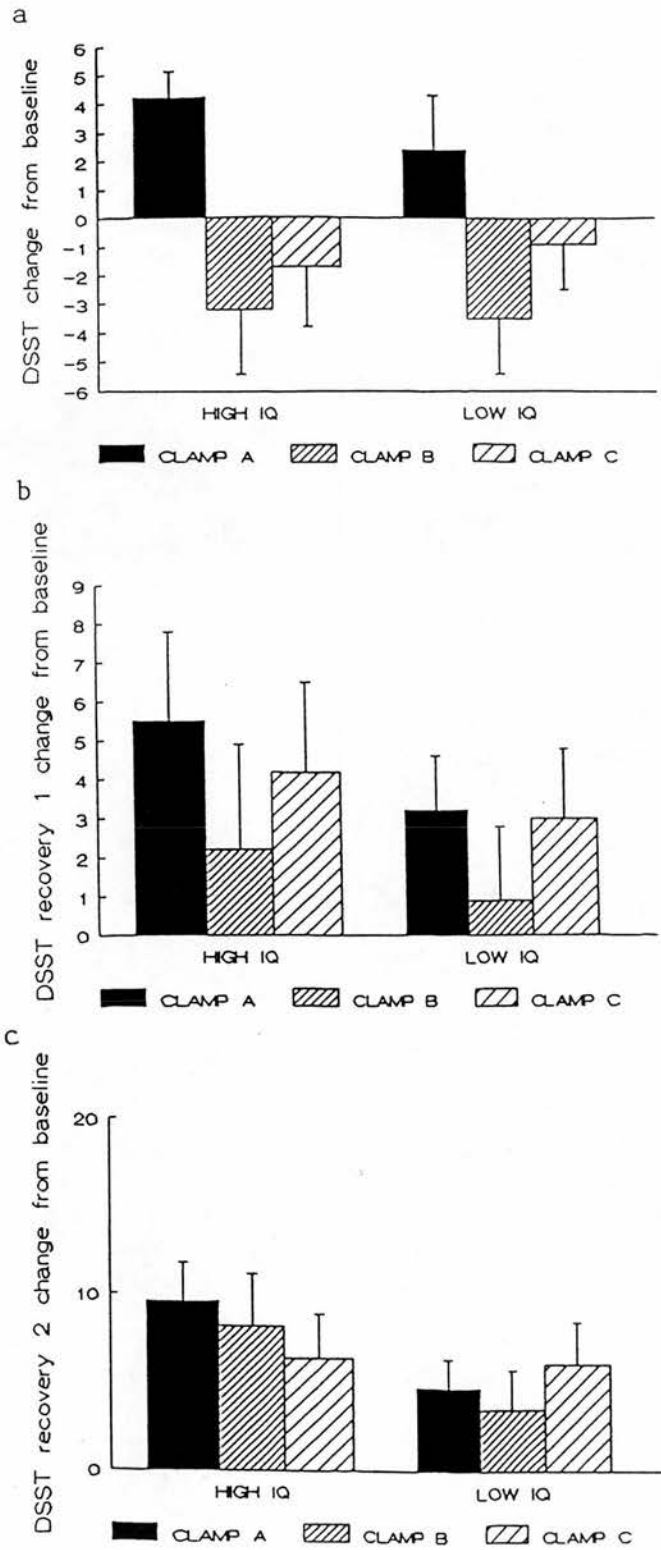




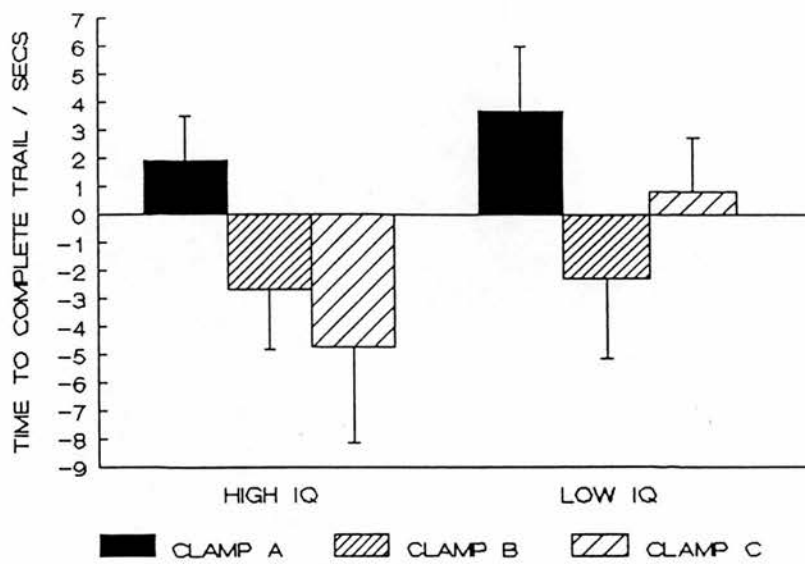
**FIGURE 3.2.3**  
**4 CHOICE REACTION TIME DECISION TIME (DT) CHANGES FROM**  
**BASELINE DURING a) hypoglycaemia, b) 15 minutes after recovery of**  
**euglycaemia c) 30 minutes after recovery of euglycaemia.**



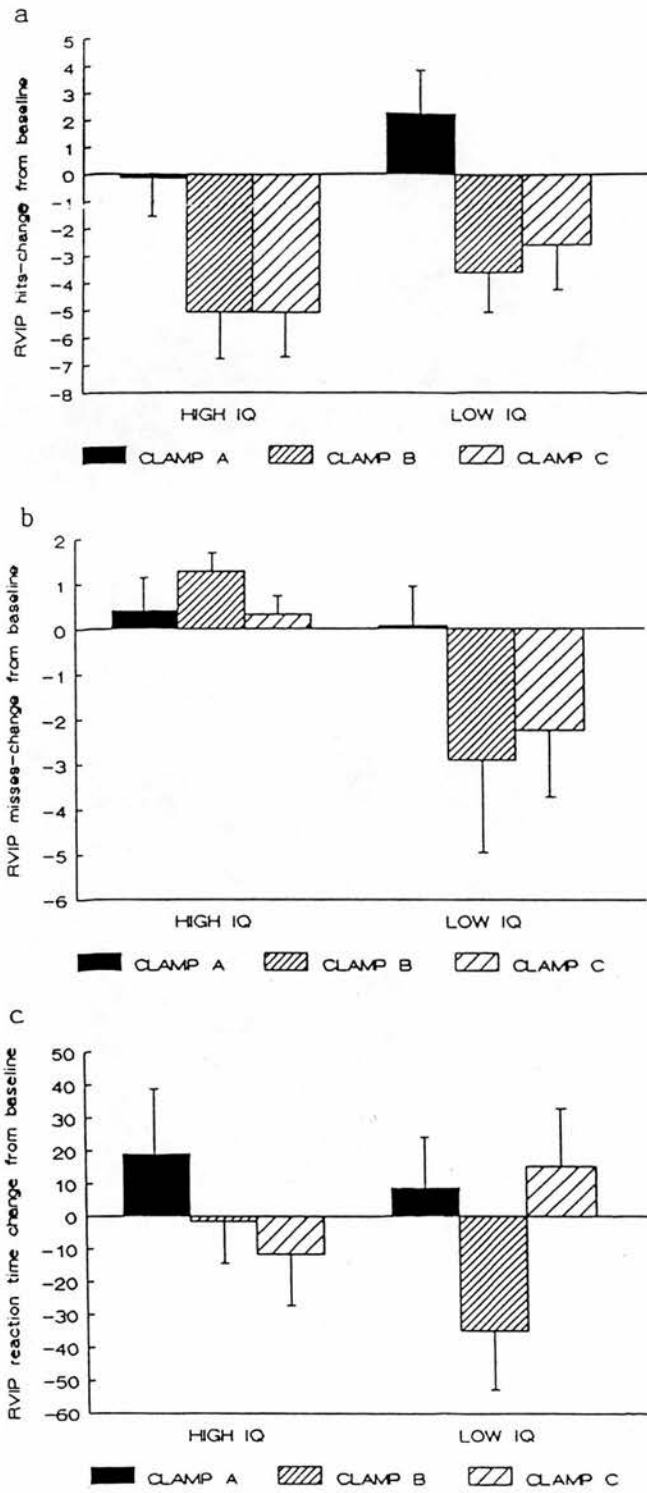
**FIGURE 3.2.3 (CONTINUED)**  
**4 CHOICE REACTION TIME MOVEMENT TIME (MT) CHANGES FROM**  
**BASELINE DURING d) hypoglycaemia, e) 15 minutes after recovery of**  
**euglycaemia f) 30 minutes after recovery of euglycaemia.**



**FIGURE 3.2.4**  
**DSST CHANGE FROM BASELINE DURING a) hypoglycaemia,**  
**b) 15 minutes after recovery of euglycaemia**  
**c) 30 minutes after recovery of euglycaemia.**



**FIGURE 3.2.5  
TMB CHANGE FROM BASELINE DURING HYPOGLYCAEMIA.**



**FIGURE 3.2.6**  
**RVIP CHANGE FROM BASELINE DURING HYPOGLYCAEMIA**  
 a) 'hits', b) 'false alarms' (misses) c) reaction time (RT).

## DISCUSSION

There is a large interindividual variation in the response of cognitive function to various metabolic insults, including acute hypoglycaemia (Herold et al 1985, Hoffman et al 1989, Maylor et al 1990). The much-posed but little-tested question arises as to whether those subjects of higher IQ have a reserve capacity enabling them to cope better under such studies as hypoglycaemia.

The hyperinsulinaemic glucose clamp provides a reproducible tool with which to study the effects of IQ on cognitive reserve. The cognitive test battery was carefully selected to examine complex cognitive processes which have been shown previously to be more affected by acute hypoglycaemia (Holmes et al 1983, Herold et al 1985, Pramming et al 1986, Hoffman et al 1989, Stevens et al 1989). All of the tests in the present study were significantly affected by hypoglycaemia with the exception of the TMB test although it has been shown previously by Hoffman et al (1989) that this test is affected by hypoglycaemia in patients with Type 1 diabetes. However, in a study comparing healthy subjects with patients who had Type 1 diabetes in which Trail Making B was used as part of the cognitive test battery, it was found that the patients with diabetes performed worse during hypoglycaemia (Wirsen et al 1991); this may explain why the performance in our healthy subjects did not deteriorate significantly in this test during hypoglycaemia.

The low IQ group had a significantly poorer performance at baseline performance which might be anticipated. Irrespective of whether absolute declines in scores or percentage changes in scores were examined, the results were very similar. Overall, during acute hypoglycaemia there were few differences in deterioration in performance between the high and low IQ groups. In the present study no relative improvements in cognitive performance were observed after 40 to 60 minutes of hypoglycaemia when compared with performance after 5 to 25 minutes of hypoglycaemia in either group. This suggests that if adaptation to



hypoglycaemia does occur as suggested in some previous studies (Kerr et al 1989, 1991a, Nagy et al 1993) although not confirmed in the studies in our laboratory (Part III, Chapter 1), it does not appear to be an IQ-dependent process. Many previous studies have observed a delay in recovery of cognitive function following hypoglycaemia (Eckert et al 1992, Blackman et al 1992, Ryan et al 1990), although we have not been able to replicate these findings using an extensive cognitive test battery and if a lag in recovery does occur it does not appear to be IQ- dependent.

Further analysis of the individual tests determined which tests were most affected by hypoglycaemia. In the 4 second PASAT, performance by the low IQ group did not deteriorate as much as the high IQ group. It is possible that this difference may be attributable to a Type 1 error, but it is also consistent with the findings of Maylor et al 1990. It is also of interest that the 4 second PASAT is a test with an empirical association to memory (Deary et al 1991a). During hypoglycaemia there is an increase in cerebral blood flow to region of the brain involved in higher cortical function, in particular to the frontal lobes (Tallroth et al 1992, see Part III Chapter 4). It is possible that the supply of glucose to the brain is already maximal in subjects with a high IQ and that there is little additional capacity to increase transport of glucose across the blood brain barrier, for example by the recruitment of additional glucose transporters. Very little is known about the behaviour of the brain glucose transporters during acute hypoglycaemia and such a mechanism may underlie the poorer performance which was observed in the high IQ subjects in the 4-second PASAT in the present study. An alternative explanation may be that subjects of higher IQ utilise different neuronal pathways than those of lower IQ even when performing the same tasks. For example, in the PASAT the higher IQ subjects may devise a method of adding up and remembering the numbers presented, resulting in more correct responses, whereas the lower IQ

subjects may treat each number as a new task and will therefore complete fewer tasks overall but will also be less susceptible to the effects of neuroglycopenia as they are utilising more simple pathways.

The subjects with a lower IQ performed more poorly during hypoglycaemia in the RVIP false alarms, which is a measure of caution. This is similar to findings from another study (see Part III Chapter 3) in which patients with Type 1 diabetes who had impaired perception of the onset of hypoglycaemia (hypoglycaemia unawareness), were observed to perform more poorly in the RVIP misses during hypoglycaemia than diabetic subjects who had normal awareness of hypoglycaemia, even though the groups were matched for IQ. Other studies, which have not examined the effects of IQ, did not show a significant deterioration in caution or accuracy during hypoglycaemia and that these functions appear to be preserved at the expense of speed of performance (Holmes et al 1883, Herold et al 1985, Cox et al 1993a). A study by Deary et al (1992) demonstrated that previous repeated severe hypoglycaemia also resulted in more false positive responses in the RVIP, even when tested during euglycaemia. These pathways are also more susceptible to the effects of repeated metabolic insults such as recurrent severe hypoglycaemia (Deary et al 1992). The poorer performance in the subjects with low IQ in the RVIP false alarms during hypoglycaemia may suggest that there may be common neuronal pathways which are more susceptible to neuroglycopenia in diabetic subjects with impaired awareness of hypoglycaemia (see Part III Chapter 3) and in all subjects who have a lower intelligence.

A larger number of subjects for the study may have been beneficial and would have minimalised the risk of a Type 1 statistical error although in the present study at least twice the number of subjects were examined compared with other hypoglycaemic clamp studies previously described (Kerr et al 1989, Wirsén et al 1991, Stevens et al 1989). The study was very labour-intensive and it would have

been very difficult to study more subjects without more extensive staffing and resources. Many cognitive tests were applied which also predisposes to a Type 1 error; however as the study was concerned with an area in which there was little previous experimental data, a large test battery was utilised predominantly as an exploratory tool. The greater deterioration in performance in the 4-second PASAT could be explained by 'floor' effects ie. this particular test was not sufficiently sensitive to demonstrate a deterioration in the subjects with a lower IQ as their performance was already poor. The degree of hypoglycaemia induced was not severe; prolonged acute hypoglycaemia is uncomfortable for the subjects and most would not be able or willing to tolerate, nor would it have been ethical to have induced a lower blood glucose for a duration of greater than 60 minutes as was used in the present study.

The aetiology of cognitive dysfunction during hypoglycaemia is probably multifactorial, resulting from a direct effect of neuroglycopenia combined with non-specific influences such as fatigue and headache which are also induced by hypoglycaemia. However, clinically the important issue is the degree of dysfunction experienced by the patient which will determine the patients' ability to perform satisfactorily at work or in other tasks such as driving. People with higher IQ's may be pursuing more demanding careers and any change in performance caused by intermittent hypoglycaemia may influence the ability of the patient to complete essential tasks. We have not been able to show that non-diabetic with higher IQ have a greater cognitive reserve when exposed to acute hypoglycaemia. Presumably this finding can be extrapolated to diabetic individual and emphasises the importance of correcting hypoglycaemia as quickly as possible in order to avoid protracted interference with daily activities.

**In summary:-**

- subjects with a higher IQ are more susceptible to the deleterious effects of acute hypoglycaemia on cognitive function than subjects of a lower IQ
- subjects with a lower IQ appear to be less cautious during acute hypoglycaemia than subjects of a higher IQ.

Part III

Chapter 3

*Study 3*

**COGNITIVE DYSFUNCTION DURING ACUTE HYPOGLYCAEMIA  
IN PATIENTS WITH TYPE 1 DIABETES AND  
IMPAIRED AWARENESS OF HYPOGLYCAEMIA**

## INTRODUCTION

The degree of cognitive dysfunction experienced during hypoglycaemia is highly variable between subjects (Herold et al 1985, Hoffman et al 1989) and appears to be greater in diabetic than non-diabetic subjects (Wirsen et al 1992), suggesting that individuals with diabetes have an increased susceptibility to neuroglycopenia. Impairment of subjective awareness of hypoglycaemia is an acquired complication of insulin-treated diabetes of long duration and affects almost 50% of patients who have had diabetes for 20 years or more (Premming et al 1991). Diabetic patients who have a history of impaired awareness of hypoglycaemia have been reported to perform more poorly on cognitive function testing (Trail-Making B) than those with normal awareness of hypoglycaemia, both at euglycaemia and during hypoglycaemia (Hepburn et al 1991b), suggesting that patients with impaired awareness of hypoglycaemia have an acquired cognitive impairment with a heightened susceptibility to neuroglycopenia. The principal aim of this study was to compare the effect of moderate hypoglycaemia (2.5mmol/l) on cognitive function in Type 1 diabetic patients who had normal awareness of hypoglycaemia with patients who had a history of impaired awareness of hypoglycaemia.



## METHODS

### Subjects

20 patients with Type 1 diabetes, 10 with normal awareness of hypoglycaemia (Group A) and 10 with a history of persistent impaired awareness of hypoglycaemia (Group B), were recruited from the Diabetic Clinic of the Edinburgh Royal Infirmary. The patients were allocated to each group a) according to their individual responses to a visual analogue scale which assessed awareness of hypoglycaemia (1 = always aware, 7 = never aware) and b) if they had noticed any change in symptomatology since diagnosis. Patients were assigned to Group A if they scored 1 on the scale and had experienced no changes in the nature or intensity of symptoms of hypoglycaemia since commencing insulin therapy. Group B patients scored 4 or more on the scale, reported predominantly neuroglycopenic symptoms during hypoglycaemia and frequently recorded blood glucose values below 2.5 mmol/l without experiencing symptoms.

The demographic details of the patients are shown in table 3.3.1. The median score on the visual analogue scale of awareness was 1.5 in Group A and 4 in Group B. The age of the patients, age of onset and duration of diabetes and frequency of previous severe hypoglycaemia did not differ significantly between the groups. One subject in each group was left-handed. Exclusion criteria for the study included a history of cerebrovascular, cardiovascular or peripheral vascular disease, previous head injury, psychiatric illness, epilepsy (including hypoglycaemia-induced convulsions), chronic alcoholism or any other systemic disease. Patients were excluded if they had diabetic retinopathy, other than early background changes, or visual impairment; none had evidence of peripheral or autonomic neuropathy. Two patients (one in each group) had recently been commenced on angiotensin converting enzyme inhibitors for hypertension. All patients were using human insulin with the exception of one patient in Group A

who was using porcine insulin. As insulin species has been shown not to have a differential effect on cognitive performance during acute hypoglycaemia (Maran et al 1993), the insulin species used was discounted in the recruitment of the subjects. Tests of intelligence level were performed on all the subjects prior to the study and the groups were matched on the basis of a measure of "general cognitive ability", assessed using the Alice Heim 4 Test (Heim 1968) and premorbid IQ, estimated using the National Adult Reading Test (Nelson 1982).

### **Experimental Procedure**

Studies were performed on two occasions, separated by at least two weeks. After a light breakfast patients were fasted until the end of the study. Administration of intermediate-acting insulin was omitted on the day of study and the morning dose of short acting insulin was reduced by 25% to reduce the risk of intercurrent hypoglycaemia. Patients were asked to perform frequent capillary blood glucose concentrations for 24 hours before the study. The study was postponed if a patient either experienced symptoms of hypoglycaemia or documented a blood glucose of less than 3.0 mmol/l.

The blood glucose concentrations were controlled using a hyperinsulinaemic glucose clamp as described in Part II.

The subjects were not informed of their blood glucose concentrations during the experiments. On one occasion (Study 1) the arterialised blood glucose concentration was stabilised and maintained at 4.5 mmol/l during the first battery of cognitive tests (T1), was then lowered to 2.5mmol/l and maintained at this level for 30 minutes during the second cognitive test battery (T2), before being restored to 4.5 mmol/l at which it was maintained for 10 minutes before and during the final cognitive test battery (T3)(fig. 3.3.1). On a separate occasion (Study 2) the blood

glucose concentration was maintained at 4.5 mmol/l throughout all three cognitive testing periods. The study conditions were counterbalanced. One of the female patients with impaired awareness was unable to participate in the euglycaemic study as she developed an exacerbation of her irritable bowel syndrome and required medication which may have affected cognitive function.

### **Cognitive and Physiological Measurements**

Blood was withdrawn for estimation of plasma catecholamines at the time points indicated in figure 3.3.1. Continuous heart-rate measurements and blood pressure were recorded as shown in figure 3.3.1.

The cognitive test battery took approximately 20 minutes to administer and consisted of four tests (as described previously in Part II) in the following order:

- 1 - Paced Auditory Serial Addition Test (PASAT) (Deary et al 1991a)
- 2 - Digit Symbol Substitution test (DSST) (Wechsler 1981)
- 3 - Trail-Making B (TMB) (Reitan et al 1974)
- 4 - Rapid Visual Information Processing (RVIP) (Deary et al 1992)

### **Statistical Analysis**

Multivariate analysis of variance (MANOVA) was used to assess the effects of hypoglycaemia on cognitive function. There were three independent variables :

- i) Awareness of hypoglycaemia, with two levels, "normal" (Group A) and "impaired" (Group B) ;
- ii) Study, with two levels, "hypoglycaemic" (Study 1) and "euglycaemic" (Study 2);
- iii) Time, with three levels, "first test period", "second test period" and "third test period" (T1, T2 and T3).

"Awareness" was a between subjects factor while "Study" and "Time" were within

subjects factors.

In Study 1 and Study 2 the first test period represents a baseline score assessed during euglycaemia (T1); the second test period (T2) represents the score during hypoglycaemia in Study 1 and a second test at euglycaemia in Study 2, and the third test (T3) period represents recovery from hypoglycaemia in Study 1 and a third test at euglycaemia in Study 2. The dependent variables were the scores derived on seven cognitive function tests : four and two second PASAT, DSST, TMB, RVIP hits, RVIP misses and RVIP reaction times.

The key variable in all the analyses is "Awareness", and the most important statistical results are the interactions representing the effect of awareness on performance under different study conditions (Awareness by Study interaction), and in particular the effect of awareness on performance at certain time epochs such as during hypoglycaemia and recovery (Awareness by Study by Time interaction).

Separate univariate analyses of variance (ANOVA) were used to assess the effects of Awareness, Study and Time on blood glucose, blood pressure and heart-rate.

Only nine patients of Group B were included in the overall MANOVA because of missing data, although further analysis of variance of the individual test scores, was performed in all 20 patients. The overall significance in the multivariate tests was calculated from the Wilks' Lamda test statistic. Significant interactions were further analysed using non-paired t-tests to determine specific differences between the groups. Non-parametric tests (Mann Whitney) were used for hormonal data which were not normally distributed. All figures indicate standard errors unless otherwise specified.

TABLE 3.3.1

## DEMOGRAPHIC DATA OF DIABETIC SUBJECTS

	Group A Normal awareness of hypoglycaemia	Group B Impaired awareness of hypoglycaemia	Significance
n	10	10	-
Age (years)	37.4±5.1	35.0±7.7	NS
Duration of diabetes (years)	17.9±7.8	12.8±4.4	NS
Age of onset of diabetes (years)	19.6±9.5	22.1±10.3	NS
BMI (kg/m <sup>2</sup> )	24.2±3.0	25.6±2.7	NS
Insulin dose (u/kg)	0.75±0.2	0.72±0.2	NS
HbA1 (%)	10.3±2.2	9.7±1.0	NS
Episodes of severe hypoglycaemia (total no.)			
0 - 5	8	6	NS
>5	2	4	NS
NART score	36.3±6.5	35.4±10.1	NS
AH4 score	86.4±15.3	78.3±13.0	NS

(Mean ± SD)

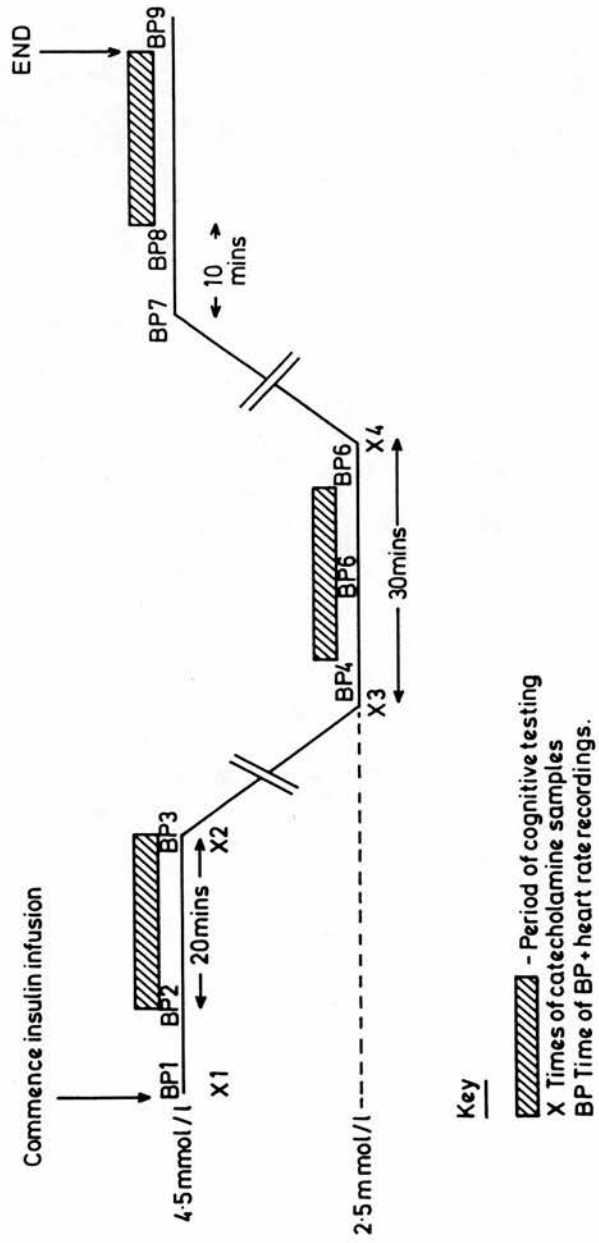


FIGURE 3.3.1 - STUDY DESIGN



## **RESULTS**

### **Blood glucose**

The blood glucose profiles of both groups are shown in figure 3.3.2. Once stable euglycaemic control had been established, the profiles were almost identical in both groups throughout the study. Testing with ANOVA revealed no significant main effect of Awareness, and the Awareness by Study and Awareness by Study by Time interactions were not significant. In effect, no overall difference between the two groups could be discerned, no differential effect of study type on blood glucose was identified between the groups, and no time epochs within studies were observed in which the blood glucose differed between groups.

### **Plasma Catecholamines**

The results of the plasma catecholamine measurements are shown in table 3.3.2. A marked variation was observed in the plasma concentrations of adrenaline and noradrenaline between subjects. Many of the measurements for noradrenaline were at the lower limit of detection of the assay, which may therefore lead to inaccuracies. During the euglycaemia study the plasma concentrations of noradrenaline and adrenaline did not differ significantly from baseline. Non-parametric analysis did not demonstrate any difference between the two groups in either study. However the increase in the levels of noradrenaline and adrenaline at their peak during hypoglycaemia, compared with basal levels was significant in both groups ( $p < 0.01$ ).

### **Blood pressure and heart rate**

The blood pressure and heart rate results are shown in figure 3.3.3. Throughout Study 1, Group A tended to have slightly higher systolic and diastolic blood pressures, although both groups demonstrated a rise in systolic blood pressure during hypoglycaemia. ANOVA showed no overall effect of Awareness, and the

Awareness by Study interactions were not significant for the changes in systolic and diastolic pressures. However, a significant difference in systolic blood pressure (but not diastolic blood pressure) was observed between the two groups at certain time epochs (Awareness by Study by Time interaction,  $p=0.02$ ). This suggests that the "aware" patients tended to have a slightly higher systolic blood pressure than the patients with impaired awareness, although further analysis at individual time points failed to identify any significant differences. Both groups also demonstrated a rise in heart rate during hypoglycaemia and the "aware" patients tended to have a slightly higher heart rate throughout the hypoglycaemia study. However, on ANOVA there was no overall effect of Awareness, and the Awareness by Study and Awareness by Study By Time interactions were not significant, which indicates that the heart rates of both groups were similar throughout the studies.

### **Cognitive tests**

The results of the individual tests for both Study 1 and Study 2 are shown in figures 3.3.4. The raw data for the scores is shown in table 3.3.3 a and b. To standardise the format of all figures, a deterioration in performance is represented by a downward deflection and an improvement as an upward deflection. It is evident that performance deteriorated during hypoglycaemia in all tests, with only minor changes in performance being observed in the euglycaemia control study.

### *Multivariate analysis:*

Results of the MANOVA of the effects Awareness, Study, Time and their interactions, on overall cognitive performance are shown in table 3.3.4. Irrespective of awareness, the cognitive test battery demonstrated a significant difference in performance between Study 1 and Study 2 during hypoglycaemia and on recovery from hypoglycaemia (Study effect  $p= 0.001$ , Study by Time interaction  $p=0.008$ ), confirming a detrimental effect of hypoglycaemia on overall

cognitive performance. A significant difference in performance in the different cognitive tests was also observed across time in the two different studies (Test by Study by Time interaction,  $p=0.009$ ), suggesting that not all of the tests were equally affected by the changes in blood glucose at all time points. For example, the pattern of change in caution in performance (RVIP misses, fig 3.3.4e) during hypoglycaemia and on recovery from hypoglycaemia was quite different from the change in performance observed with the PASAT or the DSST (Figs 3.3.4b and c). The overall MANOVA results indicate that there was an overall trend for awareness to affect performance ( $p=0.08$ ).

*Univariate analysis:*

The effect of Awareness and its interactions with Study and Time were performed using individual test scores to determine which tests were affected more than others (Table 3.3.5).

TMB (fig 3.3.4a) - A significant change in performance was noted across time when comparing Study 1 (hypoglycaemia) and Study 2 (euglycaemia) in all subjects (Study by Time interaction  $p<0.001$ ). Moreover a significant difference was observed between the groups at certain time epochs, in particular on recovery from hypoglycaemia (Awareness by Study by Time interaction  $p=0.03$ ). Further comparisons with t-tests confirmed that the patients with impaired awareness were significantly impaired following recovery from hypoglycaemia compared with the patients with normal awareness of hypoglycaemia ( $p=0.04$ ), although there were no differences in performance at baseline or during hypoglycaemia.

PASAT (4 and 2 second) (fig 3.3.4b) - Hypoglycaemia significantly affected performance in these tests, with a deterioration in scores during hypoglycaemia, followed by recovery in performance after the restoration of

euglycaemia (Table 3.3.3 - Study and Study by Time interactions  $p < 0.001$ ). However, ANOVA did not demonstrate any differences in performance between the patients with normal or impaired awareness at any time of measurement.

DSST (fig 3.3.4c) - This test was also significantly affected by hypoglycaemia, with a recovery in performance observed following restoration of euglycaemia (Table 3.3.3 - Study effect  $p=0.005$ ; Study by Time effect  $p < 0.001$ ). No differences were found between the two groups at any time point.

RVIP hits (fig 3.3.4d) - Hypoglycaemia significantly affected performance in this test in both groups (Study effect  $p=0.04$ ; Study by Time interaction  $p=0.002$ ). A significant difference in performance was observed between the two groups at certain time epochs, in particular on recovery from hypoglycaemia (Awareness by Time interaction  $p=0.05$ ). However, overall there did not appear to be a difference between groups (Awareness by Study by Time interaction non-significant). Comparison of test scores at each time point in Study 1 using unpaired t-tests, demonstrated that, although no differences in performance were evident between the groups during euglycaemia, during hypoglycaemia the patients with impaired awareness tended to perform more poorly ( $p=0.08$ ). On recovery from hypoglycaemia the scores in the patients with normal awareness returned to the baseline score whereas those patients with impaired awareness of hypoglycaemia remained significantly impaired ( $p=0.02$ ).

RVIP misses (fig 3.3.4e) - The patients with impaired awareness of hypoglycaemia tended to be less cautious than the patients with normal awareness throughout both studies (Awareness effect  $p=0.08$ ). Analysis with t-tests at individual time points demonstrated that during hypoglycaemia the patients with impaired awareness became significantly less accurate than the patients with normal awareness ( $p=0.03$ ) and they remained less accurate even following



recovery from hypoglycaemia ( $p=0.04$ ).

RVIP reaction times (fig 3.3.4f) - The patients in both groups became significantly slower during hypoglycaemia (Study effect and Study by Time interaction  $p<0.001$ ) and the patients with impaired awareness tended to be slower overall. Analysis with t-testing demonstrated that the patients with impaired awareness of hypoglycaemia also tended to become slower during hypoglycaemia ( $p=0.09$ ) and remained significantly slower following recovery from hypoglycaemia ( $p=0.05$ ).

In the euglycaemia study (Study 2) significant differences between the groups were found only in reaction time during study period 2 (T2, RVIP-RT  $p=0.04$ ) and in accuracy during study period 3 (T3, RVIP misses  $p=0.04$ ).

TABLE 3.3.2

## PLASMA CATECHOLAMINE CONCENTRATIONS

	NORADRENALINE		ADRENALINE	
	Group A Normal awareness	Group B Impaired awareness	Group A Normal awareness	Group B Impaired awareness
<b>Study 1 - (hypoglycaemia)</b>				
Basal	2.0 (1.0-3.5)	1.6 (1.1-3.4)	0.17 (0.10-0.40)	0.13 (0.10-0.20)
Euglycaemia	2.2 (1.5-3.7)	2.3 (1.4-4.4)	0.17 (0.11-0.36)	0.15 (0.10-0.49)
Start of Hypoglycaemia	2.3 (0.1-4.5)	2.6 (1.7-5.7)	0.22 (0.10-0.52)	0.15 (0.10-1.89)
End of Hypoglycaemia	2.6 (1.3-4.4)	3.0 (1.5-5.4)	0.21 (0.10-0.47)	0.20 (0.12-2.12)
Restoration of euglycaemia	2.7 (1.5-4.8)	2.9 (1.5-2.9)	0.24 (0.10-0.32)	0.18 (0.10-0.75)
<b>Study 2 - (euglycaemia)</b>				
Basal	1.7 (0.8-2.9)	1.6 (1.0-2.6)	0.18 (0.10-0.27)	0.11 (0.10-0.19)
Euglycaemia 1	2.6 (1.0-4.0)	2.2 (0.8-2.4)	0.20 (0.10-0.30)	0.10 (0.10-0.16)
Euglycaemia 2	2.3 (1.2-4.6)	1.9 (1.2-10.2)	0.16 (0.10-0.44)	0.10 (0.10-0.15)
Euglycaemia 3	2.1 (0.9-2.9)	1.7 (0.9-3.9)	0.15 (0.10-0.24)	0.12 (0.10-0.18)
Euglycaemia 4	1.9 (1.1-3.7)	1.8 (0.7-2.2)	0.16 (0.10-0.22)	0.12 (0.10-0.15)
median (range)				



TABLE 3.3.3a

## SCORES OF COGNITIVE TESTS DURING STUDY 1 (HYPOGLYCAEMIA)

	GROUP A Normal awareness	GROUP B Impaired awareness	
<b>Trail-making B / seconds</b>			
1 - 4.5 mmol/l	41.3±10.5	45.8±13.5	NS
2 - 2.5 mmol/l	62.4±21.5	56.5±23.6	NS
3 - 4.5 mmol/l	36.9±8.2	52.0±20.5	p=0.04
<b>RVIP - 'hits'</b>			
1 - 4.5 mmol/l	21.3±6.8	19.2±4.4	NS
2 - 2.5 mmol/l	17.6±7.4	12.5±4.5	p=0.08
3 - 4.5 mmol/l	22.9±6.4	15.7±6.3	p=0.02
<b>RVIP - 'misses'</b>			
1 - 4.5 mmol/l	2.2±1.6	3.4±2.6	NS
2 - 2.5 mmol/l	1.5±0.7	4.5±3.7	p=0.03
3 - 4.5 mmol/l	2.7±1.6	4.8±5.6	NS
<b>RVIP - 'reaction time' / ms</b>			
1 - 4.5 mmol/l	478±70	548±120	NS
2 - 2.5 mmol/l	542±82	632±135	p=0.09
3 - 4.5 mmol/l	484±78	567±93	p=0.05
<b>PASAT - 4-second</b>			
1 - 4.5 mmol/l	55.5±3.4	54.5±4.1	NS
2 - 2.5 mmol/l	43.9±9.4	44.1±10.2	NS
3 - 4.5 mmol/l	56.8±2.9	53.5±7.2	NS
<b>PASAT - 2-second</b>			
1 - 4.5 mmol/l	36.3±11.1	33.7±10.4	NS
2 - 2.5 mmol/l	20.3±8.3	23.7±11.3	NS
3 - 4.5 mmol/l	41.5±11.5	35.1±12.4	NS
<b>DSST</b>			
1 - 4.5 mmol/l	60.6±15.0	53.7±17.0	NS
2 - 2.5 mmol/l	46.0±11.7	43.7±11.6	NS
3 - 4.5 mmol/l	54.6±12.3	51.5±12.7	NS

mean ± SD

TABLE 3.3.3b

## COGNITIVE SCORES DURING STUDY 2 (EUGLYCAEMIA)

	GROUP A Normal awareness	GROUP B Impaired awareness	
<b>Trail-making B / seconds</b>			
1 - 4.5 mmol/l	46.2±11.1	49.8±9.2	NS
2 - 4.5 mmol/l	42.9±12.0	47.1±14.8	NS
3 - 4.5 mmol/l	41.7±12.7	47.1±16.8	NS
<b>RVIP - 'hits'</b>			
1 - 4.5 mmol/l	22.5±7.5	20.3±4.7	NS
2 - 4.5 mmol/l	24.2±6.9	19.4±6.9	NS
3 - 4.5 mmol/l	23.7±8.5	18.4±2.1	NS
<b>RVIP - 'misses'</b>			
1 - 4.5 mmol/l	1.7±2.4	3.5±3.8	NS
2 - 4.5 mmol/l	1.8±2.3	3.8±3.4	NS
3 - 4.5 mmol/l	1.7±1.1	4.5±3.9	p=0.04
<b>RVIP - reaction time / ms</b>			
1 - 4.5 mmol/l	457±65	522±120	NS
2 - 4.5 mmol/l	419±68	482±60	p=0.04
3 - 4.5 mmol/l	445±26	466±26	NS
<b>PASAT - 4-second</b>			
1 - 4.5 mmol/l	57.3.6±3.2	56.1±2.4	NS
2 - 4.5 mmol/l	57.8±1.7	56.9±2.1	NS
3 - 4.5 mmol/l	58.2±1.7	56.6±2.8	NS
<b>PASAT - 2-second</b>			
1 - 4.5 mmol/l	39.6±10.8	37.6±8.2	NS
2 - 4.5 mmol/l	42.5±10.7	39.8±6.9	NS
3 - 4.5 mmol/l	44.8±10.6	41.0±7.9	NS
<b>DSST</b>			
1 - 4.5 mmol/l	56.0±9.2	55.7±17.6	NS
2 - 4.5 mmol/l	59.7±8.6	55.3±14.9	NS
3 - 4.5 mmol/l	62.4±9.3	56.0±11.4	NS

mean ± SD

TABLE 3.3.4

**MULTIVARIATE ANALYSIS OF VARIANCE OF COGNITIVE TEST SCORES AND INTERACTIONS WITH AWARENESS (AW),  
STUDY TYPE (IE HYPO- OR EUGLYCAEMIC) AND TIME ACROSS STUDY (TIME)**

<b>FACTOR</b>	<b>SIGNIFICANCE</b>	<b>EXPLANATION OF RESULTS</b>
AW	p=0.08	A trend towards an overall effect of awareness : the unaware group tend to perform worse
STUDY	p=0.001	An overall difference between Study 1 and Study 2 ie hypoglycaemia has a significant effect on performance
AW BY STUDY	NS	No overall difference in performance between Study 1 and Study 2 between the 2 groups
TEST BY STUDY	p=0.003	Certain cognitive tests respond differently to hypoglycaemia compared with prolonged euglycaemia
TESTS BY TIME	p=0.01	Significant difference in cognitive performance in different tests at certain time points.
STUDY BY TIME	p=0.008	Difference in performance at certain time points in Study 1 vs Study 2
AW BY STUDY BY TIME	NS	No significant effect of awareness on differences in overall cognitive performance between Study 1 and Study 2 at comparable time points
TEST BY STUDY BY TIME	p=0.009	Individual cognitive tests respond differently to different study conditions at certain time points.
AW BY STUDY BY TEST BY TIME	NS	No overall difference in the effect of awareness on performance on tests under different study conditions at comparable time points

'AW' - awareness of hypoglycaemia

TABLE 3.3.5

UNIVARIATE ANALYSIS OF VARIANCE OF INDIVIDUAL COGNITIVE TESTS AND INTERACTIONS WITH AWARENESS ACROSS TIME AND EFFECT OF STUDY

COGNITIVE TEST	STUDY	AW	AW BY TIME	STUDY BY TIME	AW BY STUDY BY TIME
PASAT - SLOW	p < 0.001	NS	NS	p < 0.001	NS
PASAT - FAST	p < 0.001	NS	NS	p < 0.001	NS
DSST	p = 0.005	NS	NS	p < 0.001	NS
RVIP HITS	p = 0.04	NS	p = 0.05	p = 0.002	NS
RVIP MISSES	NS	p = 0.08	NS	NS	NS
RVIP RT	p < 0.001	p = 0.09	NS	p < 0.001	NS
TMB	NS	NS	NS	p < 0.001	p = 0.03

'AW' - awareness of hypoglycaemia

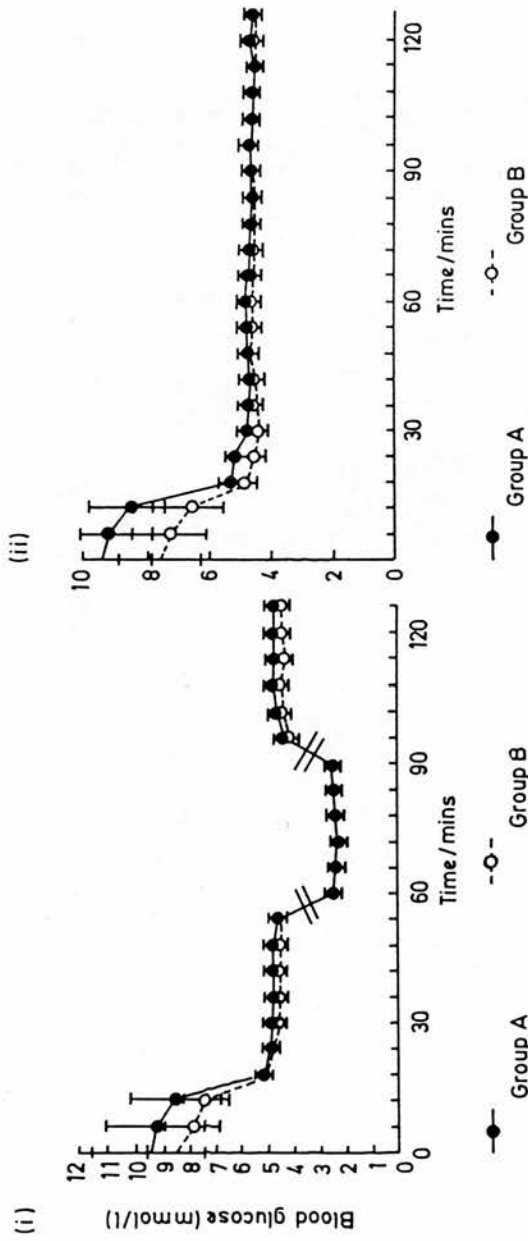
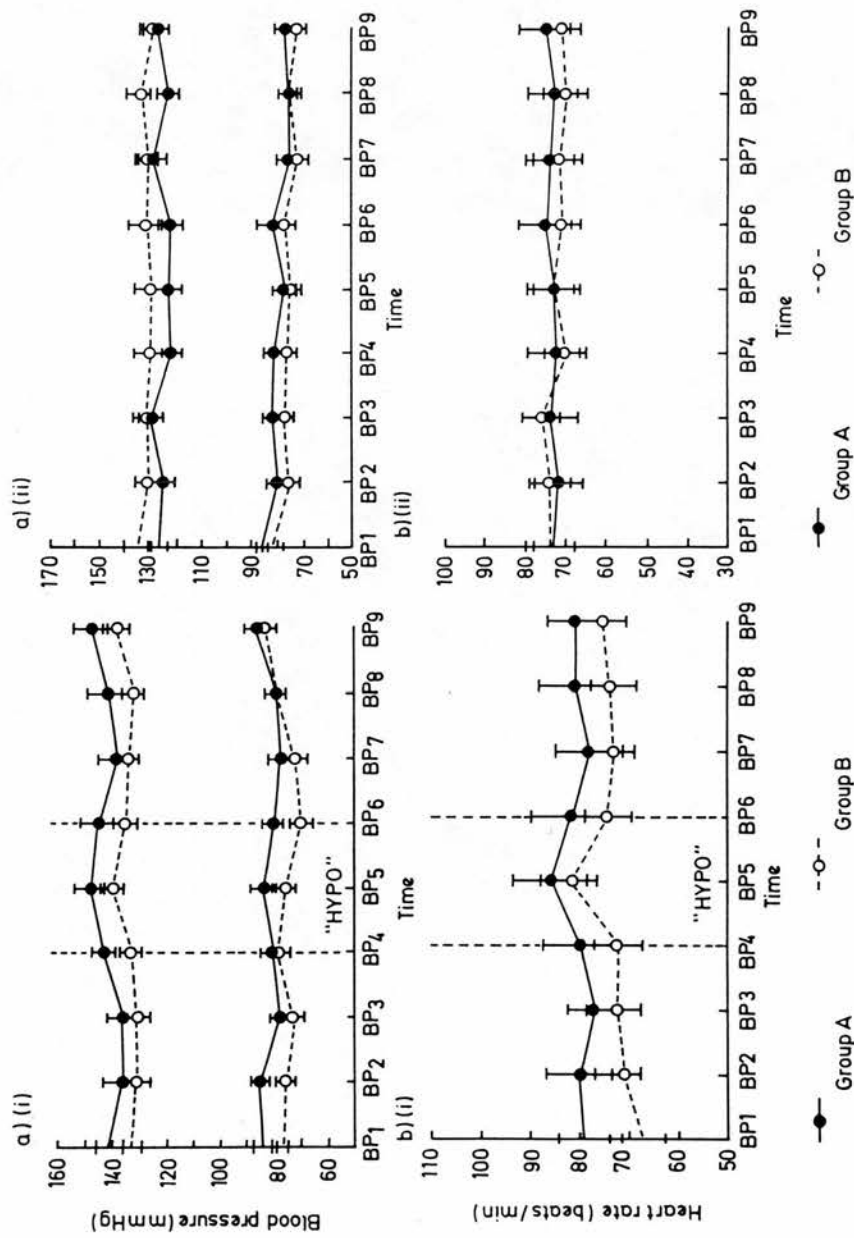


FIGURE 3.3.2  
 BLOOD GLUCOSE PROFILES FOR i) STUDY 1 (hypoglycaemia) i) STUDY 2 (euglycaemia).  
 GROUP A (normal awareness) - solid line, solid circles, GROUP B (impaired awareness) - dotted line, open circles.  
 Mean  $\pm$  SEM. Vertical dashed lines indicate period of hypoglycaemia.



**FIGURE 3.3.3**  
**BLOOD PRESSURE AND HEART RATE PROFILES DURING i) STUDY 1 (hypoglycaemia) ii) STUDY 2 (euglycaemia).**  
**GROUP A (normal awareness) - solid line, solid circles; GROUP B (impaired awareness) - dotted line, open circles. Mean  $\pm$  SEM**  
**a) Blood pressure :the systolic blood pressure is the upper line and the diastolic blood pressure is the lower line.**  
**b) Heart-rate**  
**Vertical dashed lines indicate period of hypoglycemia.**



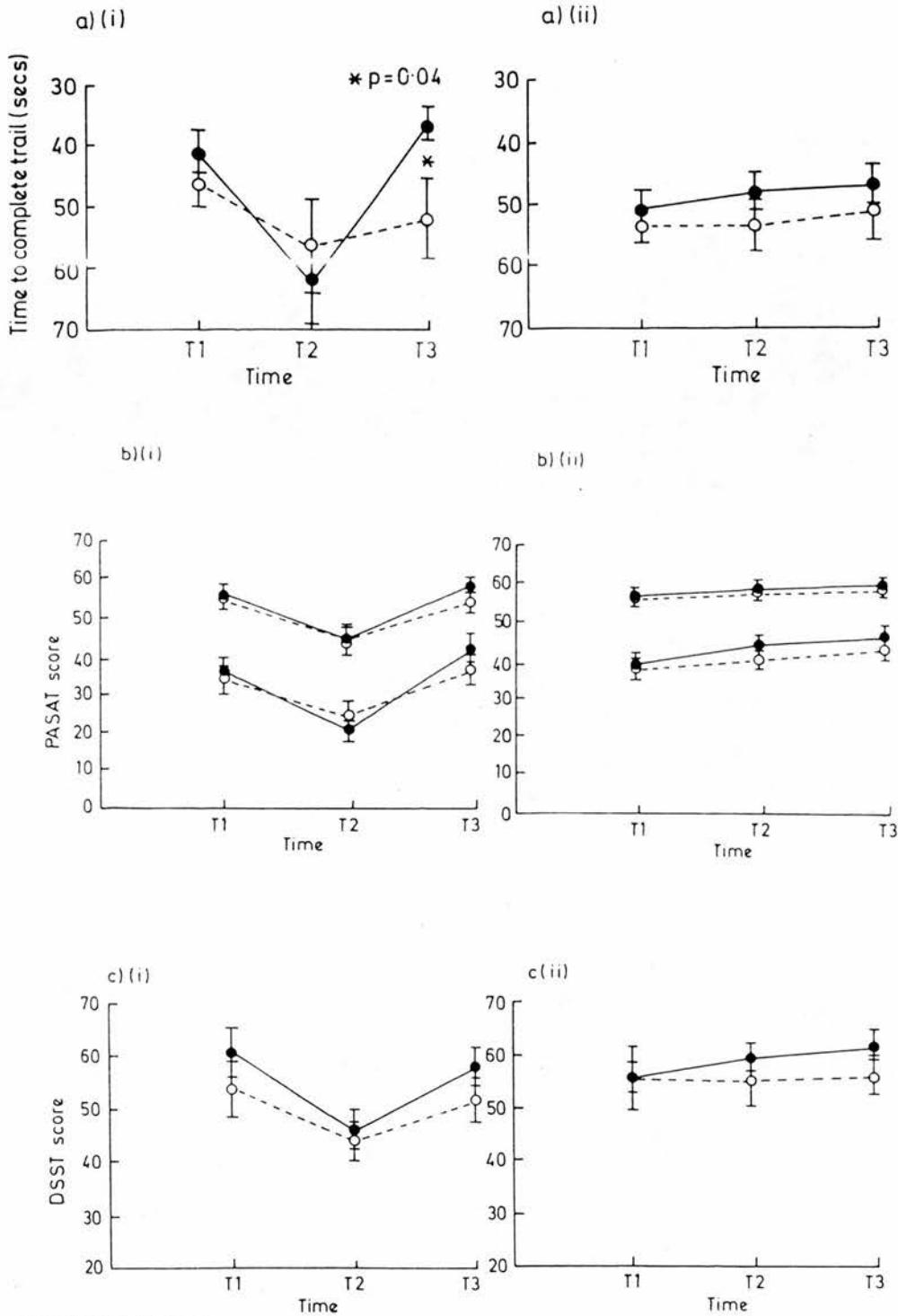


FIGURE 3.3.4

**COGNITIVE FUNCTION TEST RESULTS DURING:**

**i) STUDY 1 (hypoglycaemia) ii) STUDY 2 (euglycaemia). MEAN  $\pm$  SEM**

**GROUP A (normal awareness) - solid lines, solid circles.**

**GROUP B (impaired awareness) - dotted lines, open circles.**

a) TMB time for completion (secs).

b) PASAT test scores. The upper lines indicate the PASAT at 4 second intervals and the lower line indicate the PASAT at 2 second intervals.

c) DSST scores in 90 seconds.

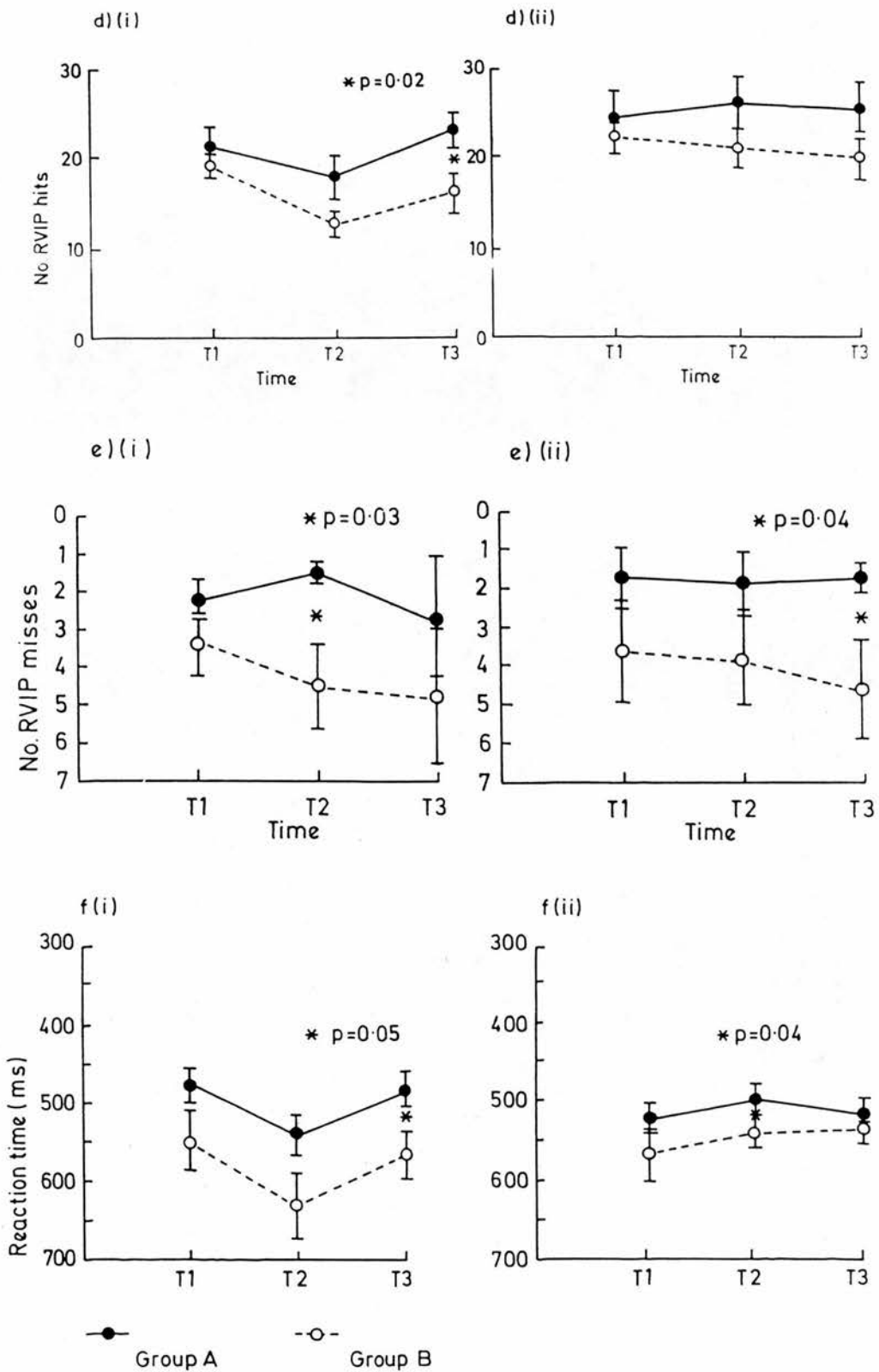


FIGURE 3.3.4 (CONTINUED)

- d) RVIP "Hits".
- e) RVIP "misses".
- f) RVIP reaction times (ms).

## DISCUSSION

Progressive impairment in cognitive function during acute hypoglycaemia of increasing severity is well recognised using different tests of cognitive function and all of the cognitive function tests employed in this study examined complex cognitive processes which have been shown previously to be most affected by acute hypoglycaemia (Holmes et al 1983, Herold et al 1985, Pramming et al 1986, Hoffman et al 1989, Stevens et al 1989). This study of Type 1 diabetic patients confirmed that deterioration of complex cognitive functions becomes evident during controlled hypoglycaemia.

A previous study which addressed the effects of acute hypoglycaemia on cognitive function in patients with diabetes (Herold et al 1985), suggested that those patients who experienced predominantly neuroglycopenic rather than autonomic symptoms during hypoglycaemia appeared to have a greater deterioration in reaction times, although the study was not designed to examine the relationship between either symptoms or awareness of hypoglycaemia and cognitive function. A subsequent study of acute hypoglycaemia in diabetic patients who had impaired awareness of hypoglycaemia demonstrated a significantly poorer performance in the Trail Making B test than "aware" diabetic controls, both at baseline euglycaemia and during hypoglycaemia (venous blood glucose 1.7mmol/l) (Hepburn et al 1991b), although the effect on recovery of cognitive function following hypoglycaemia was not examined. However in that study the patients were not matched for intelligence or previous history of severe hypoglycaemia (Hepburn et al 1991b).

In the present study patients were selected on the basis of a clinical history of subjective impairment of awareness of hypoglycaemia and not on the response to experimental hypoglycaemia. This approach was necessary because hypoglycaemia induced in a laboratory, particularly when using a hyperinsulinaemic glucose clamp, does not simulate the ability of the patient to

detect the onset of hypoglycaemia in normal daily life. Although it is acknowledged that retrospective estimates of hypoglycaemia may be inaccurate, it was important to match the groups for history of exposure to severe hypoglycaemia as this factor can affect cognitive function (Deary et al 1993a) and would confound the results. Although in the present study the overall relationship between awareness of hypoglycaemia and cognitive dysfunction during hypoglycaemia was manifested only as a trend towards a greater deterioration, the combination of cognitive tests that were employed was very sensitive in detecting changes during hypoglycaemia. The overall effect of awareness may have reached significance if a larger number of patients had been studied. Further analysis of the individual tests was therefore considered to be justified as they appeared to be affected differently depending on the study conditions (viz: the Test by Study by Time interaction was significant overall). For example the pattern for deterioration of cognitive function on the DSST was different from the TMB. ANOVA revealed an overall effect of awareness on the TMB and RVIP tests: TMB scores were affected differently between the groups by the effect of hypoglycaemia at comparable time points (Awareness by Study by Time interaction). For RVIP hits an overall effect of awareness was also identified at certain time points, irrespective of the study conditions for the RVIP hits. Direct comparison of scores between the two groups confirmed that patients with impaired awareness of hypoglycaemia had a poorer performance for RVIP hits during hypoglycaemia, and also a delayed recovery in their performance both for the RVIP hits and TMB, compared with those patients who had normal awareness of hypoglycaemia. Not all of the tests were affected equally by awareness, even though the tests were sensitive to the effects of hypoglycaemia, suggesting that neuroglycopenia may affect some neuronal pathways more than others in patients who have developed impaired awareness of hypoglycaemia.

An overall trend towards an effect of Awareness irrespective of Time or Study was

observed for RVIP misses and reaction time. Direct comparisons between the groups in the hypoglycaemia study (Study 1) revealed that the patients with impaired awareness were significantly less cautious, scoring more false alarms during hypoglycaemia (RVIP misses). They also tended to have slower responses during hypoglycaemia (RVIP RT), and during the recovery phase were significantly impaired compared to aware group (RVIP RT  $p=0.05$ ). In the euglycaemia study, all scores tended to improve with time which indicates that a "learning/practice" process was occurring and confirms that the deterioration in performance during the hypoglycaemia study could not be attributed to fatigue. However in the euglycaemia study the patients with impaired awareness became less cautious, scoring more false alarms towards the end of the study (increased RVIP misses at T3). They also appeared to "learn" more slowly than the aware group with respect to RVIP reaction times (RVIP RT at T2) although they did demonstrate a "catch up" effect later, as shown by the absence of a significant difference in reaction times at T3. These results suggest that overall degree of caution and speed of performance (measured by RVIP misses and RVIP reaction times) may be impaired in patients with impaired awareness of hypoglycaemia and that this function deteriorates further during hypoglycaemia. However, in the patients with normal awareness of hypoglycaemia caution was observed to increase during hypoglycaemia so that they scored fewer misses in the RVIP. This finding is in keeping with the conclusions of previous studies of patients with Type 1 diabetes with and non-diabetic subjects (Holmes et al 1983, Holmes et al 1984, Herold et al 1985, Cox et al 1991 and 1993a) in which accuracy of performance was maintained at the expense of speed of performance.

In the present study the patients with impaired awareness of hypoglycaemia performed less well than the aware patients during hypoglycaemia (2.5mmol/l) in one test alone (RVIP misses). However the degree of cognitive dysfunction may depend on the severity of hypoglycaemia induced; more abnormalities may have



become apparent at lower blood glucose concentrations which would surmount the glycaemic threshold for the induction of cognitive dysfunction (Premming et al 1986, Driesen et al 1991). In addition, at baseline euglycaemia the scores on the TMB did not differ between the diabetic groups, in contrast to the study by Hepburn et al (1991b) in which the patients were neither matched for IQ nor for history of previous exposure to severe hypoglycaemia, which probably directly affects cognitive function (Langan et al 1991, Deary et al 1993a). The method of induction of hypoglycaemia also differed from the present study so that direct comparisons are not applicable (Heine 1993). Although the hyperinsulinaemic glucose clamp technique is "unphysiological", at present it is the only method available which will allow prolonged testing of cognitive function at a constant and controlled level of hypoglycaemia.

It has been suggested that a "diabetic encephalopathy" may result from a combination of chronic hyperglycaemia and recurrent severe hypoglycaemia (Khardori et al 1986, Pozzessere et al 1988, Pozzessere et al 1991, Ryan et al 1992, Dejgaard et al 1991). It is possible that some patients who develop impaired awareness of hypoglycaemia have a form of diabetic encephalopathy. In addition, the same metabolic disturbances may increase susceptibility of the brain to neuroglycopenia resulting in a progressive decline in intellectual function and more pronounced impairment of cognitive function during acute hypoglycaemia. This hypothesis is supported by the evidence from a study in which cognitive function appeared to be more affected in diabetic patients than in non-diabetic controls during acute hypoglycaemia (Wirsen et al 1992).

In the present study the RVIP reaction times tended to be slower at all times during hypoglycaemia in the diabetic patients with impaired awareness. However RVIP reaction time is probably not an accurate measure of true reaction time as the patients are not instructed to complete the task as quickly as possible. However



this abnormality of cognitive function may indicate that the diabetic patients with impaired awareness of hypoglycaemia are more susceptible to the effects of the chronic metabolic disturbances of diabetes both on central and peripheral nerve function. In addition, the patients with impaired awareness of hypoglycaemia scored more RVIP misses at all times irrespective of the prevailing blood glucose concentration, suggesting that permanent neurological damage may have developed in these patients.

The plasma catecholamine concentrations were measured in view of a potential change in cognitive function occurring through a stimulatory effect on cerebral function. The effects of catecholamines and adrenoceptor blocking drugs, such as beta blockers, on cerebral function are controversial (Solomon et al 1983, Herrick et al 1989, Madden et al 1986, Deary et al 1991b). The glycaemic thresholds for the secretion of counterregulatory hormones in response to hypoglycaemia are influenced by prevailing glycaemic control, the frequency of exposure to hypoglycaemia and the duration of diabetes (Simonson et al 1985, Amiel et al 1988, Heller and Cryer 1991), and are impaired in patients who have chronic changes in their awareness of hypoglycaemia (Hepburn et al 1991b). These variables may account for the wide variation in the plasma catecholamine responses to hypoglycaemia which was observed in the present study and also for the skewed distribution within the patient groups. Cognitive testing may also increase the plasma catecholamines (Frankenhaeuser 1971) and make interpretation of the data difficult. In the present study the plasma catecholamine responses to acute hypoglycaemia of the two groups were compared using non-parametric tests and no significant differences were found at any time point. This would refute the possibility that increased plasma concentrations of catecholamines had provided an effective "stimulation" or "arousal" during hypoglycaemia in the aware group so resulting in their better performance. Although there was a rise in catecholamine levels during hypoglycaemia there were no differences between the groups which

was surprising in view of the data reported by Hepburn and colleagues (1991b) which showed that the threshold for release of counterregulatory hormones occurred at a lower blood glucose in the patients with impaired awareness and that the magnitude of the response was also diminished in comparison with the patients with normal awareness. In the present study the plasma catecholamine concentrations were greatly skewed by one patient with impaired awareness who had very poor glycaemic control with a total glycosylated haemoglobin of around 15%. It is likely that at a blood glucose of 2.5 mmol/l she was well above her threshold for release of counterregulatory hormones and therefore perceived the degree of hypoglycaemia as much more profound resulting in a greater magnitude of catecholamine secretory response. In addition, as noted previously, many of the nor-adrenaline concentrations fell at the lower end of detection of the assay which may have introduced inaccuracies.

Previous studies have alleged that cognitive function improves during prolonged hypoglycaemia in non-diabetic and insulin-dependent diabetic subjects suggesting that cerebral adaptation to neuroglycopenia may occur (Kerr et al 1989 and 1991a) although the study described in Part III Chapter 1 did not support these observations. In the present study the same order of the cognitive tests was maintained to avoid any potential effect on the tests scores during hypoglycaemia. The two tests that differed with respect to awareness of hypoglycaemia were the last to be administered. Although it is unlikely that cerebral adaptation developed during hypoglycaemia in the aware group of diabetic patients in the light of the previous data described, it is not possible to exclude its development so resulting in a better performance in the later cognitive tests.

Some studies have suggested that the glycaemic thresholds for changes in cognitive function are higher in subjects with strict glycaemic control ie cognitive function is preserved until lower blood glucose concentrations (Jones et al 1991, Ziegler et al

1992), whereas other studies have shown that there is no change in threshold (Widom and Simonson 1990) or in fact that cognitive dysfunction may even occur at a higher blood glucose in patients with strict glycaemic control (Amiel et al 1991). In the present study the groups were matched for prevailing glycaemic control to avoid the possibility of this confounding variable.

Some studies have suggested that recovery of cognitive function is delayed following normalisation of blood glucose both in non-diabetic and diabetic subjects (Herold et al 1985, Ryan et al 1990, Blackman et al 1992, Eckert et al 1992). To our knowledge comparisons between groups on the basis of chronic changes in awareness of hypoglycaemia have not been made previously. Heller et al (1987) demonstrated a delayed recovery in reaction times after hypoglycaemia (arterialised blood glucose 2.5mmol/l) in insulin-treated diabetic patients who did not develop symptoms of hypoglycaemia during the study, compared with symptomatic diabetic patients and non-diabetic subjects but the groups were not matched for duration of disease, glycaemic control or IQ. This delayed recovery would also be consistent with a diminished cerebral reserve to resist the effects of neuroglycopenia.

Accurate assessment of the symptoms of hypoglycaemia was not possible in the present study, as the cognitive test batteries were prolonged and SPET scans were also performed (see Part III Chapter 4) and, in order to avoid interruption, prohibited regular questioning about symptoms. In addition, cognitive testing *per se* induces symptoms such as anxiety which would interfere with interpretation of the symptom scores. However, we observed that most patients in both groups had few symptoms of hypoglycaemia at an arterialised blood glucose of 2.5 mmol/l, suggesting that the threshold for symptomatic responses was lower than that for cognitive dysfunction. This observation is consistent with previous reports (Pramming et al 1986, Mitrakou et al 1991). The glycaemic thresholds at which

cognitive function deteriorated during hypoglycaemia was examined in diabetic subjects using four-choice reaction time (Maran et al 1992). Those patients who had loss of awareness of hypoglycaemia exhibited cognitive dysfunction at similar blood glucose concentrations as non-diabetic subjects, despite having no symptoms of hypoglycaemia. These observations are of practical importance to diabetic management. Diabetic patients who have impaired awareness of hypoglycaemia, should be instructed that although they may feel entirely "normal" on recovery from an episode of hypoglycaemia, their cognitive function may remain impaired for a considerable time. Tasks such as driving or operating machinery should not be resumed for a period of at least 30-45 minutes after restoration of blood glucose to normal, and activities involving intellectual input should not be continued immediately otherwise performance is likely to be suboptimal.

In summary:-

Patients with impaired awareness of hypoglycaemia experience:

- greater cognitive dysfunction (in some tests) than patients with normal awareness of hypoglycaemia
- cognitive dysfunction persists despite restoration of blood glucose concentrations to normal.

Part III

CHAPTER 4

*Study 4*

**CHANGES IN CEREBRAL BLOOD FLOW DURING ACUTE  
HYPOGLYCAEMIA IN PATIENTS WITH TYPE 1 DIABETES  
AND IMPAIRED AWARENESS OF HYPOGLYCAEMIA**



## **INTRODUCTION**

Impaired awareness of hypoglycaemia occurs in 50% of patients who have a duration of diabetes of 20 or more years (Pramong et al 1991) and has been discussed in Part I, Chapter 1. The mechanism which results in impaired awareness of hypoglycaemia is not known but one possible mechanism which has been suggested is a deficiency of the glucose sensor mechanism (Frier 1993) which may be located in the brain (Biggers et al 1989, Skov and Pryds 1992) or possibly in the liver (Donovan et al 1993). In the previous study, Part III Chapter 3, it was observed that diabetic patients with impaired awareness of hypoglycaemia experience greater and more prolonged cognitive dysfunction in response to hypoglycaemia when compared with patients with normal awareness. The mechanism responsible for these differences is not clear but could result from differences in delivery, uptake and metabolism of glucose by cerebral tissue.

Technological advances have enabled more detailed analysis of cerebral metabolism and blood flow using Single Photon Emission Tomography (SPET) and this technique has been used to study a variety of neurological diseases (Launes et al 1989, Holman et al 1991, Grünwald et al 1991). Before the advent of this sophisticated scanning technique, crude estimates of cerebral blood flow had provided conflicting results with respect to the effect of hypoglycaemia. Some studies had described that there were no changes in cerebral blood flow (Eisenberg and Seltzer 1962, Kety et al 1948) whereas others had described an increase in cerebral blood flow during acute hypoglycaemia induced as part of insulin-shock treatment (Della Porta et al 1964). More recent studies have demonstrated an increase of approximately 20% in cerebral blood flow during hypoglycaemia (Neil et al 1987, Tallroth et al 1992, Kerr et al 1993) which persists after the restoration of euglycaemia. Studies of cerebral blood flow using SPET have also been performed both in non-diabetic subjects and in patients with Type 1 diabetes during acute hypoglycaemia (Tallroth et al 1992, 1993), and at euglycaemia in subjects who



had a history of recurrent severe hypoglycaemia (MacLeod et al in press).

The aim of this study was to try and identify any differences in rCBF during acute hypoglycaemia between diabetic patients with impaired awareness of hypoglycaemia and patients with normal awareness of hypoglycaemia, which may explain the differences in cognitive function during acute hypoglycaemia described earlier (Part III Chapter 3). Any difference in blood flow between patients with and without impaired awareness of hypoglycaemia may also give an indication as to which region of the brain may be responsible for sensing changes in blood glucose concentrations, leading to stimulation of the normal symptomatic and counterregulatory hormonal responses.

## **METHODS**

This study was combined with the study described in Part III chapter 3. The selection method and clinical characteristics of the subjects and the protocol for induction of hypoglycaemia has been described previously (Part III Chapter 3). In the present study SPET scans were only performed during Study 1 (ie hypoglycaemia study day) at the times shown in figure 3.4.1. The first scan was performed during euglycaemia after completion of the cognitive test battery (T1), and the second scan was performed during hypoglycaemia (2.5 mmol/l) after completion of the second cognitive test battery.

### **SPET Imaging Technique**

A cannula for injection of the 250 MBq bolus of isotope was inserted at the beginning of the study at the same time as the insertion of the cannulae for manipulation of the blood glucose. A single slice multi-detector dedicated head scanner (Multi-X 810, Strichman Medical Equipment Inc., Boston, USA) was used to examine subjects after repeated injections of half-dose fractions of <sup>99m</sup>Technetium Exametazime. The maximum in-slice resolution of the scan is given as 7.5mm (full width, half maximum) by 15 mm slice thickness, when using 572 hole collimators. The sensitivity of the instrument has been measured as 520 counts per second in a head sized phantom containing 1 KBq/ml (Ebmeier et al 1991). During injection of the isotope the patient lay on the imaging table with eyes covered and ears plugged to reduce sensory input. The noise in the study laboratory was kept to a minimum for five minutes after the start of the injection. The patient's head was then positioned in a moulded head rest with the help of two crossed light beams. To restrict movement, the head was fixed with pressure pads over the zygomatic arches.

During the first scan in the present study counts were acquired in two slices parallel to the orbito-meatal line (OML), at 40 and 60 mm above the OML. After

completion of the first scan, hypoglycaemia was induced as described previously. The second dose of 250 MBq was then injected during the same conditions as described above. During the second scan, the whole brain was scanned in parallel transaxial slices at 1 cm intervals from the OML upwards.

### **Image Analysis**

Local count densities were examined with a region of interest approach. Standard templates were derived from a neuroanatomical atlas (Talairach et al 1988) and fitted by aligning the outer border of the template with the 40% isocontour line of the brain activity map. This method avoids arbitrary definition of regions of interest centred on local hot spots, which could introduce a bias. The power of such analysis would allow detection of regional activation of approximately 5% in a group of 10 subjects. The count densities were corrected by determining the relative contributions of tracer from each of the injections. The normalised count for a particular region was the regional scan density resulting from each injection, divided by the whole slice density for that injection of tracer.

### **Cognitive Tests**

The cognitive tests were administered as described in Part III Chapter 3.

### **Statistical Analysis**

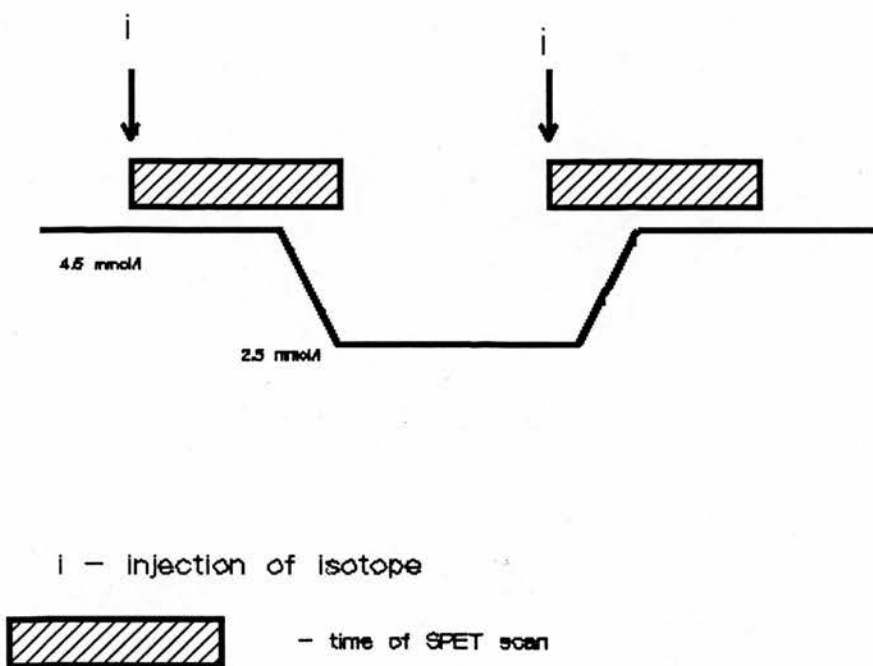
Univariate analysis of variance (ANOVA) was used to assess the effects of hypoglycaemia on tracer uptake in each region of cerebral blood flow.

There were two independent variables:

- i) Awareness with two levels, "normal" (Group A) and "impaired" (Group B)
- ii) Scan condition , with two levels, "euglycaemia" (baseline scan) and "hypoglycaemia" (second scan).

The 'awareness' variable is important in determining any differences in cerebral blood flow between the two groups and the interaction of 'awareness by scan condition' describes differences between the groups at different blood glucose concentrations.

Comparisons in regional tracer uptake and changes in tracer uptake were performed between the groups using unpaired t-tests while the effects of hypoglycaemia *per se* were examined using paired t-tests. Spearman's rho was used to correlate psychometric performance during euglycaemia and hypoglycaemia for those regions in which there was a significant change in blood flow.



**FIGURE 3.4.1**  
**STUDY DESIGN - SHOWING TIMES OF INJECTION OF ISOTOPE AND**  
**DURATION OF SPET SCAN**

## RESULTS

Results of blood glucose concentrations, cognitive tests, blood pressure, heart rate and symptom scores have been described previously (Part III, Chapter 3). Table 3.4.1 shows the normalised regional count densities during euglycaemia and hypoglycaemia for each cerebral hemisphere and each patient group. The results of the univariate analysis of variance for the individual regions are shown in table 3.4.2 and the further t-test analyses results are shown in table 3.4.3. Analysis of variance demonstrated a significant effect of awareness on count density in the right occipital region (lower slice) irrespective of blood glucose conditions (awareness effect  $p=0.03$ ) although further analysis with t-tests only showed a trend towards increased count density in the group with normal awareness at baseline ( $p=0.09$ ). ANOVA did demonstrate a significant effect of hypoglycaemia on regional count density in the following areas : right putamen (lower slice,  $p=0.04$ ), right thalamus (lower slice,  $p=0.01$ ), and both superior pre-frontal regions (left  $p=0.007$ , right  $p=0.01$ ). The paired t-test analyses confirmed these effects, demonstrating increased count densities during hypoglycaemia in the superior pre-frontal region and right thalamus and decreased count density in the right putamen (Table 3.4.3).

The PASAT was the only cognitive test to correlate with changes in tracer uptake and the results are shown in table 3.4.4. There was a significant positive correlation between accuracy on the 2-second PASAT and increased uptake in the right and left superior prefrontal cortex. There was also a similar correlation observed in the 4-second PASAT in the right putamen (Table 3.4.4).



TABLE 3.4.1

## NORMALISED COUNT DENSITIES OF THE REGIONS OF INTEREST

HEMISPHERE	NORMAL AWARENESS				IMPAIRED AWARENESS			
	LEFT		RIGHT		LEFT		RIGHT	
	Baseline	Hypoglycaemia	Baseline	Hypoglycaemia	Baseline	Hypoglycaemia	Baseline	Hypoglycaemia
<b>Lower slice</b>								
Frontal	1.04±0.02	1.04±0.03	1.07±0.01	1.05±0.02	1.03±0.01	1.04±0.02	1.03±0.01	1.04±0.02
Ant. temporal	1.10±0.02	1.09±0.03	1.16±0.02	1.09±0.04	1.08±0.02	1.10±0.03	1.13±0.01	1.18±0.06
Post. temporal	1.06±0.02	1.08±0.05	1.09±0.02	1.07±0.03	1.09±0.01	1.08±0.02	1.11±0.01	1.08±0.03
Occipital	1.07±0.02	1.10±0.05	1.08±0.02	1.08±0.08	1.03±0.01	1.03±0.02	1.03±0.01	1.05±0.03
Calcarine	1.26±0.03	1.17±0.03	1.27±0.02	1.23±0.06	1.24±0.02	1.24±0.05	1.27±0.02	1.03±0.02
Post. cingulate	0.92±0.02	0.96±0.05	1.01±0.03	1.01±0.03	0.95±0.04	0.97±0.05	1.06±0.03	1.24±0.04
Ant. cingulate	1.17±0.03	1.10±0.07	1.17±0.03	1.20±0.05	1.20±0.01	1.18±0.07	1.20±0.02	0.95±0.04
Caudate	0.91±0.04	0.89±0.04	0.89±0.04	0.94±0.08	1.00±0.01	0.85±0.07	0.91±0.04	0.89±0.05
Putamen	1.07±0.02	1.13±0.04	1.05±0.02	0.99±0.04	1.14±0.02	1.09±0.05	1.07±0.01	0.97±0.04
Thalamus	1.06±0.02	1.07±0.0	1.04±0.02	1.14±0.04	1.08±0.01	1.01±0.04	1.00±0.02	1.08±0.04
<b>Upper slice</b>								
Frontal	1.01±0.01	1.06±0.02	1.03±0.02	1.08±0.02	1.00±0.01	1.00±0.01	1.00±0.01	1.06±0.03
Ant. cingulate	1.19±0.02	1.14±0.03	1.19±0.06	1.19±0.06	1.18±0.03	1.18±0.03	1.18±0.07	1.29±0.05
Parietal	1.04±0.01	1.06±0.03	1.05±0.01	1.05±0.01	1.05±0.01	1.05±0.01	1.05±0.01	1.05±0.01
Occipital	1.05±0.02	1.05±0.02	1.05±0.02	1.05±0.02	1.05±0.02	1.08±0.04	1.04±0.01	1.07±0.03
Post. cingulate	1.28±0.02	1.27±0.06	1.29±0.07	1.26±0.02	1.27±0.03	1.21±0.04	1.26±0.03	1.35±0.04

mean ± SE

TABLE 3.4.2

**UNIVARIATE ANALYSIS OF VARIANCE OF  
NORMALISED REGIONAL COUNT DENSITIES**

	Scan Condition		EFFECT Awareness		Awareness by Scan	
	p value		p value		p value	
	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT
<b>Lower slice</b>						
Frontal	NS	NS	NS	NS	NS	NS
Ant. cingulate	NS	NS	NS	NS	NS	NS
Ant. temporal	NS	0.07	NS	NS	NS	NS
Post. temporal	NS	NS	NS	NS	NS	NS
Occipital	NS	NS	0.09	0.03	NS	NS
Calcarine	NS	NS	NS	NS	NS	NS
Post. cingulate	NS	0.06	NS	NS	NS	NS
Caudate	0.06	NS	NS	NS	NS	NS
Putamen	NS	0.04	NS	NS	NS	NS
Thalamus	NS	0.01	NS	NS	NS	NS
<b>Upper slice</b>						
Frontal	0.007	0.01	NS	NS	NS	NS
Ant. cingulate	NS	NS	NS	NS	NS	NS
Parietal	NS	NS	NS	NS	NS	NS
Occipital	NS	NS	NS	NS	NS	NS
Post. cingulate	NS	NS	NS	NS	NS	NS

TABLE 3.4.3

**T-TEST RESULTS OF COMPARISONS OF REGIONAL COUNT DENSITIES  
WHERE ANOVA EFFECT P < 0.05.**

<b>Awareness Effect</b>	<b>ANOVA p value</b>	<b>T-test p value</b>	<b>Comments</b>
Right occipital (lower slice)	p=0.03	p=0.09 baseline p=0.1 hypoglycaemia	Count density increased in normal awareness

<b>Scan Conditions Effect</b>	<b>ANOVA p value</b>	<b>T-test p value</b>	<b>Comments</b>
Right putamen (lower slice)	0.04	0.03	Count density reduced in hypoglycaemia
Right thalamus (lower slice)	0.01	0.008	Count density increased in hypoglycaemia
Left frontal (upper slice)	0.007	0.006	Count density increased in hypoglycaemia
Right frontal (upper slice)	0.012	0.012	Count density increased in hypoglycaemia

TABLE 3.4.4

**CORRELATION COEFFICIENTS BETWEEN RESULTS OF THE PASAT AND CHANGE IN TRACER UPTAKE  
FROM BASELINE TO HYPOGLYCAEMIA**

	Right		Superior prefrontal		Left		Right putamen	
	PASAT-2	PASAT-4	PASAT-4	PASAT-2	PASAT-2	PASAT-4	PASAT-2	PASAT-4
Baseline	0.07	0.23	0.23	-0.03	-0.03	-0.08	-0.21	-0.33
Hypoglycaemia	<b>0.59*</b>	0.37	0.37	<b>0.50*</b>	<b>0.50*</b>	0.22	0.44	<b>0.62*</b>
Difference	0.38	0.39	0.39	<b>0.48*</b>	<b>0.48*</b>	0.00	0.10	<b>0.61*</b>

$x^*$  - indicates significant correlation where  $p \leq 0.05$ .

## DISCUSSION

Tallroth and colleagues, using  $^{133}\text{Xenon}$  SPET demonstrated an increased cerebral blood flow especially to the frontal and parietal lobes. By use of an alternative technique,  $^{133}\text{Xenon}$  inhalation, a global increase in cerebral blood flow was demonstrated during hypoglycaemia (Kerr et al 1993). Hyperinsulinaemia *per se* was also shown to increase cerebral blood flow but to a more limited degree. These studies in human subjects are consistent with the findings in animal studies although delayed hypoperfusion rather than hyperperfusion has been demonstrated in animals, following the restoration of euglycaemia (Abdul-Rahman et al 1980, Siesjo and Abdul-Rahman 1979, Bryan 1988).

The changes in cerebral blood flow demonstrated by the present study reflect changes observed during euglycaemia in patients with diabetes and a history of previous severe hypoglycaemia (MacLeod et al in press). In that study, it was demonstrated that patients with diabetes had increased frontal lobe blood flow during euglycaemia compared with non-diabetic subjects, and, that in patients with a history of severe hypoglycaemia the frontal blood flow was augmented compared with diabetic patients who had not previously been exposed to severe hypoglycaemia. Other neurophysiological and neuropsychological studies have demonstrated that the frontal lobes appear to be particularly susceptible to the effects of hypoglycaemia (Holmes et al 1983, Pramming et al 1986). Therefore this increase in blood flow to the frontal lobes in response to hypoglycaemia may serve as a protective mechanism which has evolved to prevent a deterioration in cerebral function. The mechanism by which this occurs may involve the increased availability or function of glucose transporters, or the recruitment of capillaries as has been suggested previously (Blomqvist et al 1991, McCall et al 1986, Pelligrino et al 1990, Skov and Pryds 1992). Interestingly it has also been demonstrated that there appear to be increased numbers of Glut 1 transporters in

the posterior regions of the brain, compared with frontal regions (Brant et al 1993) and it could be surmised that the increased flow to the frontal areas may be necessary to overcome this relative deficiency of transporters. The results from the present study support this hypothesis: the greater the increase in frontal blood flow, the smaller the degree of cognitive dysfunction during hypoglycaemia, as measured by the PASAT. This would suggest that cerebral blood flow changes diminish the effect of acute hypoglycaemia on cognitive function.

The effect of awareness of hypoglycaemia on regional blood flow was less marked and insufficient to explain the differences in cognitive function between the groups during hypoglycaemia. Unfortunately it was not possible to perform three scans during the study in order to assess any differences in cerebral blood flow during the recovery period, as this would have exposed the patients to an excessive amount of radiation. As has already been discussed, impaired awareness of hypoglycaemia probably has a multifactorial aetiology and it is unlikely that a single area of abnormal cerebral blood flow would underlie this phenomenon. In addition, the resolution of the SPET technique would not be adequate at identifying subtle changes in cerebral blood flow, especially in the brain-stem. As the t-test analysis did not demonstrate a significant effect of hypoglycaemia, this suggests that the changes in blood flow were small and below the power of resolution of the scan. Further analysis using other techniques such as PET may provide answers in the future.



PART III

CHAPTER 5

*Study 5*

**MOOD CHANGES DURING ACUTE HYPOGLYCAEMIA IN  
NON- DIABETIC SUBJECTS**

## INTRODUCTION

In clinical practice patients with diabetes frequently report feeling miserable or tearful during or after an episode of acute hypoglycaemia and patients who have experienced many episodes of severe hypoglycaemia have been reported to be more anxious and to score lower on "happiness" ratings (Wredling et al 1992). Gonder-Frederick and colleagues assessed mood changes associated with fluctuations in blood glucose concentrations in patients with insulin-dependent diabetes (Gonder-Frederick et al 1989). They demonstrated that low blood glucose concentrations tended to be associated with negative mood states, primarily 'nervousness'. However there have been few dynamic studies performed during a single episode of acute hypoglycaemia to assess the nature and progression of changes in mood.

Moods are emotion-like experiences that are more persistent than emotional reactions, and lack reference to specific objects, by contrast with motivations, such as hunger, and emotions, such as fear (Matthews 1992). A great deal of research has been conducted on self-reported mood checklists in order to reveal their psychometric structure. Recently, some consensus has been achieved by accounts of mood which posit two or three dimensions. This contrasts with earlier research which assessed as many as 12 separate dimensions of mood (Nowlis 1965).

Matthews' review (1992) uses four mood dimensions in order to describe psychometric accounts of mood: 1) energetic arousal, 2) tense arousal, 3) hedonic tone and 4) general arousal. Of those offering two-dimensional solutions, some researchers characterise mood only in terms of energetic and tense arousal (Thayer 1989, Cox and Mackay 1985, Watson and Tellegen 1985), whereas an alternative two dimensional scheme includes hedonic tone and general arousal (Russell 1979, Diener et al 1985). In addition, McConville and Cooper (1992) suggest that hedonic tone and 'state extraversion' might offer the most parsimonious account of

mood. Three dimensional mood structures assess energetic arousal, tense arousal and hedonic tone (Matthews et al 1990, Sjoberg et al 1992). This three dimensional structure will be used in the present study, though it should be noted that they are not orthogonal; hedonic tone is moderately positively correlated with energetic arousal and negatively correlated with tension (at around 0.3 for both measures, Matthew 1992).

As may also be said of personality and ability dimensions, psychometric research cannot validate the structure of mood. At best, psychometric analysis may reveal a structure that is validated by other means. One approach of the validation of mood dimensions is to attempt to discover their biological bases, by means of experimental manipulations. Thayer (1989) has reviewed studies of drug manipulations of mood and demonstrated that no straight forward account of neurotransmitters and mood states can be offered at present. Manipulations such as carbohydrate ingestion, exercise, sleep deprivation and relaxation have been shown to affect energy and tension differently but have not afforded much advance in understanding the structure of mood (Thayer 1989, Johnson 1982, Matthews et al 1990).

The present investigation introduces a further biological method of manipulating mood states - namely a controlled hypoglycaemic episode using the hyperinsulinaemic glucose clamp technique (De Fronzo et al 1979). We hypothesised that subjects who experience hypoglycaemia will have different responses to the two types of arousal: as a result of the autonomic activity induced by hypoglycaemia we expect tense arousal to increase; and as a result of neuroglycopenia we expect energetic arousal to decrease. If such a state of 'tense tiredness' (Thayer 1989) can be demonstrated, ie. a state in which two types of arousal can be made to move in opposite directions, this will provide some validity

for the inclusion of two dimensions of arousal in addition to hedonic tone. Additionally, mood research may benefit from the addition of a biological procedure in which the physiological mechanisms are relatively well understood.

## **METHODS**

This study was performed in conjunction with *study 1* and the subjects, experimental protocol and study design are as described in Part III chapter 1.

Hypoglycaemia (arterialised blood glucose 2.5 mmol/l) was induced and maintained for a period of sixty minutes on two occasions (Studies B and C) and on a third occasion euglycaemia was maintained throughout (4.5 mmol/l) (see figure 3.1.1). The euglycaemic condition (A) served as a control/placebo condition for the other two conditions (B and C)

### **Assessment of Mood and Symptoms**

The UWIST Mood Adjective Checklist (UMACL) [Matthews et al 1990] was used to assess mood (described in Part II). Each subject was requested to complete the questionnaire at each of the seven time points shown in figure 3.1.1. The adjectives were graded by intensity on a scale of 1 to 4 and were classified into three groups : hedonic tone, tense arousal and energetic arousal (see appendix). Each dimension is indexed by eight items and higher scores indicated a high "happiness" rating for hedonic tone, a high "anxiety" rating for tense arousal and a high "energy" rating for energetic arousal. The maximum score which could be attained for each of the three mood components was 32 and the minimum score was four. Symptoms were assessed as described previously at the same time points as the assessment of mood (Part III chapter 1).

### *Statistical Analysis*

Analysis of variance (ANOVA) was used to assess the effects of hypoglycaemia on mood. In this study the two hypoglycaemia studies (B and C) served as replication of the hypoglycaemia condition and the scores obtained during the hypoglycaemia studies were meaned for the purpose of analysis. There were two main independent variables:

1 - Condition, a within subjects factor with two levels, "euglycaemia" (condition A), " hypoglycaemia" (mean [Condition B + Condition C]); the effect of *condition*, therefore, incorporates any effect of hypoglycaemia on mood.

2 - Time, a within subjects factor with seven levels, according to each of the time points of administration of the questionnaire.

The three dependent variables were the three UWIST mood factors. The three factors hedonic tone, energetic arousal, and tense arousal were analysed separate analyses of variance. In the present study conservative *post hoc* tests were not thought to be appropriate as the study was predominantly exploratory and, in order to investigate the time course of changes rather than the mere presence or absence of such changes in mood, several time points at which mood was measured were included. It was anticipated that the actual changes between many of these time points would be small and also highly variable. For example the changes between the scores at the end of euglycaemia and the beginning of hypoglycaemia would be much greater than the changes between the scores at baseline and the end of the initial euglycaemia. However, in the ANOVA the mood scores at each time point are treated equally resulting in a highly conservative analysis.

The overall significance in the multivariate tests was calculated from the Wilks' Lamda test statistic. Significant results were further analysed using t-tests to determine the significance of differences between the study conditions.



## RESULTS

### Symptom Scores

Results of the symptom scores at the 7 time points have been described previously (Part III Chapter 1). In summary, significant increments in autonomic and neuroglycopenic symptom scores were recorded reaching a maximum at the end of the period of hypoglycaemia.

### Mood Questionnaires

The results of the mood questionnaire factors in the different experimental models are shown in figure 3.5.1 a-c and the analyses of variance in table 3.5.1. The mood profiles were very similar in both the hypoglycaemia conditions B and C. Table 3.5.2 shows the comparisons (using t-tests) between the hypoglycaemia condition (ie mean {B+C} ) and the euglycaemia placebo condition A.

Hedonic Tone (Figure 3.5.1 a) In the euglycaemic control condition (A) the scores for hedonic tone did not change throughout the condition. However, in the hypoglycaemia studies hedonic tone decreased significantly during hypoglycaemia, but returned towards normal during recovery from hypoglycaemia (ANOVA Condition by Time interaction  $p=0.016$ ; Condition effect  $p=0.027$ ). Further analysis using t-tests demonstrated a significant difference from the control condition after 30 and 60 minutes of hypoglycaemia ( $p=0.007$  and  $p=0.001$ ).

Tense arousal (Figure 3.5.1 b) Little change in the tense arousal scores during the euglycaemic control condition was observed; during hypoglycaemia there was a significant increase in tension (ANOVA Condition by Time interaction  $p=0.001$ ; Condition effect  $p=0.001$ ). Further analysis with t-tests confirmed a significant increase in tension scores from the beginning of hypoglycaemia which did not return to normal until 30 minutes after restoration of euglycaemia ("hypo"  $p=0.001$ , "hypo+30 mins"  $p<0.001$ , "hypo+60 mins"  $p<0.001$ ,

"euglycaemia+15 mins"  $p=0.012$ ).

Energetic arousal (Figure 3.5.1 c) During the euglycaemia control condition there was a reduction in energy scores with a nadir at the fifth time point; however during hypoglycaemia there was a more profound reduction in energy scores during hypoglycaemia, although this just failed to reach significance on testing with ANOVA (Condition by Time interaction  $p=0.099$ ; Condition effect  $p=0.056$ ). Analysis with t-tests did indicate a lower energy score after 30 minutes of hypoglycaemia which persisted until the end of the condition ("hypo+30 mins"  $p=0.039$ , "hypo+60 mins"  $p=0.014$ , "euglycaemia+15 mins"  $p=0.045$ , "euglycaemia+30 mins"  $p=0.009$ ).

TABLE 3.5.1

**UNIVARIATE ANALYSIS OF VARIANCE FOR THE  
MOOD SCORES ACROSS TIME**

<b>Mood component</b>	<b>Condition</b>	<b>EFFECT</b>	
		<b>Time</b>	<b>Condition by Time</b>
Hedonic tone	0.027	0.003	0.016
Tense arousal	0.001	<0.001	0.001
Energetic arousal	0.056	<0.001	0.099

TABLE 3.5.2

**T-TEST SIGNIFICANCE AT SPECIFIED TIME POINTS COMPARING  
THE EFFECT OF EUGLYCAEMIA vs HYPOGLYCAEMIA ON MOOD**

<b>Mood component</b>	<b>Time of testing</b>	<b>Significance of t-test {A vs mean [B+C]}</b>
<b>Hedonic tone</b>	Baseline (0)	NS
	Euglycaemia (E)	NS
	Hypoglycaemia (H)	NS
	Hypo + 30 mins (H+30)	0.007
	Hypo + 60 mins (H+60)	0.001
	Restoration euglycaemia(E+15)	NS
	End (E+30)	NS
<b>Tense arousal</b>	Baseline (0)	NS
	Euglycaemia (E)	NS
	Hypoglycaemia (H)	0.001
	Hypo + 30 mins (H+30)	<0.0005
	Hypo + 60 mins (H+60)	<0.0005
	Restoration euglycaemia(E+15)	0.012
	End (E+30)	NS
<b>Energetic arousal</b>	Baseline (0)	NS
	Euglycaemia (E)	NS
	Hypoglycaemia (H)	NS
	Hypo + 30 mins (H+30)	0.039
	Hypo + 60 mins (H+60)	0.014
	Restoration euglycaemia(E+15)	0.045
	End (E+30)	0.009

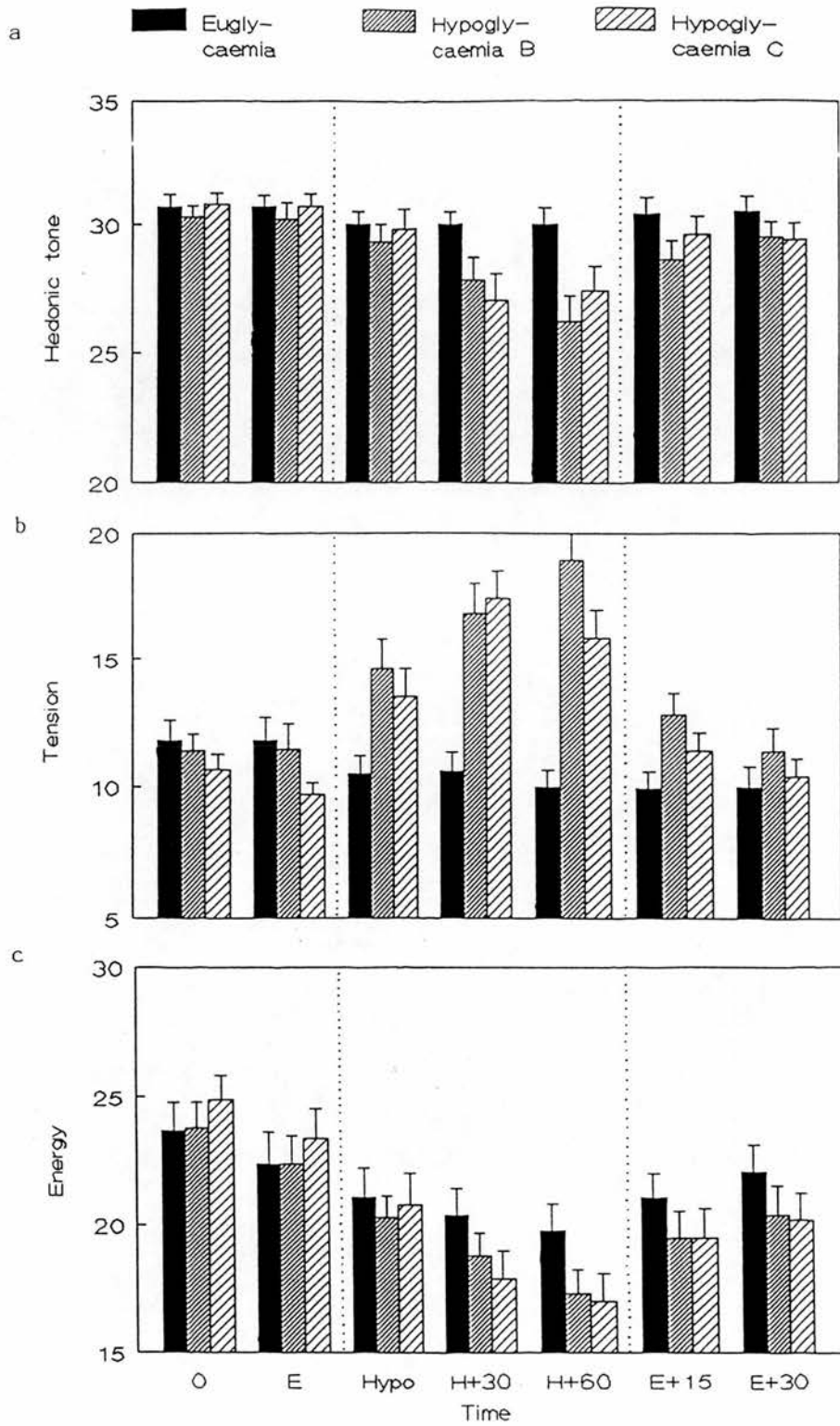


FIGURE 3.5.1

**CHANGES IN MOOD DURING ACUTE HYPOGLYCAEMIA**

**a - Hedonic tone**

**b - Tense arousal**

**c - Energetic arousal**

**Solid area - Study A; close hatched area - Study B; wide hatched area - Study C**

## DISCUSSION

Previous studies have shown that for individual diabetic patients treated with insulin, an idiosyncratic relationship exists between mood and blood glucose concentrations (Gonder-Frederick et al 1989). There appears to be much variation in the type of mood change experienced at high blood glucose concentrations with some patients reporting positive mood states (eg confident and cheerful) while others report negative mood states (eg angry and sad). However, low blood glucose concentrations appear to be mostly associated with negative mood states such as 'nervousness' (Gonder-Frederick et al 1989) although the 'mood scales' utilised did not differentiate between the different components of mood as described by Thayer (1989) and Matthews (1992).

In the present study a fall in hedonic tone, a rise in tense arousal and a fall in energetic arousal were observed during acute insulin-induced hypoglycaemia. These observations of increased negative mood states are consistent with the earlier results reported by Gonder-Frederick et al (1989). Acute insulin-induced hypoglycaemia results in a variety of counterregulatory neuronal and hormonal events (see Part I Chapter 1) and it is possible that some of these events may be responsible for the changes in mood described. As discussed earlier, the psychometric structure of mood has been agreed although there are few well-understood biological interventions of mood and hypoglycaemia provides one possible method to investigate the biological / physiological basis for changes in mood.

Thayer's model of mood (1989) includes the two components 'tense arousal' and 'energetic arousal' which act in opposite directions to produce the state of 'tense tiredness' which indicates declining physical resources together with subjective tension. Tense arousal is a negative mood tone associated with feelings of tension and anxiety or fearfulness and often combined with muscular tension. Energetic



arousal is a positive affective mood associated with decision-making and action. This state of 'tense tiredness' is thought to be important as it may be relevant to certain clinical states such as depression. In the present study the observed changes in mood during hypoglycaemia, with an increase in tension and a fall in energetic arousal fulfil this model of 'tense-tiredness'.

In the present study it is not possible to identify the separate biological bases for the changes in each mood factor. It is possible that activation of the autonomic nervous system may result in changes in mood resulting in increased tension or anxiety; however it is most likely that the mood changes result from a combination of central and peripheral neuroglycopenia and the counterregulatory hormonal changes associated with hypoglycaemia. As the time course for the changes in mood differed for energetic arousal compared with hedonic tone and tense arousal, this suggests that different biological factors are responsible. It would be of interest in further larger studies to attempt to correlate the changes in counterregulatory hormonal responses, and physiological changes such as haemodynamic responses and tremor, with the changes in each of the mood components.

In conclusion, hypoglycaemia provides a useful biological model with which to investigate changes in mood. In clinical practice these fluctuations in mood experienced during hypoglycaemia are important both to patients with diabetes and their relatives and friends, and may potentially interfere with social and work interactions. The effects of recurrent episodes of acute hypoglycaemia on mood are not known and require further assessment to establish whether these changes may become irreversible with time. In addition changes in other mood factors during acute hypoglycaemia such as anger would be of interest and of great importance to individual patients, particularly with regard to personal relationships

and interactions. This study provides further evidence for a detrimental effect of hypoglycaemia on psychological function.

In summary hypoglycaemia results in:

- lower hedonic tone which was rapidly restored to normal with the restoration of euglycaemia;
- higher tense arousal which was less rapidly restored to normal
- lower energy which persisted for at least 30 minutes after restoration of euglycaemia

PART IV

CHAPTER 1

*Study 6*

**THE FREQUENCY AND MORBIDITY OF HYPOGLYCAEMIA IN PATIENTS  
WITH TYPE 1 (INSULIN-DEPENDENT) DIABETES WHO HAVE  
IMPAIRED AWARENESS OF HYPOGLYCAEMIA**

## INTRODUCTION

Hypoglycaemia is a common and potentially dangerous side-effect of treatment with insulin in diabetic patients with a significant morbidity (Paz-Guevera et al 1975, Deckert et al 1978, Hepburn et al 1989, MacLeod et al 1993, Frier 1993). When severe hypoglycaemia is defined as the need for external assistance to effect resuscitation, retrospective estimates of the frequency of severe hypoglycaemia in unselected diabetic populations range from 1.1 to 1.6 episodes per patient per year (Prarming et al 1990, Reichard et al 1991, MacLeod et al 1993). Alterations in awareness of hypoglycaemia have been recognised for many years in patients with Type 1 diabetes and have been observed to predispose to a greater frequency of severe hypoglycaemia (Maddock and Trimble 1928, Cooke 1934, Lawrence 1941, Maddock and Krall 1953, Balodimos and Root 1959). The aetiology of impaired awareness of hypoglycaemia is probably multifactorial and has been discussed previously (Part I Chapter 1).

In a retrospective survey (Prarming et al 1990) almost 50% of patients with Type 1 diabetes were estimated to have experienced a change in the symptoms of hypoglycaemia after 20 years of insulin treatment, and this acquired defect has been shown to be associated with a higher frequency of severe hypoglycaemia (Hepburn et al 1990, Prarming et al 1990, MacLeod et al 1993). Severe hypoglycaemia produces profound neuroglycopenia which may impair the patient's subsequent recollection of the episode, and therefore retrospective assessments may underestimate the true frequency of severe hypoglycaemia experienced by affected patients. In the present study the frequency of severe hypoglycaemia in patients with Type 1 diabetes with normal awareness of hypoglycaemia was documented prospectively over 12 months and compared with that of a matched group of patients who had persistent impairment of awareness of hypoglycaemia.

## **METHODS**

### **Definition of impaired awareness of hypoglycaemia**

The symptoms which patients usually experienced during hypoglycaemia, were documented and each symptom assessed on a visual analogue scale of 1 to 7 (see Appendix) (1="not present", 7="present a great deal"); the symptoms were subdivided into autonomic, neuroglycopenic and non-specific groups (table 4.1.1). Awareness of hypoglycaemia was defined as "normal" if the patient had not experienced any subjective alteration since the diagnosis of diabetes and described the development of predominantly autonomic warning symptoms associated with the onset of acute hypoglycaemia (Hepburn et al 1991a). The patients with *normal awareness* of hypoglycaemia also scored between 1 and 2 on a visual analogue scale of 1 to 7 which was used to record usual awareness of hypoglycaemia (1="always aware of the onset of hypoglycaemia"; 7="never aware of the onset of hypoglycaemia"). Patients were considered to have *impaired awareness* if they had noticed a definite change in their warning symptoms of hypoglycaemia for at least two years, associated with a reduction in autonomic symptom scores so that predominantly neuroglycopenic symptoms were now experienced (table 4.4.1) and they had scored more than 4 on the visual analogue scale assessing awareness.

### **Patient Groups**

Sixty patients with Type 1 diabetes who were attending the diabetic outpatient review clinic of the Royal Infirmary of Edinburgh were recruited and divided into two groups on the basis of their self-reported awareness of hypoglycaemia:

Group 1 - 31 patients with normal awareness of hypoglycaemia.

Group 2 - 29 patients with impaired awareness of hypoglycaemia.

On questioning, the two groups had not differed in their symptom profiles of hypoglycaemia at the time of diagnosis of diabetes, but the patients with impaired awareness now experienced significantly fewer autonomic symptoms than those

patients with normal awareness (table 4.1.1). As the scores of symptoms experienced during hypoglycaemia at the time of diagnosis rely entirely on patient recall and may be inaccurate, it is not possible to compare these derived scores directly with the scores attained for their current symptom profile. Where possible, confirmation of impaired awareness was obtained by questioning spouses, partners or other close relatives about the patients' recent history of hypoglycaemia and their symptomatic responses.

### **Patient Characteristics**

The characteristics of each patient group are shown in table 4.1.2. The groups were matched for age, duration of diabetes, age of onset and glycaemic control at the start of the survey. Details of complications of diabetes were ascertained by physical examination including direct ophthalmoscopy, and also from clinical records. The presence of microalbuminuria was assessed by urinary albumin/creatinine ratios or if frank proteinuria (Albustix positive) was present, the magnitude was quantitated by 24 hour urine collections. Both groups had few diabetic complications and did not differ in the incidence or severity of retinopathy, neuropathy, or nephropathy (Table 4.1.2). Autonomic function tests were performed in all patients (Ewing et al 1985) and scores indicated that three (10.3%) of the 29 patients with impaired awareness had abnormal autonomic function and three (9.7%) of the 31 patients with normal awareness had abnormal autonomic function. Total glycated haemoglobin was measured using high-speed liquid chromatography based on an ion exchange reversed-phase partition method (Hi Auto A1c HA 8121) (non-diabetic range for our laboratory 4.5-8%) at intervals during the study (non-diabetic range for our laboratory 4.5-8%) and did not differ between the groups at the beginning or at the end of the study.

The number of daily injections of insulin did not differ between the groups; over



70% of both groups were taking a twice daily regimen (figure 4.1.1). Four patients in each group were using animal insulin and all the other patients had been using human insulin for at least five years prior to the study. The pattern of home blood glucose monitoring did not differ significantly between the groups; 52% of patients with normal awareness and 62% with impaired awareness monitored capillary blood glucose regularly, at least on alternate days.

### **Protocol for survey**

Patients were asked to document all episodes of hypoglycaemia within 24 hours of its occurrence and details about each hypoglycaemic episode were documented: time of day of episode, activity at time of hypoglycaemia (including sleeping), any obvious predisposing factors (eg delayed or missed meals, strenuous exertion), the treatment required, the need for external help and any resultant morbidity, such as physical injury, loss of consciousness, convulsions or accidents (see Appendix). *Severe* hypoglycaemia was defined as any episode requiring external assistance or resulted in coma. Witnessed accounts (by relatives, friends or colleagues) of each episode of hypoglycaemia were recorded wherever possible. The patients were asked to monitor capillary blood glucose either visually or using a meter on a regular basis and complete three 10 point diaries during each three month period (see Appendix). After enrolment patients were reviewed at three monthly intervals and glycaemic control was reappraised at each visit and any necessary adjustments in insulin doses were made. If considered to be appropriate, changes in insulin regimen were made in patients experiencing severe hypoglycaemia: two of the patients with impaired awareness of hypoglycaemia were changed to multiple injection insulin regimens to try and avoid hypoglycaemia; no changes in glycaemic control were observed as a result of these changes.

A questionnaire documenting "fear" of hypoglycaemia (Cox et al 1987) was undertaken at the beginning of the study to identify any differences in attitude and

behavioural responses towards hypoglycaemia. The patients' driving history and the number and nature of any previous road traffic accidents were documented.

### **Statistical Analysis**

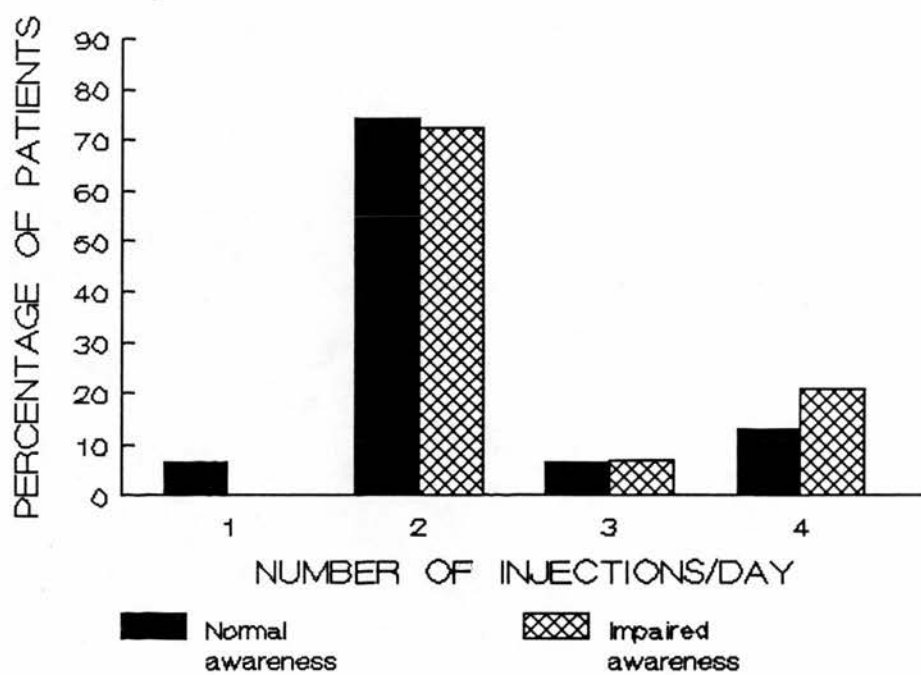
Demographic data were analysed using t-tests for unpaired samples. All other data which were not normally distributed were analysed using Wilcoxon rank sum tests and where applicable, Chi-squared tests were used to analyse group differences.

TABLE 4.1.1

SYMPTOM SCORES (MEDIAN) OF PATIENTS WITH TYPE 1 DIABETES RECALLED AT TIME OF DIAGNOSIS AND AT START OF STUDY

	AT DIAGNOSIS		PRESENT		p
	Group 1	Group 2	Group 1	Group 2	
Autonomic score (max =49)	16	14	14	10	p < 0.001
Neuroglycopenic score (max =49)	17	19	17	20	NS
Non-specific score (max =28)	6	8	6	5	NS

Group 1 - normal awareness  
 Group 2 - impaired awareness



**FIGURE 4.1.1**  
**FREQUENCY OF INSULIN ADMINISTRATION IN PATIENTS WITH**  
**TYPE 1 DIABETES**

## RESULTS

### Frequency and Time of Day of Hypoglycaemia

The frequency of all episodes of hypoglycaemia is shown in table 4.1.3. Approximately 85% of all episodes of severe hypoglycaemia were verified by witnesses. The patients with impaired awareness of hypoglycaemia experienced significantly more episodes of severe hypoglycaemia than the patients with normal awareness, but the frequency of mild episodes did not differ significantly between the two groups.

The number of episodes of severe hypoglycaemia occurring either while awake or asleep are shown in figure 4.1.2 and the times of occurrence throughout the day of the episodes of severe hypoglycaemia are shown in figure 4.1.2. The periodicity of severe hypoglycaemia differed between the two groups. In the patients with normal awareness, 60% of all episodes occurred between midnight and 08.00h, and 53% of all episodes occurred during sleep, compared with the patients with impaired awareness in whom 31% of all episodes occurred between midnight and 08.00h and 31% of episodes occurred during sleep. The patients with normal awareness of hypoglycaemia therefore experienced a significantly greater proportion of episodes of severe hypoglycaemia during the night (usually during sleep) compared with those with impaired awareness ( $p=0.05$ ). The patients with impaired awareness of hypoglycaemia experienced a significantly greater proportion of episodes during the evening before retiring to bed compared with the patients with normal awareness of hypoglycaemia. No differences in the identifiable causes of hypoglycaemia could be ascertained between the two groups. In the patients with normal awareness 60% of the episodes of severe hypoglycaemia could not be explained, as were 54% of the episodes in the patients with impaired awareness. The methods by which severe hypoglycaemia was treated did not differ proportionately between the groups; 14% of episodes ( $n=2$ )

in patients with normal awareness required glucagon compared with 21% (n=17) of episodes in patients with impaired awareness; *all* other episodes were treated with oral carbohydrate.

### **Morbidity**

Only five episodes of severe hypoglycaemia required hospital treatment. On two different occasions the same patient required admission for treatment, on one occasion suffering a fractured neck of femur and on the other a head injury. Two of the other three episodes occurred in another patient who had impaired awareness. Five (33%) of the fifteen episodes of severe hypoglycaemia in the patients with normal awareness of hypoglycaemia resulted in loss of consciousness compared with 29 (35%) of the 82 episodes in the patients who had impaired awareness. Only two patients, both of whom had impaired awareness of hypoglycaemia experienced convulsions during hypoglycaemia; one experienced nocturnal convulsions associated with hypoglycaemia on five separate occasions and the other experienced hypoglycaemic convulsions on two occasions, also during the night. Neither patient had evidence of idiopathic epilepsy, not associated with hypoglycaemia.

### **Fear of Hypoglycaemia**

Responses to the "fear" questionnaire indicated that patients with normal awareness of hypoglycaemia worried less about hypoglycaemia (median score 31.5) than patients with impaired awareness (median score 41)( $p=0.008$ ). However despite their increased concern and anxiety, the patients with impaired awareness had not modified their behaviour (median score 30) in an attempt to avoid hypoglycaemia, when compared with the patients with normal awareness (median score 29).



### **Home blood glucose monitoring**

Similar numbers of blood glucose measurements were made by both groups with a mean number of 90 recordings per patient. The mean number (in 12 months) of home blood glucose measurements with values less than 3.0 and 2.5 mmol/l respectively and which were not accompanied by symptoms of hypoglycaemia were significantly fewer in the patients with normal awareness of hypoglycaemia compared with the patients with impaired awareness (blood glucose values < 3.0 mmol/l  $p < 0.001$ ; blood glucose < 2.5 mmol/l  $p < 0.0001$ ) (Figure 4.1.4).

### **Driving Experience**

The questionnaire assessed overall driving experience since commencing treatment with insulin, and was not restricted to the study period. Twenty four patients with normal awareness of hypoglycaemia held a valid driving licence (restricted to three years); eight had previously experienced hypoglycaemia while driving but none of the episodes had resulted in road traffic accidents. Eleven of these patients had been involved in minor motor vehicle accidents at other times which were not related to hypoglycaemia. Only 11 patients with impaired awareness of hypoglycaemia currently held valid driving licences; five patients with impaired awareness had ceased driving voluntarily because of concern about the risk of causing accidents during hypoglycaemia, and no longer held driving licences: one patient's licence had been revoked by the licensing authority. However, of those patients who had impaired awareness and had continued to drive, five had experienced hypoglycaemia while driving. Only one such patient had been involved in a road traffic accident, which was not related to hypoglycaemia and was the fault of a third party. Comparison of the accident rates of both groups using chi-squared tests showed that the patients with impaired awareness tended to have fewer driving-related accidents overall ( $0.05 < p < 0.1$ ).

TABLE 4.1.2

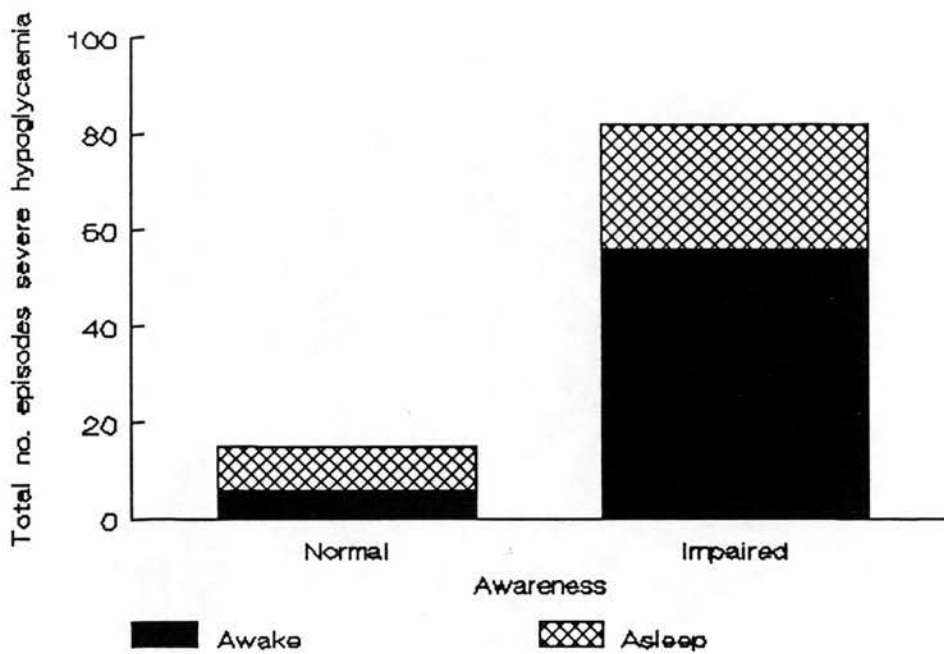
## DEMOGRAPHIC DATA OF PATIENTS WITH TYPE 1 DIABETES

	Group 1 (Normal awareness)	Group 2 (Impaired awareness)	Significance
n	31	29	
Gender distribution (M/F)	18/13	17/12	NS
Age (years)	43.9±10.6	48.4±11.7	NS
Age of onset (years)	25.3±10.3	27.8±10.8	NS
Duration of IDD (years)	18.6±7.6	21.0±8.1	NS
Daily insulin dose (units/kg)	0.63±0.09	0.69±0.10	NS
HbA1 (%) start of study	10.0±1.2	10.2± 1.5	NS
end of study	10.2±1.4	10.0±1.2	NS
<hr/>			
(mean±SD)			
<b>Complications /number of patients (percentage)</b>			
Microalbuminuria	4 (12.9)	4 (13.7)	NS
Proteinuria (<0.3g/l)	0	1 (3.4)	NS
<b>Retinopathy</b>			
Background	23 (74.4)	26 (89.6)	NS
Proliferative	8 (23.6)	3 (10.4)	NS
Awareness on linear analogue scale (median)	5	1.5	p<0.0005

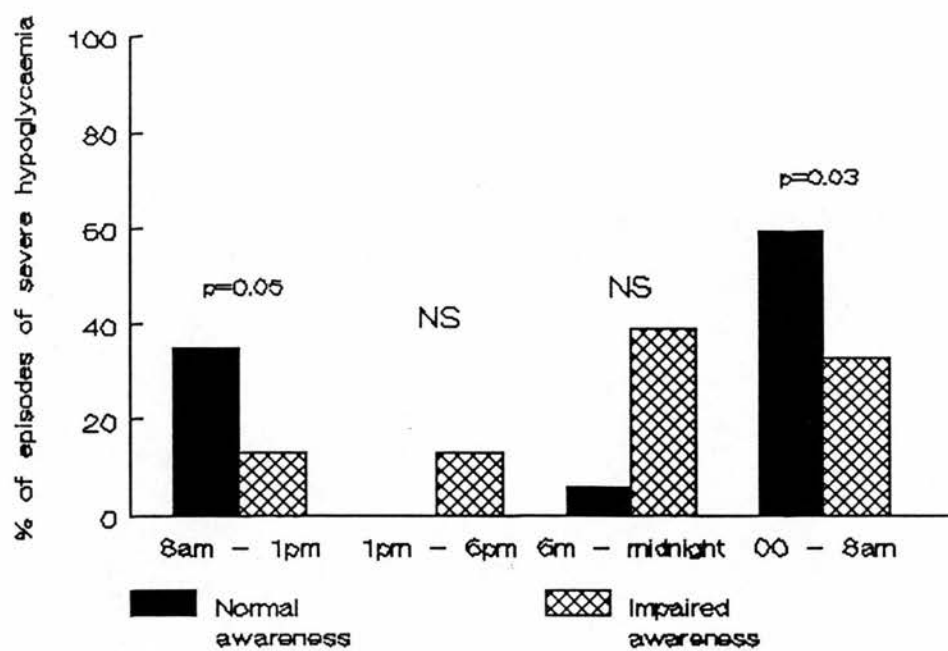
TABLE 4.1.3

**FREQUENCY OF HYPOGLYCAEMIA IN PATIENTS WITH  
TYPE 1 DIABETES.**

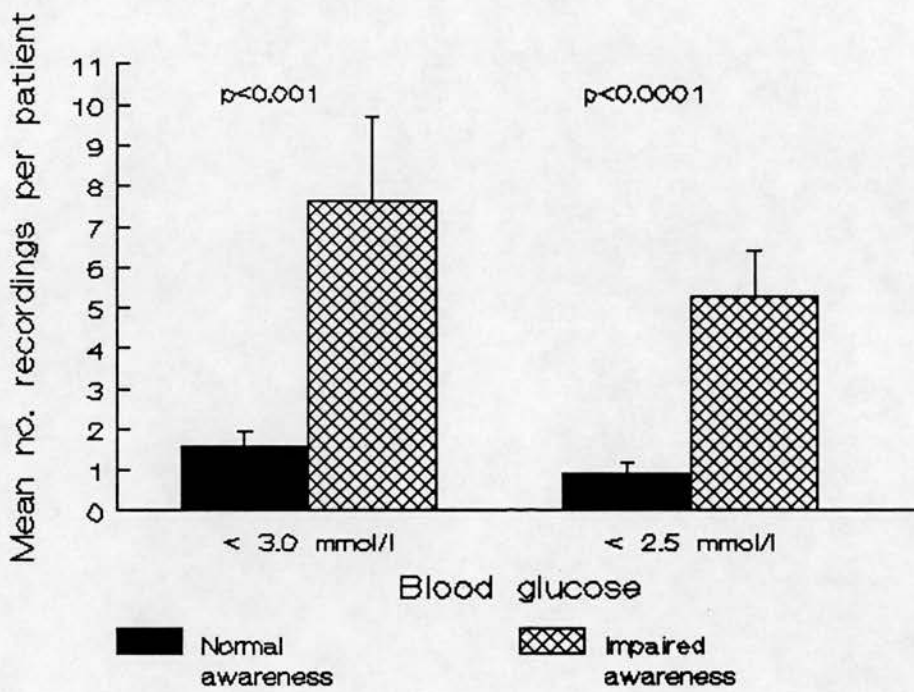
	Group 1 (Normal awareness)	Group 2 (Impaired awareness)	
n	31	29	
<b>Severe</b>			
Total no. episodes severe hypoglycaemia	15	82	p < 0.001
Number (percentage) of patients experiencing > 1 severe episode	8 (25.8)	19 (65.5)	p < 0.01
No. episodes/patient/year year	0.48	2.83	p < 0.0001
<b>Mild</b>			
Total no. episodes mild hypoglycaemia	73	137	NS
Number (percentage) of patients experiencing > 1 mild hypo	25 (80.1)	18 (62)	NS
No. episodes/patient/year	2.8	4.7	NS



**FIGURE 4.1.2**  
**TOTAL NUMBERS OF EPISODES OF SEVERE HYPOGLYCAEMIA**  
**OCCURRING IN ONE YEAR IN PATIENTS WITH AND WITHOUT**  
**IMPAIRED AWARENESS OF HYPOGLYCAEMIA.**  
**(Hatched areas indicate those episodes occurring during sleep)**



**FIGURE 4.1.3**  
**TIME OF DAY OF EPISODES OF SEVERE HYPOGLYCAEMIA**



**FIGURE 4.1.4**  
**MEAN NUMBER OF EPISODES PER PATIENT OF**  
**ASYMPTOMATIC HYPOGLYCAEMIA.**  
 (Left hand panel - hypoglycaemia defined as <3.0mmol/l; right hand panel  
 - hypoglycaemia defined as <2.5mmol/l)



## DISCUSSION

Accurate assessment of the frequency of severe hypoglycaemia in insulin-treated diabetic patients is confounded by potential incomplete self-reporting of episodes, possibly in some instances because of transient amnesia caused by neuroglycopenia. In addition, other events such as transient ischaemic attacks or syncopal episodes may be misattributed to hypoglycaemia, and diabetic patients with poor glycaemic control often experience symptoms of hypoglycaemia within a hyperglycaemic range and so over-estimate the true frequency of hypoglycaemia (Maddock and Krall 1953, Boyle et al 1988, Clarke et al 1991, Polonsky et al 1992).

Prospective assessment with recordings of events by patients, supported by objective witnessed accounts where possible is the most accurate method of collecting establishing the frequency with accuracy. Despite this approach, it is possible that the frequency may still be an underestimate particularly in the patients with impaired awareness of hypoglycaemia. Furthermore in the present prospective survey treatment regimens were reviewed to try to prevent further episodes of severe hypoglycaemia. Two of the patients with impaired awareness who had recorded episodes of severe hypoglycaemia as well as multiple episodes of asymptomatic biochemical hypoglycaemia were commenced on multiple injection regimens which was associated with a reduction in the frequency of both asymptomatic and severe hypoglycaemia over the subsequent six months. This therapeutic change was considered to be essential but will have modified the estimated true frequency of severe hypoglycaemia, introducing an underestimate in the group with impaired awareness of hypoglycaemia. It is also possible that episodes of hypoglycaemia occurring during sleep, which were principally nocturnal, were not always detected in some of the patients in either group who were

not awakened by hypoglycaemia. However despite these possible inaccuracies in reporting, the frequency of severe hypoglycaemia was observed to be almost six fold higher in Type 1 diabetic patients with impaired awareness of hypoglycaemia. Although the frequency of mild hypoglycaemia did not differ between the groups, such estimates are recognised to be inaccurate. Pramming et al (1990) have shown discrepancies between prospective and retrospective estimates, with poor recall by patients at intervals of more than one week. In the present study, patients with impaired awareness frequently recorded low random blood glucose concentrations which were unaccompanied by symptoms, and this was significantly more common than in the group with normal awareness.

The proportion of all patients who had experienced hypoglycaemia was shown to be greater in the present study, which may reflect a tendency to underestimate the frequency of hypoglycaemia in retrospective studies (Goldgewicht et al 1983). In the present study the overall incidence of severe hypoglycaemia in all of the patients was estimated at 1.6 episodes patient<sup>-1</sup> year<sup>-1</sup> which is very similar to frequencies estimated previously, both retrospectively (MacLeod et al 1993) and prospectively (Pramming et al 1991). The use of a similar definition of severe hypoglycaemia is essential to compare studies; in several previous studies (Casparie et al 1985, Nilsson et al 1991, DCCT 1991) the incidence of hypoglycaemia was reported to be lower than the present study because only episodes requiring resuscitation with parenteral glucagon or intravenous glucose or admission to hospital had been documented. If such a definition had been used in the present study the incidence of severe hypoglycaemia in the patients with normal awareness would have been much lower at 0.06 episodes patient<sup>-1</sup> year<sup>-1</sup> and in the patients with impaired awareness at 0.23 episodes patient<sup>-1</sup> year<sup>-1</sup>, although the relative difference in frequency between the two groups

would be preserved. Using this modified and less complete definition the incidence of severe hypoglycaemia for the patients with normal awareness in the present study would then be consistent with rates reported retrospectively in other earlier studies (Nilsson et al 1988, Mulhauser et al 1985). In the Diabetes Control and Complications Trial (DCCT) in which data was collected prospectively the incidence of severe hypoglycaemia in patients receiving intensive insulin therapy was noted to be 0.62 episodes patient<sup>-1</sup> year<sup>-1</sup> (DCCT Research Group 1993). However there were important differences in the study population which was much younger, had a shorter duration of diabetes and most importantly patients with a history of recurrent severe hypoglycaemia or hypoglycaemia with no warning symptoms were excluded (DCCT Research Group 1990). In addition all the patients participating in the intensive therapy group were seen at monthly intervals in order to achieve strict glycaemic control. These differences would explain the higher overall frequency observed in the present study in an unselected diabetic population.

In assessing the frequency of severe hypoglycaemia many studies have not differentiated between the patient groups on the basis of awareness of hypoglycaemia. In the present study the percentage of patients with normal awareness who had experienced severe hypoglycemia (25.8%) was similar to previous reports (DCCT Research Group 1991, MacLeod et al 1993), but the proportion of patients with impaired awareness who had experienced severe hypoglycaemia was significantly higher with two thirds (65.5%) reporting severe hypoglycaemia. This proportion is comparable to that obtained by Hepburn et al (1990) in our department in Edinburgh, in a retrospective assessment of a group of 69 patients with impaired awareness of hypoglycaemia from a different population of 302 insulin-treated diabetic

patients.

Although some studies have not shown an increased incidence in severe hypoglycaemia in intensively treated patients (Mulhauser et al 1985, Nilsson et al 1988) others have reported an increased incidence associated with strict glycaemic control (Barbosa and Johnson 1983, Casparie and Elving 1985, DCCT Research Group 1993). Both of the diabetic groups in the present study exhibited a similar quality of glycaemic control throughout the study, and the groups were also matched for age, duration of diabetes and age at onset of diabetes. Differences in the frequency of severe hypoglycaemia can not be attributed to disparity in the quality of glycaemic control.

In the present study severe hypoglycaemia occurred at all times of day in the patients with impaired awareness but more often in the evening after the evening meal when the patients were at home. The estimate of the proportion of severe episodes of hypoglycaemia occurring between midnight and 8 am was lower in the patients with impaired awareness (31%) than those patients with normal awareness (60%); 53% of all episodes of severe hypoglycaemia occurred during sleep in the patients with normal awareness compared with 31% of all episodes in the patients with impaired awareness of hypoglycaemia. In the DCCT, 43% of all episodes of severe hypoglycaemia occurred between midnight and 8am and 55% of all episodes occurred during sleep, although the comparative frequencies in patients with normal awareness and impaired awareness of hypoglycaemia were not reported. The present study confirms this increased risk of severe hypoglycaemia occurring during sleep and reflects that even in patients with normal awareness of hypoglycaemia during waking hours, perception of hypoglycaemia during sleep may be impaired thereby predisposing to more profound levels of hypoglycaemia and concomitant neuroglycopenia so



resulting in need for external help for resuscitation of the patient.

Previous retrospective studies have suggested that the majority of episodes of severe hypoglycaemia can be attributed to excessive insulin dosage or to patient error, with about a quarter being unexplained (Potter et al 1982, Goldgewicht et al 1983, Casparie and Elving 1985, MacLeod et al 1993). However in the present study only about half of the episodes were explicable and this difference may be a consequence of the different methods of collection of data.

In the present study those patients with impaired awareness of hypoglycaemia experienced a four-fold increased risk of asymptomatic biochemical hypoglycaemia. It has been shown that frequent episodes of hypoglycaemia may impair counterregulation and symptomatic responses to acute hypoglycaemia (Cryer 1992, Cryer 1993, Dagogo-Jack et al 1993). Asymptomatic nocturnal hypoglycaemia has also been shown to influence awareness of hypoglycaemia and reduce the counterregulatory responses to subsequent episodes of hypoglycaemia (Veneman et al 1993). Therefore those patients experiencing more asymptomatic episodes of biochemical hypoglycaemia are at further risk of reduction of symptomatic and counterregulatory responses to hypoglycaemia which may predispose them to more episodes of severe hypoglycaemia. Thus an "acute-on-chronic" exacerbation of pre-existing impaired awareness may occur.

Patients with impaired awareness of hypoglycaemia have been shown previously to be more worried about hypoglycaemia than patients with normal awareness, although they do not appear to modify their behaviour accordingly (Hepburn et al 1992) and severe hypoglycaemia has been shown

to occur more frequently in patients who experienced difficulty in controlling their diabetes (Goldgewicht 1983). Similarly patients exposed to recurrent severe hypoglycaemia have an increased level of anxiety and feel more unhappy (Wredling et al 1992). The results of the present study are consistent with these previous findings.

In the present survey, severe hypoglycaemia was associated with moderate injuries requiring admission to hospital on only two occasions in a single patient who had impaired awareness of hypoglycaemia. However no history of hypoglycaemia-related driving accidents was elicited in any of the patients, and the patients with impaired awareness actually reported significantly fewer driving related accidents. This was probably a consequence of altered driving behaviour in such patients who take increased care when driving, and many drivers who have experienced recurrent severe hypoglycemia had ceased driving voluntarily so reducing their risk of motor vehicle accidents. This phenomenon has been reported previously (Eadington et al 1989, Stevens et al 1989).

The present study confirms that diabetic patients who have the chronic form of impaired awareness of hypoglycaemia have a six fold increase in frequency of severe hypoglycaemia. This problem requires regular reinforcement of education of the causes and risks of hypoglycaemia, and continuous reappraisal of the targets of glycaemic control, in order to reduce the frequency of this potentially dangerous complication of insulin therapy.



In summary:-

Patients with impaired awareness of hypoglycaemia :

- have a six-fold increased risk of severe hypoglycaemia
- experience severe hypoglycaemia at all times of day in comparison to those subjects with normal awareness who experience predominantly nocturnal episodes
- frequently experience asymptomatic biochemical hypoglycaemia
- fear hypoglycaemia but do not modify their behaviour accordingly
- do not appear to be at increased risk of road traffic accidents.

PART IV

CHAPTER 2

*Study 7*

**PERSONALITY AND BEHAVIOURAL CHANGES IN PATIENTS WITH  
LONG DURATION TYPE 1 DIABETES AND RECURRENT EXPOSURE  
TO SEVERE HYPOGLYCAEMIA**

## INTRODUCTION

A higher standard in the quality of glycaemic control has resulted from improved treatment policies and has contributed to increased longevity and reduced frequency and morbidity of complications of patients with insulin-treated diabetes. As a result most patients with Type 1 diabetes now survive for more than 30 years on insulin therapy. Diabetes mellitus is known to affect cognitive function and in patients with Type 1 diabetes mellitus, cognitive dysfunction is probably multifactorial in origin (Ryan 1991). However accumulating evidence suggests that recurrent severe hypoglycaemia has a progressive, deleterious effect on psychological and cerebral functions (Lawrence et al 1942, Murphy and Purtell 1943, Wilder 1943, Jones 1947, Fineberg and Altschul 1951, Bale 1973, Wredling et al 1990, Langan et al 1991, Deary et al 1993a). Changes in mood, personality and social function have been described in diabetic patients with a history of recurrent severe hypoglycaemia (Wredling et al 1992) but the "natural history" of these changes has not been described. Quantitative measurement in this field of investigation is difficult and this clinical study was to provide descriptive data, with some preliminary, semi-quantitative analysis, of changes in behaviour, personality and intelligence which have been observed in a group of five patients with Type 1 diabetes of long duration. All of the patients had experienced multiple episodes of severe hypoglycaemia before the development of pronounced changes in personality and cognitive function, and it is surmised that this recurrent exposure to severe neuroglycopenia may have contributed to the abnormalities observed.

## PATIENTS AND METHODS

A multi-faceted approach was taken, incorporating both carer-based and hospital-based accounts in conjunction with information from standard questionnaires, in order to obtain a balanced view of this complex problem. Investigation was carried out by utilising four methods of approach :

- 1) carers' descriptive accounts of personality and behaviour changes in the patients;
- 2) clinical case histories from hospital records and carers;
- 3) carer-based, semi-quantitative measurements of changes in behaviour and personality.
- 4) measurement of premorbid and present IQ.

Five patients with Type 1 (insulin-dependent) diabetes, all of whom attended the diabetic clinic at the Royal United Hospital, Bath, were studied and their spouses were questioned. All of the spouses had independently identified changes in cognitive function and personality of varying severity in their partners. These carers have formed a self-help group to address some of the problems they are experiencing with their partners. The establishment of this formal group drew attention to the existence of this small cohort of patients who had similar disabilities and in whom severe hypoglycaemia appeared to be a primary causal factor. All of the patients had exhibited strong motivation to achieve good glycaemic control and had attended diabetic out-patient clinics on a regular basis.

Some of the patients were so disabled intellectually that reliable information concerning their past history and personality had to be obtained from their spouses and from hospital case records. The presence and severity of diabetic complications had been ascertained by Dr JP O'Hare and Dr JP Reckless their local medical specialists and further information was extracted from hospital

records. A retrospective assessment was made of the number of episodes of severe hypoglycaemia and of ketoacidosis. An episode of severe hypoglycaemia was defined as one which could not be self-treated and required help from another person, irrespective of whether coma had occurred.

The carers were asked to complete a series of questionnaires about the individual patients. The questionnaires, have been well validated previously and are widely used in clinical psychology. They are intended to be answered by the patients themselves, but in the present study, because the patients had significant disabilities, the carers were asked to complete the questions by proxy, responding as if they were the patient. This practice of obtaining information from 'significant others' is widely recognised to be of value in psychiatric interviews. The following questionnaires: "cognitive function", "Nottingham Heath Profile" and "Eysenck Personality Questionnaire" were completed twice under the supervision of a single investigator (AEG) :

- a) to assess the patient's premorbid state (*before* exposure to multiple episodes of hypoglycaemia); ie to assess "how the patients used to be".
- b) to assess the present situation ie to assess "how the patients are now".

**Cognitive function** : a 35 item questionnaire (Taylor 1990) was used in which questions were directed at changes in memory, concentration and comprehension with particular reference to everyday activities:

- eg
- How often do you forget where things are normally kept or look for them in the wrong place?
  - How often do you have difficulty doing calculations ?
  - How often do you have difficulty planning things out in advance?

Possible answers to these questions were "very often", "quite often",

"occasionally", "very rarely" or "never", which scored from 4 to 0 respectively, with a higher score indicating a greater number of problems.

**Nottingham Health Profile Part 2** (Hunt et al 1980) : this questionnaire identifies problems in areas of employment, domestic tasks, home life, social life, sex life and hobbies. Statements such as "I am having difficulty concentrating at work" or "I am going out less often these days" are answered by either "yes" or "no", and a greater number of affirmative responses correlates with increased difficulties in that particular area. This questionnaire has been validated as a good indicator of "social interaction".

**Eysenck Personality Questionnaire** (Eysenck and Eysenck 1986) : this assessment is used extensively to investigate personality with particular reference to three dimensions:

- a) *neuroticism* - a tendency to negative mood states;
- b) *extraversion* - a tendency to being sociable, lively or outgoing;
- c) *psychoticism* - a tendency to being "tough-minded", insensitive, with a liking for "unusual" things and a disregard for danger.

In addition each carer was asked to complete a General Health Questionnaire (GHQ) both for their spouse and also for themselves, recording how they felt at the time of questioning. The GHQ identifies psychiatric morbidity, in particular any tendency to anxiety and minor depressive states. The cut off score for the presence of psychiatric morbidity was taken as 4/5 (Goldberg and Williams 1988). The carers were also asked to identify specific problems that had been encountered with their spouses.

### **Measurement of IQ**

Those patients who were able to comply with formal IQ testing were assessed using



the *National Adult Reading Test* (NART)(Nelson 1982) and the *Wechsler Adult Intelligence Scale - Revised* (WAIS-R)(Wechsler 1981). The following subtests of the WAIS-R were used for assessing verbal IQ : "information", "digit span", "vocabulary", "comprehension" and "similarities", and for performance IQ: "picture completion", "block design", "object assembly" and "digit symbol". NART is an assessment of "best" global cognitive function ever attained, irrespective of time. When this pre-morbid assessment of IQ is compared with present IQ measured by WAIS-R, it can identify any significant deterioration occurring over a period of years. The NART IQ estimates were adjusted by seven points in the present study as the NART tends to overestimate the WAIS-R IQ (Langan et al 1991). The patients were monitored by carers to ensure that they were not hypoglycaemic at the time of testing.

### **Statistics**

Changes between pre-morbid and present cognitive abilities, affirmative responses in the Nottingham Health Profile, and personality traits were assessed using the Wilcoxon signed rank test.

## RESULTS

### Patient Details

Individual case histories are described below and the characteristics of the patients are shown in tables 4.2.1 and 4.2.2.

### Case Reports

#### *Case 1*

This 66 year old man developed Type 1 diabetes in 1945 when aged 20 years. Shortly after commencing insulin therapy, he experienced nocturnal convulsions which at that time was diagnosed as epilepsy. In 1968, nocturnal convulsions associated with hypoglycaemia occurred while in hospital. Frequent hypoglycaemic episodes occurred during his initial years of insulin treatment and at least two hypoglycaemic episodes resulted in coma and a fractured skull and on another occasion a fractured femur. Hypoglycaemia unawareness developed after 11 years of diabetes at which time he also experienced frequent episodes of hypoglycaemia. Several random blood glucose values were low during diabetic clinic attendances and the patient had increased his insulin dose on several occasions without seeking medical advice. He has been a heavy smoker and has chronic respiratory disease.

Over 20 years ago "cerebral retardation" was first observed by his wife and medical attendants and this has progressed to the extent that he can no longer be left alone for long or overnight. His wife remarked "he is not the man I married". The patient and his wife had enjoyed a close relationship, sharing ideas, problems and emotions, but this had deteriorated because of an increasing lack of communication. The patient was an engineer but had to retire (aged 51 years) because of lack of concentration and poor memory. After his wife developed

breast cancer and co-existing non-insulin dependent diabetes, he has been described as "manipulative", particularly before being admitted to hospital for respite care. Although he eventually acknowledged that his wife has developed diabetes he still denies that she has malignancy. Motivation to pursue any previous interests has been lost and he is unable to handle any financial affairs. On occasions he has become "lost" in the local neighbourhood. His insight this has become progressively limited.

### *Case 2*

This 51 year old man developed Type 1 diabetes when aged 17 years. He coped well initially with self-management of his diabetes. Random blood glucose values were occasionally in the range of 2.0-3.0 mmol/l and the advent of the measurement of glycated haemoglobin revealed levels within the non-diabetic range indicating strict glycaemic control. Loss of hypoglycaemia awareness developed after 30 years of diabetes. He has experienced more than 100 episodes of severe hypoglycaemia, five of which have required hospital admission because of coma between 1967 and 1990. During severe hypoglycaemia he becomes violent and aggressive making it impossible for his wife to treat him effectively.

His wife first noticed psychological changes when the patient was in his late 40's. He developed fluctuating moods and was very anxious about his work performance. He doubts his ability to cope with his job as an electrician and is contemplating early retirement. Although the patient's ability to participate in social and household activities has declined, communication with his wife remains satisfactory and he has retained considerable insight into his condition.

### *Case 3*

This 62 year old man developed Type 1 diabetes at the age of 30 years. He had previously been a pilot and navigator in the RAF but diabetes necessitated a change of employment to work as an estate agent and subsequently a council senior valuer. Random blood glucose values have been variable with several high and low readings. Nocturnal hypoglycaemia became frequent (particularly between 1970 and 1980) and worsened when he was threatened with dismissal from work because of poor performance. During 20 years of treatment, he has suffered more than 20 episodes of hypoglycaemic coma, although most have been treated by his wife. On several occasions transient hemiparesis occurred during severe hypoglycaemia, but cerebral angiography was normal. He developed hypoglycaemia unawareness when aged 60 years.

After 20 years of diabetes the patient's wife became concerned about an obvious deterioration in her husband's cognitive abilities, which was associated with lack of confidence, diminished motivation and reduced conversational skills. The time taken to complete household tasks has increased considerably. Interests in the arts have been retained but his ability to concentrate has decreased and he often sleeps through a concert. The patient appreciates that he has cognitive problems, but is reluctant to discuss them with his wife.

### *Case 4*

This 50 year old man developed Type 1 diabetes when aged 10 years and his mother imposed very strict control of his diabetes during childhood and adolescence. During this time he had one episode of hypoglycaemic coma requiring admission to hospital and missed schooling because of his diabetes. In adult life he had three admissions to hospital in hypoglycaemic coma but has, in addition, suffered about 10 episodes of severe hypoglycaemia per year requiring

the intervention of his wife and emergency medical services. Since the advent of glycated haemoglobin, most of his values have been in the non-diabetic range. Within the last decade he developed impaired awareness of hypoglycaemia with a predominance of neuroglycopenic symptoms.

He trained as a printing apprentice then became a lecturer at a technical college. One year before the present assessment his wife noticed impairment of his memory increasing irritability. The patient has experienced difficulty in coping with his job but formal memory assessment revealed only minor abnormalities. He resigned as church treasurer because he no longer felt able to cope with the work or responsibility. Personal communication with his wife has remained excellent, and he is able to discuss problems such as the prospect of taking early retirement.

#### *Case 5*

This 55 year old man developed Type 1 diabetes at the age of 32 years. On two occasions (1982 and 1984) he required hospital admission for stabilisation of his diabetes because of fluctuating blood glucose values. Several glycated haemoglobin values have indicated very strict control. During the last 10 years he has experienced about six episodes of severe hypoglycaemia. The development of severe glaucoma with deterioration of vision necessitated his early retirement at the age of 48 years. He developed impaired symptomatic awareness of hypoglycaemia about four years ago.

About 10 years ago the patient's wife noticed changes in his behaviour and intellectual abilities. Initially he appeared to have a poorer short term memory and seemed devious, concealing dates of diabetic clinic appointments and then pretending that he had attended. In recent years he has become garrulous, particularly in company, although the content of his conversation often lacks logic

and rational discussion has become difficult. He can no longer be left to supervise his grandchildren as he actively encourages them to misbehave. His wife is now reluctant to leave him alone more than two hours. His personal hygiene and interest in his appearance have deteriorated while previously he attended to these aspects with great care. His visual impairment and diabetic cheiroarthropathy have compounded all of the above problems. A psychiatric assessment did not identify any abnormality. He has good insight but rarely admits to his problems except during outbursts of anger.

### **Carers' Assessment**

All of the patients' carers had noticed behavioural changes in their partners before the patients themselves had been aware of any difficulties. Two of the patients (cases 2 and 4) have only recently been affected so that communication between husband and wife is still "open" and satisfactory. However the other three carers all commented that conversation and rational discussion with their spouses had become very limited; all of the patients appeared to have "good days" and "bad days". There are occasions when the patients may appear and behave "normally" in company (for example during diabetic out-patient clinic attendance) and this had previously interfered with attempts to convince professional attendants of their problems. Two of the carers had given up their own jobs to spend more time looking after their partners. Another carer who has a stressful job, felt that the demands being made at home had compromised her work and have necessitated her resignation. All of the couples have had to reduce their social activities either because the patients tire rapidly or because the patients display a complete lack of interest. The carers have tried not to expose their partners to situations which are stressful or potentially embarrassing to both. With the exception of cases 2 and 4 all carers had found that holidays away from home were stressful and were seldom appreciated by the patients. All of the carers had attempted to continue pursuing their personal interests and maintain attendance at social events. Carer 1 found



respite care to be very helpful and carer 3 employs a person to look after her husband, so that she can have weekends away from home.

The carers' group was well informed about financial benefits such as attendance allowance. They expressed that their principal desire was to have medical recognition of the extent and nature of their problems and to help others with similar problems. The psychological support obtained from other members of the group was considered to be of great value. With the exception of carer 2, the carers did not find that management of the patients' diabetes, including the treatment of hypoglycaemia, presented any significant difficulties. The major concerns of the carers were how the patients would be looked after if the carer was ill or if the patient could no longer be left alone at any time.

When asked about changes in their own mood, four out of the five carers said they had become more irritable, depressed and anxious. Their responses to the General Health Questionnaire are shown in table 4.2.3.

### **Results of Personality and Cognitive Assessments**

1. **Cognitive function questionnaire** - (table 4.2.4). Carers' estimates of premorbid and present cognitive function indicated that the patients had significant changes in cognitive abilities ( $p=0.04$ ), suggesting that increased difficulties with concentration and memory in daily activities had occurred.

2. **Nottingham Health Profile** - (table 4.2.5). In the premorbid assessment only one carer identified any problems with their spouse in the spheres of employment, household activities, home-life, social life and hobbies. However, in the assessment of current problems at the time of questioning, all carers could identify

some difficulties with their spouses in the areas of employment, household activities, home life, hobbies and sex life and four of the five carers identified problems with the patients' social life.

3. **Eysenck Personality Questionnaire** - Answers from four out of five carers revealed that their spouse showed an increase in neuroticism and all five carers observed a decrease in extraversion. Table 4.2.6 and figure 4.2.1 indicate that comparison of pre-morbid and present scores showed a tendency towards an increase in neuroticism and a decrease in extraversion although psychoticism was not greatly changed. The "lie" score which attempts to measure a tendency on the part of some subjects to "fake good", was unchanged which suggests that carers did not have an unrealistic "ideal" view of their spouses premorbid personality. Overall, this suggests that the patients appeared to have a tendency to experience more "negative" mood states and had become less sociable following multiple episodes of severe hypoglycaemia.

4. **General Health Questionnaire** - (table 4.2.3) Carers' assessments suggested that psychiatric morbidity, in particular a tendency to anxiety, was present in all the patients and was evident also in three of the five carers.

5. **Intelligence** - (table 4.2.7) Only three of the five patients were able to cooperate fully in the testing of both NART and WAIS-R; two of these patients exhibited a marked decline both in verbal and performance IQ (ie difference between the NART and WAIS-R scores. Patient 5 refused to do the NART and patient 1 refused to do one section of both the verbal and performance sections in the WAIS-R.

TABLE 4.2.1

## CLINICAL CHARACTERISTICS OF PATIENTS WITH TYPE 1 DIABETES AND COGNITIVE DYSFUNCTION.

PATIENT	AGE/(yrs)	DIABETES(yrs) Age of onset	DIABETES(yrs) Duration	SMOKING HABIT	Retinopathy	DIABETIC COMPLICATIONS Neuropathy Nephropathy	PVD	OTHER MEDICAL DISORDERS
1	66	20	47	YES	Background	Dysaesthesia Autonomic	Present	Epilepsy, COAD
2	51	17	34	NO	Background	Dysaesthesia	-	Lipodystrophy (related to injection sites)
3	62	30	32	NO	Background	Dysaesthesia Autonomic	Present	
4	50	10	40	NO	Proliferative	-	-	Memory assessment - not demented
5	56	32	24	EX	-	Sensorimotor	-	Severe glaucoma

Abbreviations :

PVD = peripheral vascular disease

COAD = chronic obstructive airways disease

TABLE 4.2.2

FREQUENCY AND MORBIDITY ASSOCIATED WITH SEVERE HYPOGLYCAEMIA IN PATIENTS WITH TYPE 1 DIABETES

PATIENT	NUMBER OF SEVERE EPISODES OF HYPOGLYCAEMIA	HYPOGLYCAEMIA AWARENESS SCORE*	DURATION OF CEREBRAL DYSFUNCTION/(yrs)	COMMENTS
1	> 30	1	15	Skull fracture caused by hypoglycaemia-induced convulsions
2	> 100	2.5	2	
3	> 20	2.5	17	Hemiparesis during hypoglycaemia - cerebral angiography normal
4	10/year	5	1	
5	6	3	12	

\* Awareness scored on visual analogue scale of 1 to 7

1 = never aware of hypoglycaemia

7 = always aware of hypoglycaemia

TABLE 4.2.3

**RESULTS OF CARERS' ASSESSMENTS OF PRESENT PSYCHIATRIC MORBIDITY IN THEMSELVES AND THEIR SPOUSES USING THE GENERAL HEALTH QUESTIONNAIRE**

PATIENT NO.	PATIENT SCORE	CARER SCORE
1	*16	*13
2	* 8	*14
3	*11	3
4	* 9	*11
5	*17	1

\* - suggests psychiatric morbidity

TABLE 4.2.4

**RESULTS OF CARER'S ASSESSMENTS OF CHANGES IN PATIENTS' COGNITIVE FUNCTION (higher scores equate with impairment of function)**

	PRE-MORBID SCORE	PRESENT SCORE	
Patient no:			
1	55	123	
2	24	75	
3	40	85	
4	31	60	
5	21	128	
Median	31	85	p = 0.04

TABLE 4.2.5

**RESULTS OF CARER'S ASSESSMENTS OF PATIENTS' SOCIAL  
FUNCTIONING USING THE NOTTINGHAM HEALTH PROFILE (scored  
as number of affirmative replies to problems in each area)**

	PREMORBID SCORE median	PRESENT SCORE median	p value
Areas affected:			
Work	0	3	0.07
Household activities	0	3	0.04
Home life	0	3	0.07
Social life	0	3	0.1
Hobbies	0	2	0.04
Sex life	0	1	0.07

TABLE 4.2.6

**RESULTS OF CARERS' ASSESSMENTS OF PATIENTS PRESENT AND  
PREMORBID PERSONALITIES USING  
THE EYSENCK PERSONALITY QUESTIONNAIRE**

	PREMORBID SCORE median	PRESENT SCORE median	p value
Neuroticism	8	14	.08
Extraversion	8	2	.07
Psychoticism	4	8	.22
"lie score"	5	4	.14



TABLE 4.2.7

RESULTS OF IQ - NART (ADJUSTED) AND WAIS-R FOR PATIENTS WITH TYPE 1 DIABETES

PATIENT	FULL IQ		VERBAL IQ		PERFORMANCE IQ		COMMENTS
	NART	WAIS-R	NART	WAIS-R	NART	WAIS-R	
1	103	-	101	-	104	-	Omitted 2 subtests of WAIS-R
2	109	90	109	94	107	87	
3	116	133	116	132	112	122	
4	104	88	104	84	104	96	
5	-	82	-	75	-	93	Refused to do NART

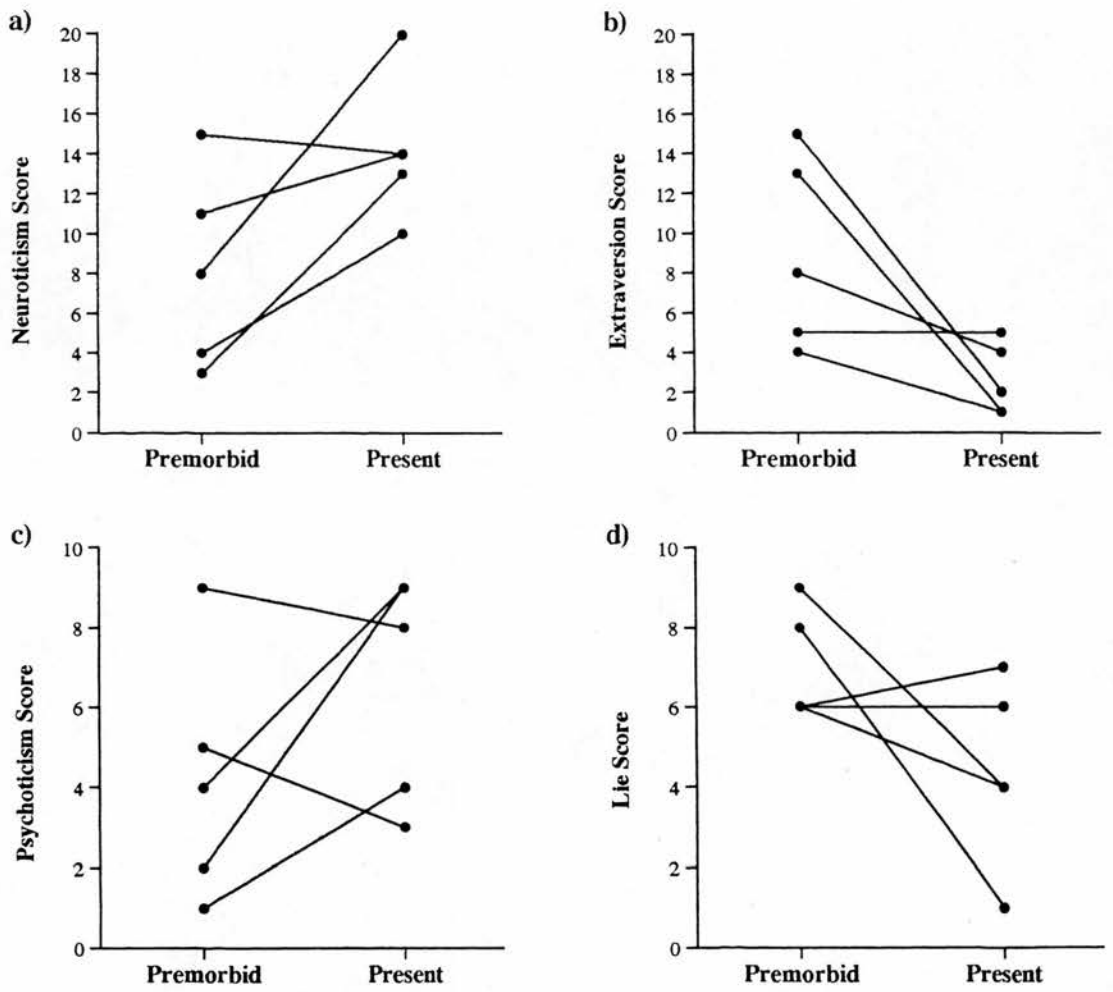


FIGURE 4.2.1  
 DIAGRAM TO SHOW CHANGES IN PERSONALITY IN INDIVIDUAL PATIENTS AS ASSESSED BY CARERS USING EYSENCK PERSONALITY QUESTIONNAIRE : a) Neuroticism b) Extraversion c) Psychoticism d) "lie" score

## DISCUSSION

The pathogenesis of cerebral dysfunction in diabetes may be multifactorial. Premature vascular disease may present as multi-infarct dementia and patients with diabetic neuropathy have been shown to exhibit changes in cognitive function (Ryan et al 1992). It is possible that metabolic disturbances such as hyperglycaemia and hypoglycaemia interact with co-existing vascular disease in promoting cognitive and psychological dysfunction in diabetic patients (Ryan et al 1993). This putative multifactorial aetiology, in association with the difficulties of assessing changes in cognitive function and personality in brain damaged patients, presents difficulties for investigation in this area, and few studies have been reported. In the present study of a small self-selected group of diabetic patients it was felt that a "multi-faceted" approach to assessment would be the most practical method to address and define the nature of these problems.

The present report describes five patients with Type 1 diabetes, who had a history of multiple episodes of severe hypoglycaemia and minimal evidence of vascular disease at the time their cognitive problems commenced. Changes in cognitive function were identified and significant difficulties were revealed with particular reference to daily activities and social function in association with psychiatric morbidity and personality changes. The contribution of other medical disorders is difficult to assess. In patient 1 possible chronic epilepsy, and obstructive airways disease may have contributed to the impairment of cognitive function through effects of medication (Thompson and Trimble 1982, Meador et al 1990), cerebral ischaemia and hypoxia, and other chronic diseases, such as glaucoma resulting in blindness (patient 5), may have compounded the changes in personality that were observed. Although some of these patients had developed peripheral neuropathy, the cognitive changes predated the development of overt neuropathy, although this does not preclude early insidious damage or the development of a central neuropathy (Dejgaard et al 1991). Glycaemic control in many of these patients

appeared to have been very strict for several years despite the attempts of their medical attendants to encourage relaxation of glycaemic control, which is recognised to predispose to an increased frequency of severe hypoglycaemia (DCCT 1991, DCCT 1993). In addition the period of strict glycaemic control took place at a time when this was not a universal policy in diabetic management. It is feasible that a contributory factor to the induction of cognitive dysfunction was the exposure to recurrent severe hypoglycaemia in many of these patients.

Hypoglycaemia of varying severity is a common side effect of insulin therapy in diabetic patients. The frequency of mild to moderate hypoglycaemia is difficult to estimate with accuracy unless monitored prospectively, but retrospective estimates of the frequency of episodes of severe hypoglycaemia are more robust (Pramong et al 1991) and have ascertained with reliability (MacLeod et al 1993). It has been estimated that at some time during treatment with insulin 30% of diabetic patients experience a hypoglycaemic coma, while 10-20% of patients experience coma once a year, and 3% of patients suffer severe recurrent hypoglycaemia (Gale 1986). The patients in the present cohort probably fall within this last group. Chronic impairment of awareness of hypoglycaemia is associated with an increased frequency and morbidity from severe hypoglycaemia (Hepburn et al 1990; see Part IV Chapter 1). Most of the patients in the present study had some degree of reduced awareness of hypoglycaemia which probably contributed to their history of frequent severe hypoglycaemia, although the fact that many of the severe episodes of severe hypoglycaemia pre-dated the onset of changes in awareness of hypoglycaemia would also support the hypothesis that impaired awareness of hypoglycaemia *per se* results from recurrent exposure to hypoglycaemia (Clarke et al 1991). The morbidity of some of the episodes of hypoglycaemia was significant with three of the patients giving a history of transient hemiplegia, skull fracture or convulsions, all of which may have contributed to the subsequent

development of cerebral dysfunction.

Personality and cognitive changes occurring as a result of recurrent hypoglycaemia have been described previously in patients with diabetes (Murphy and Purtell 1943, Wilder 1943, Jones 1947, Fineberg and Altschul 1951, Bale 1973, Wredling et al 1990, Langan et al 1991), but data is limited. Changes in IQ following severe hypoglycaemia have been documented in patients with Type 1 diabetes (Langan et al 1991, Deary et al 1993a). Langan and colleagues (1991) found a difference in the IQ decrement of 5.8 points in performance IQ in insulin-treated diabetic patients who had experienced five or more episodes of severe hypoglycaemia compared with no IQ decrement in patients without recurrent hypoglycaemia. The same diabetic patients had lower WAIS-R performance and verbal IQ scores when compared with a matched control group of 100 non-diabetic subjects (Deary et al 1993a) but after controlling for the frequency of severe hypoglycaemia, the performance IQ was similar in the diabetic and the control groups while a significant difference in verbal IQ persisted. This suggests that performance IQ may be reduced by previous exposure to severe hypoglycaemia while the lower verbal IQ in the diabetic patients may be attributable to other factors such as the social impact of the disorder. In the present study, only three of the five patients were able to cooperate fully with cognitive testing which suggests that some form of personality/cognitive impairment *per se* may be present. NART is not an accurate measure of pre-morbid IQ in subjects of high intelligence and therefore the results obtained from patient 3 are unreliable (Nelson 1982). The other patients did however exhibit changes in both verbal and performance IQ suggesting that not only may hypoglycaemia have had an effect on their performance IQ but their lower verbal IQ may reflect some of the social problems which they had encountered.

The patients in this study represented a group which had been selected through

their carers. The carers constitute an educated and articulate group who are able to identify the nature of the problems and voice their anxieties. This introduces an additional selection bias; the patients may not be typical of the diabetic population as a whole, and comparison with an "appropriate" control group is unlikely to be meaningful. Most of these patients had a high pre-morbid intelligence and had held jobs which required training and skills. In such patients the cumulative effects of recurrent cerebral injury, of whatever aetiology, may be more apparent than in a group of patients who have a lower pre-morbid intelligence and more limited cognitive abilities. In addition the effect of "chronic disease" per se in causing cognitive and personality changes is unknown. A further problem with studying patients who have such changes in personality and cognitive function is that data obtained from the patients may not be accurate and the spouses may provide different information. In addition it has to be acknowledged that information from the carers may be biased as they have been exposed to the problem for many years, so encouraging their desire to promote greater awareness and acknowledgement that cognitive dysfunction and personality change may be complications of diabetes and severe hypoglycaemia.

The questionnaires had previously been validated for use on patients and required only minor changes for adaptation to use with the carers. Very few pre-existing questionnaires have addressed a carer's assessment or opinion of any changes evident in the patients with whom they live. Premorbid personality assessment is also extremely difficult and at present no validated measures of this entity exist. A well validated personality questionnaire was therefore used and adapted for each carer's assessment of premorbid personality, as patients are unlikely to identify their own changes in personality with accuracy. However the results of the personality questionnaires do exhibit discriminant validity, because, while the neuroticism scores increased and the extraversion scores decreased, the



psychoticism scores were unchanged and the "lie score" actually decreased.

Loss of employment through dismissal or inability to cope with a job, was a major problem for the patients in the present study. Patients with demanding occupations requiring intellectual skills may be disproportionately affected. Early retirement has invoked personal resentment and loss of self esteem, which may compound the established neuropsychological disability. A Swedish study (Wredling et al 1992) of diabetic patients with a history of recurrent severe hypoglycaemia found that such patients exhibited higher anxiety and lower happiness ratings compared to a control group of diabetic patients who had no history of severe hypoglycaemia. In that study (Wredling et al 1992) the patients had rated their own degree of neuroticism which was not found to differ between the groups, in contrast to the present study.

The frequency of these type of cognitive and behavioural changes in the insulin-treated diabetic population is unknown. It is possible that many patients have more subtle impairments and most physicians with large diabetic clinics may be able to identify patients with similar disabilities. The spectrum of cognitive dysfunction may be wide, and in the majority of patients cognitive and personality changes may be relatively minor or may be detectable only by close relatives. The present group probably represent a more extreme end of the spectrum of disability with severe cognitive dysfunction resulting in adverse effects on the lifestyle of the patients and their carers, with family and social life suffering considerably. Many of the spouses of such patients confess to enduring a "living bereavement", observing their partners to have lost their intellect, charisma and sense of humour and to have developed a child-like dependence akin to the problems associated with premature senility or Alzheimer's disease. This forgotten minority of carers are in need of support, and deserve medical recognition of the chronic problems with which they have to cope. In the UK,

there are at present no guidelines for carers to assist with the management of patients with insulin-treated diabetes who have any form of mental handicap. The activities of the group of carers in Bath highlighted the necessity for the production of such information and this has been recognised by the British Diabetic Association. It is important that in every diabetic patient the quality of glycaemic control is optimised to avoid severe hypoglycaemia, a goal which is much more difficult to achieve in patients who have sustained personality changes and cognitive deficits. Prospective studies both in newly diagnosed diabetic patients and patients with long-standing diabetes, to examine changes in personality and cognitive dysfunction, are required to define the nature of such problems with greater precision, particularly in those at increased risk of severe hypoglycaemia. This was unfortunately not addressed by the Diabetes Control and Complications Trial (DCCT 1993) in which diabetic patients with hypoglycaemia unawareness were excluded.

PART V

**CONCLUSIONS AND FUTURE RESEARCH**

The conclusion of the Diabetes Control and Complications Trial in the USA (DCCT 1993) has provided unequivocal evidence that strict glycaemic control in patients with Type 1 diabetes, retards the progression of diabetic microangiopathy. These findings have major implications for the management of patients with Type 1 diabetes, both in the day to day clinical management of such patients and in the organisation and delivery of diabetic care. However, in order to achieve the levels of glycaemic control in the DCCT, monthly outpatient review visits were required and frequent medical contact with the patients was maintained, which clearly would be a major imposition on limited specialist resources if strict glycaemic control was to be attempted in *all* patients with Type 1 diabetes. Even with close medical supervision the incidence of severe hypoglycaemia was increased threefold in those patients on receiving intensive insulin therapy. Therefore, the institution of intensive insulin therapy without frequent reassessment and close supervision of the patient may lead to an unacceptably high incidence of severe hypoglycaemia. The DCCT *neither* included patients with impaired awareness of hypoglycaemia *nor* patients with a previous history of recurrent severe hypoglycaemia (DCCT 1990). In such patients, who are already at an increased risk of severe hypoglycaemia (Hepburn et al 1990), if intensive insulin therapy predisposes to further hypoglycaemia, the resultant morbidity from such episodes may well outweigh the potential benefits obtained from intensive insulin therapy.

If the recommendations from the DCCT (DCCT 1993) are to be implemented on a wide scale it is mandatory that we continue to research and establish the risks and complications of hypoglycaemia in patients with Type 1 diabetes, particularly those at increased risk of hypoglycaemia. The studies outlined in the present thesis have attempted to address some of these problems.

## **Acute Hypoglycaemia and Cognitive Function**

The studies described in this thesis provide further information about the nature and degree of cognitive dysfunction experienced during acute hypoglycaemia. Two of these studies were performed in non-diabetic subjects, and although the results cannot be directly applied to patients with insulin-treated diabetes, it was essential that these studies were performed before attempting to address the problems in diabetic patients. From the results of these studies it appears that short term cerebral adaptation to hypoglycaemia does not occur in non-diabetic humans and that IQ only plays a minor role in determining the degree of cognitive dysfunction experienced during acute hypoglycaemia in non-diabetic individuals. Both of these studies should be repeated in larger cohorts of subjects and in patients with insulin-treated diabetes. In addition, more specific areas of cerebral functioning require further investigation eg. the effect of hypoglycaemia on visual function.

Patients with insulin-treated diabetes who have impaired awareness of hypoglycaemia appear to be at a greater risk of cognitive dysfunction during acute hypoglycaemia. This is important in the management of insulin-treated diabetes; it emphasises the need for individualisation of treatment regimens in order to minimise the risks and morbidity associated with hypoglycaemia. Insulin regimens need to be tailored to the patients' physical activities and working conditions, and also the potential risk to employment which may result in patients with excessively strict glycaemic control and chronic impaired awareness of hypoglycaemia will need to be assessed. There is a need for the continued education of all health professionals involved in the care of diabetic patients in order that the necessary information can be relayed to the patients.

Mood changes during acute hypoglycaemia have not previously been studied. The

hyperinsulinaemic hypoglycaemic glucose clamp not only provides an important research tool to investigate mood states, but also demonstrates the deleterious effects of hypoglycaemia on patients' feeling of wellbeing, which may influence their desire and willingness to achieve strict glycaemic control.

Changes in cerebral blood flow during hypoglycaemia may be an adaptive mechanism to preserve cerebral function. However the interactions between cerebral blood flow, cerebral glucose transporters and glucose metabolism remain unclear and further analysis of functional brain glucose uptake and metabolism is required, possibly using alternative techniques such as positron emission tomography (PET).

### **Recurrent Hypoglycaemia and Cognitive Function**

Patients with insulin-treated diabetes who suffer from any form of cognitive impairment pose a problem to their health professionals as well as to their carers. Adjustment of insulin doses and monitoring blood glucose require patience and skill in patients with normal cognitive ability, and will be therefore be that much more difficult in patients with impaired ability. The study of the carers from Bath illustrates many of these problems and demonstrates the need for greater understanding and support for such carers. In addition there is accumulating evidence to suggest that recurrent severe hypoglycaemia is deleterious to cognitive function. Although the DCCT (DCCT Research Group 1993) and SDIS (Reichard et al 1991) showed no effect on cognitive function, there are as yet no large studies which have attempted to follow patients prospectively for *more than 10 years*, particularly those at greatest risk of hypoglycaemia (eg those with impaired awareness), and to assess any changes in cognitive function.



The prevention of hypoglycaemia coupled with near normal glycaemic control is the ultimate goal in the management of patients with diabetes. Further refinements in the administration of insulin may help achieve this, although until such treatment is readily and easily available the risk to benefit ratio of complications resulting from poor glycaemic control and hypoglycaemia must be assessed carefully in each diabetic patient treated with insulin.

## APPENDIX

## SUBJECT EXPLANATION SHEET FOR *STUDY 1*, *STUDY 2* AND *STUDY 5*

### INFORMATION FOR SUBJECTS

#### Study - To examine whether the brain can adapt to a low blood sugar level

This study is designed to examine adaptive changes in brain function made in response to a low blood sugar.

During the study, we will lower your blood sugar by giving you an infusion of short acting (soluble) insulin and on an infusion of sugar solution (dextrose) through a cannula (drip) in to one of the veins in your forearm. Insulin is a naturally occurring chemical (or hormone) which is produced in the pancreas, which lowers blood sugar and is used to treat diabetes. The insulin infusion will be continued throughout the study and the infusion of dextrose will be varied to control the blood sugar level. The blood sugar will be lowered to 2.5mmol/l and restored to normal after one hour. the level of the blood sugar will be carefully controlled by frequent measurement of blood sugar and adjustment of the rate at which dextrose is given. Symptoms such as sweating, tremor, palpitations, warmness, lightheadedness, dizziness and inability to concentrate may develop at the low level of blood sugar.

Your IQ will be tested before the study by some simple tests. There will be 3 separate parts to the study performed on different days in random order. On one occasion, we will perform tests of your intellectual function with a normal blood sugar. On the other two occasions we will perform tests of your intellectual function with a lower sugar, doing the tests at different times throughout the study, to examine whether the length of time which the blood sugar is low influences the brain function. You will not be aware of the order of the tests.

Blood pressure and heart rate will be recorded during the studies. We will also take blood samples through a separate cannula in a different vein to measure your blood sugar level. The study will last approximately 2½ hours. We will ask you to attend on one occasion before the studies commence to familiarise yourself with the type of tests of intellectual function we will be performing.

This is a research study which is of no personal benefit to yourself. If you decide at any time that you no longer want to participate and wish to withdraw, the study will be terminated immediately.

## PATIENT EXPLANATION SHEET FOR *STUDY 3*

### INFORMATION FOR PATIENTS

Study - To examine whether the brain function of diabetic patients who have symptomatic loss of awareness of hypoglycaemia are more affected by a low blood sugar level.

This study is designed to examine the effect of inability to perceive the symptoms of low blood sugar on how the brain is able to cope with mental tests during a period of having a low blood sugar.

During the study, we will lower your blood sugar by giving you an infusion of short acting (soluble) insulin and on an infusion of sugar solution (dextrose) through a cannula (drip) in to one of the veins in your forearm. Insulin is a naturally occurring chemical (or hormone) which is produced in the pancreas, which lowers blood sugar and is used to treat diabetes. The insulin infusion will be continued throughout the study and the infusion of dextrose will be varied to control the blood sugar level. The blood sugar will be lowered to 2.5mmol/l and restored to normal after one hour. the level of the blood sugar will be carefully controlled by frequent measurement of blood sugar and adjustment of the rate at which dextrose is given. Symptoms such as sweating, tremor, palpitations, warmness, lightheadedness, dizziness and inability to concentrate may develop at the low level of blood sugar although some patients will be selected for this study because they have lost some or all of their usual symptoms for hypoglycaemia.

Before the study we will measure your IQ with simple tests. We will then perform tests of your intellectual function before, during and after a period of a low blood sugar level.

Blood pressure and heart rate will be recorded during the studies. We will also take blood samples through a separate cannula in a different vein to measure your blood sugar level. The study will last approximately 2 hours. We will ask you to attend on one occasion before the studies commence to familiarise yourself with the type of tests of intellectual function we will be performing.

This is a research study which is of no personal benefit to yourself. If you decide at any time that you no longer want to participate and wish to withdraw, the study will be terminated immediately.

## PATIENT EXPLANATION SHEET FOR STUDY 4

### INFORMATION FOR PATIENTS PARTICIPATING IN THE HYPOGLYCAEMIA/SPET SCANNING STUDY

This study is designed to examine the effect of lowering blood sugar below normal levels (hypoglycaemia) on blood flow in the brain.

During the study we will lower your blood sugar below normal by giving you an infusion of short-acting (soluble) insulin and an infusion of sugar solution (dextrose) through a cannula (drip) into one of your forearm veins. Insulin is a naturally occurring chemical (or hormone) which is produced in the pancreas, which lowers blood sugar and is used to treat diabetes. The insulin infusion will be continued throughout the study and the infusion of dextrose will be varied to control the blood sugar level, lowering the blood sugar to 2.5 mmol l<sup>-1</sup> and then restoring it to normal after 30 minutes. The level of the blood sugar will be carefully controlled by frequent measurements of blood sugar and adjustment of the dextrose infusion rate. Symptoms such as sweating, tremor, palpitations, warmth, lightheadedness, dizziness, and inability to concentrate may develop, although some patients will be selected for this study because they have lost some or all of their usual symptoms of hypoglycaemia.

Before the blood sugar is lowered, the blood flow through the brain will be measured using a scanner machine which detects gamma rays which will be released from a small amount of radioactive tracer injected into an arm vein (through a cannula). One scan will be performed when the blood sugar is normal and this will be repeated when the blood sugar has been lowered below normal. The total radiation dose is approximately equivalent to a kidney X-ray (intravenous urogram).

In order to take the scan the subject lies on a couch, and the scan is taken by a camera behind the head. It is exactly like having one's picture taken by a camera but it takes much longer (about 40 minutes in all) and it is necessary to lie as still as possible. Most people find it quite comfortable but a member of staff will be close by at all times and you can talk to them and stop the scan if you are becoming physically uncomfortable. Considerable experience has been obtained with these scans both in Edinburgh and Glasgow and the same method is used all over the world. The tracer used in the scan gives off energy like X-rays. It has been used for many years and is licensed for safe use in medicine.

In addition during the study we will do a few simple tests to assess any changes in intellectual function during hypoglycaemia. On another day we will ask you to return and undergo these tests while your blood sugar is maintained at a normal level using a combined infusion of sugar and insulin; however in this day we will need to do the brain scan.

As well as measuring the blood flow in the brain using the scanner, we will measure your heart rate and blood pressure several times during the study. We will also take blood samples through a separate cannula in a different vein to measure your blood sugar level and some other important hormones. The study will last approximately 4 hours.

This is a research study which is of no personal benefit to yourself. If you decide at any time that you no longer want to participate and wish to withdraw, the study will be terminated immediately, and this will not prejudice any treatment you are receiving for your diabetes.

Dr. Ann Gold

Dr. Ken MacLeod

Dr. David Hepburn

Dr. Brian Frier

Department of Diabetes  
Royal Infirmary, Edinburgh.

**INSULIN INFUSION CHART FOR HYPERINSULINAEMIC  
GLUCOSE CLAMP**

**INSULIN PRIME REGIMEN**

<b>TIME (min)</b>	<b>INSULIN INFUSION RATE (mU/m<sup>2</sup>/min)</b>	<b>RATE (mls/hr)</b>
0-1	150	.....
1-2	140	.....
2-3	127.6	.....
3-4	113.6	.....
4-5	101.2	.....
5-6	90.2	.....
6-7	80.2	.....
7-8	71.4	.....
8-9	63.6	.....
9-10	60	.....



**SYMPTOM QUESTIONNAIRE USED IN CLAMP STUDIES**

**SYMPTOM SCORES**

NAME : .....

TIME : .....

	NOT AT ALL					A GREAT DEAL	
	1	2	3	4	5	6	7
CONFUSION	-	-	-	-	-	-	-
SWEATING	-	-	-	-	-	-	-
DROWSINESS	-	-	-	-	-	-	-
WEAKNESS	-	-	-	-	-	-	-
DIZZINESS	-	-	-	-	-	-	-
WARMNESS	-	-	-	-	-	-	-
DIFFICULTY SPEAKING	-	-	-	-	-	-	-
POUNING HEART	-	-	-	-	-	-	-
INABILITY TO CONCENTRATE-	-	-	-	-	-	-	-
SHIVERING	-	-	-	-	-	-	-
DOUBLE VISION	-	-	-	-	-	-	-
BLURRED VISION	-	-	-	-	-	-	-
HUNGER	-	-	-	-	-	-	-
NAUSEA	-	-	-	-	-	-	-
ANXIETY	-	-	-	-	-	-	-
TIREDNESS	-	-	-	-	-	-	-
TINGLING LIPS	-	-	-	-	-	-	-
TREMBLING	-	-	-	-	-	-	-

## UWIST MOOD ADJECTIVE LIST USED IN CLAMP STUDIES

### Matthews Self-Assessment Scale

This mood scale is designed to assess your mood at this moment in time. That means you are required to answer the items exactly as you feel just now - don't answer them according to how you normally feel. It is your present state of mind that is important here.

Please circle the answer (1, 2, 3, or 4) that you feel applies to you, for each of the adjectives listed (e.g. If you feel slightly anxious, then circle number 2 on that item).

Remember to answer all of the items. Work quickly, and don't take too long to think about each item.

	DEFINITELY	SLIGHTLY	SLIGHTLY NOT	DEFINITELY NOT
<i>Cheerful</i>	1	2	3	4
<i>Anxious</i>	1	2	3	4
<i>Vigorous</i>	1	2	3	4
<i>Nervous</i>	1	2	3	4
<i>Happy</i>	1	2	3	4
<i>Unenterprising</i>	1	2	3	4
<i>Jittery</i>	1	2	3	4
<i>Dissatisfied</i>	1	2	3	4
<i>Sluggish</i>	1	2	3	4
<i>Contented</i>	1	2	3	4
<i>Calm</i>	1	2	3	4
<i>Sad</i>	1	2	3	4
<i>Restful</i>	1	2	3	4
<i>Active</i>	1	2	3	4
<i>Satisfied</i>	1	2	3	4
<i>Tired</i>	1	2	3	4
<i>Depressed</i>	1	2	3	4
<i>Alert</i>	1	2	3	4
<i>Tense</i>	1	2	3	4
<i>Composed</i>	1	2	3	4
<i>Energetic</i>	1	2	3	4
<i>Sorry</i>	1	2	3	4
<i>Relaxed</i>	1	2	3	4
<i>Passive</i>	1	2	3	4

# PASAT RECORD SHEET

**P.A.S.A.T RECORD FORM**

NAME : ..... TEST NO. : .....

TIME : .....

2	...	...	...
7 (9)	...	9 (11)	2 (8)
3 (10)	...	7 (16)	7 (9)
4 (7)	...	6 (13)	5 (12)
8 (12)	...	5 (11)	9 (14)
1 (9)	...	8 (13)	2 (11)
5 (6)	...	1 (9)	3 (5)
6 (11)	...	4 (5)	9 (12)
9 (15)	...	1 (5)	7 (16)
1 (10)	...	2 (3)	4 (11)
3 (4)	...	6 (8)	5 (9)
6 (9)	...	3 (9)	7 (12)
4 (10)	...	7 (10)	6 (13)
3 (7)	...	5 (12)	8 (14)
2 (5)	...	8 (13)	1 (9)
7 (9)	...	3 (11)	3 (4)
8 (15)	...	9 (12)	1 (4)
5 (13)	...	1 (10)	9 (10)
9 (14)	...	4 (5)	2 (11)
4 (13)	...	8 (12)	5 (7)
2 (6)	...	6 (14)	6 (11)

NO. CORRECT (MAX = 60)

TRIAL 1 (4secs) .....

TRIAL 2 (2secs) .....

# DIGIT SYMBOL SUBSTITUTION TEST

7. DIGIT  
SYMBOL

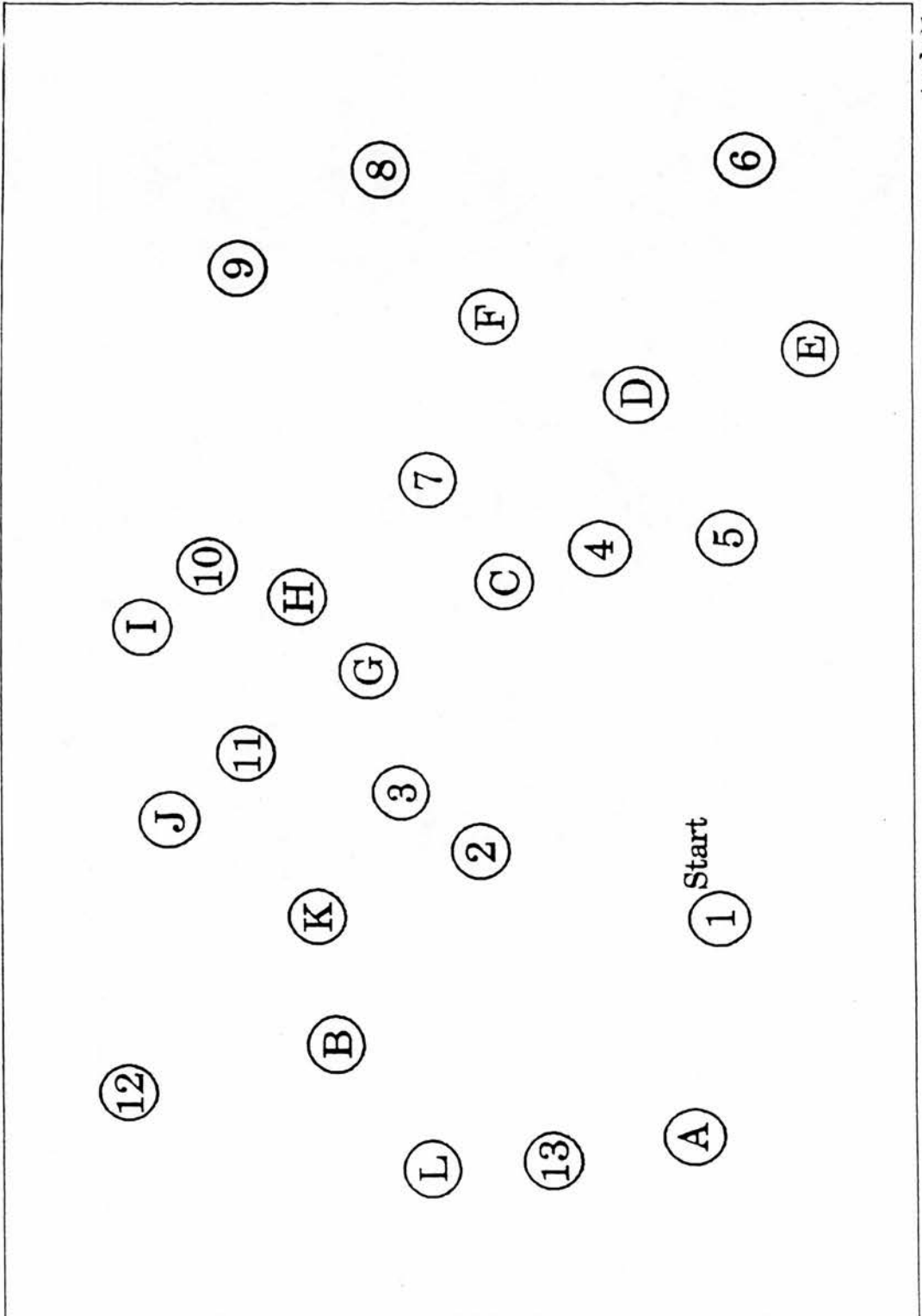
1	2	3	4	5	6	7	8	9
-	I	J	L	U	O	^	X	=

SCORE

SAMPLES

2	1	3	7	2	4	8	1	5	4	2	1	3	2	1	4	2	3	5	2	3	1	4	6	3
1	5	4	2	7	6	3	5	7	2	8	5	4	6	3	7	2	8	1	9	5	8	4	7	3
6	2	5	1	9	2	8	3	7	4	6	5	9	4	8	3	7	2	6	1	5	4	6	3	7
9	2	8	1	7	9	4	6	8	5	9	7	1	8	5	2	9	4	8	6	3	7	9	8	6

TRAIL MAKING B TEST (1 EXAMPLE)



tmb(0)1

## PATIENT EXPLANATION SHEET FOR *STUDY 7*

### HYPOGLYCAEMIA SURVEY- Patient Explanation Sheet

#### Study of patient awareness of hypoglycaemia or "hypos"

After a few years of treatment with insulin some patients find that their "hypos" change so that the warning symptoms of hypoglycaemia , or low blood sugar , which they experience are altered or fewer symptoms occur and the symptoms become much less intense. A previous study which asked patients to recall the nature of their hypos over the previous few months showed that patients with altered symptoms experienced a greater number of hypos than patients with normal symptoms. In particular, the patients with altered symptoms, or loss of awareness of hypoglycaemia, appeared to have hypos of greater severity and were therefore more susceptible to personal injury or accidents. In order to gain accurate information about this problem, we will ask patients to record their hypos as they occur rather than asking them to try to remember details of events which happened several weeks or months earlier. This will help us to build up information about why some patients experience a loss of their symptoms of hypoglycaemia and how much this affects their lifestyle. Before commencing this survey we will ask you some questions about the type of symptoms which you usually experience during a "hypo".

During the period of the survey (12 months) you will be asked to fill in a record form for each hypo as well as recording blood glucose on a regular basis as you do at present . We will also continue to check for the development of any complications associated with your diabetes (eg eye , foot , or kidney problems) as we do on a routine basis at the clinic. If you do have a severe hypo it would be most helpful if a relative , friend or other observer who is with you at the time would also complete the questionnaire, as witnesses are often able to give a more detailed account of what has happened during a hypo.

I will review patients personally in the clinic on regular basis to collect blood glucose data and any reports of hypos which have occurred.

If at any time you wish to withdraw from the study you are free to do so. All information provided will be treated as confidential .

Dr. Ann E. Gold , Research Registrar.



## SYMPTOM ASSESSMENT FOR *STUDY 7 (1)*

13. When you started on treatment with insulin, which of the following symptoms did you have during a hypo ? :

Please tick which affect you and in addition indicate with a cross which of these symptoms are the most important warning to you that a hypo is occurring.

	Not at all						A great deal
	1	2	3	4	5	6	7
Poor concentration	...	...	...	...	...	...	...
Headache	...	...	...	...	...	...	...
Nausea	...	...	...	...	...	...	...
Sweating	...	...	...	...	...	...	...
Confusion	...	...	...	...	...	...	...
Trembling	...	...	...	...	...	...	...
Sleepiness	...	...	...	...	...	...	...
Tingling of lips	...	...	...	...	...	...	...
Itchiness	...	...	...	...	...	...	...
Dizziness	...	...	...	...	...	...	...
Anxiety	...	...	...	...	...	...	...
Pounding heart	...	...	...	...	...	...	...
Shivering	...	...	...	...	...	...	...
Weakness	...	...	...	...	...	...	...
Hiccups	...	...	...	...	...	...	...
Hunger	...	...	...	...	...	...	...
Blurred vision	...	...	...	...	...	...	...
Double vision	...	...	...	...	...	...	...
Convulsions	...	...	...	...	...	...	...

## SYMPTOM ASSESSMENT FOR *STUDY 7 (2)*

14. At the present which of the following symptoms do you have during a hypo :

Please tick which symptoms apply to you and in addition indicate with a cross which of these symptoms are the most important to you as warning of a hypo.

	Not at all						A great deal
	1	2	3	4	5	6	7
Double vision	...	...	...	...	...	...	...
Anxiety	...	...	...	...	...	...	...
Sleepiness	...	...	...	...	...	...	...
Confusion	...	...	...	...	...	...	...
Sweating	...	...	...	...	...	...	...
Hunger	...	...	...	...	...	...	...
Pounding Heart	...	...	...	...	...	...	...
Nausea	...	...	...	...	...	...	...
Weakness	...	...	...	...	...	...	...
Hiccups	...	...	...	...	...	...	...
Shivering	...	...	...	...	...	...	...
Convulsions	...	...	...	...	...	...	...
Tingling of lips	...	...	...	...	...	...	...
Dizziness	...	...	...	...	...	...	...
Itchiness	...	...	...	...	...	...	...
Trembling	...	...	...	...	...	...	...
Poor concentration	...	...	...	...	...	...	...
Headache	...	...	...	...	...	...	...
Blurred vision	...	...	...	...	...	...	...



# HYPOGLYCAEMIA RECORD SHEET FOR *STUDY 7 (2)*

OBSERVATIONS OF RELATIVE OR FRIEND : To be completed by observer if possible

DID YOU NEED HELP FROM SOMEONE ELSE?    YES/NO

WHO GAVE YOU ASSISTANCE?:-

- RELATIVE            ....            (Please tick)
- FRIEND             ....
- DOCTOR            ....
- OTHER  
eg nurse, ambulanceman etc

Date of hypo .....    Time (24 hour clock).....

How are you related to the patient? : eg friend, spouse, neighbour, professional attendant eg, doctor, nurse

DID YOU OBSERVE ANY NOTICEABLE CHANGE IN BEHAVIOUR OR MOOD? :  
(eg aggressive, drowsy, tearful, refused to take food:-  
(please give details)

WERE YOU AWARE OF THE ONSET OF THIS HYPO? :-    Yes/No

WERE YOUR SYMPTOMS? :-

- (1) OF NORMAL INTENSITY            Yes/No
- (2) OF REDUCED INTENSITY            Yes/No
- (3) ABSENT                                Yes/No

COMPARED TO PREVIOUS "HYPOS" DID YOUR SYMPTOMS DEVELOP?:-

- FASTER?            ...
- SLOWER?            ...
- AT THE SAME RATE?    ...

DID THE PATIENT BECOME :  
(1) UNCONSCIOUS?            Yes/No  
(2) SUFFER INJURY?            Yes/No  
(3) HAVE A SEIZURE OR CONVULSION?    Yes/No

(If yes please give details)

Do you know of any reason why the patient had this hypo? :

Any other comments? :

Date of this report .....







## ABSTRACTS

Gold, A.E., MacLeod, K.M., Deary, I.J., Frier, B.M. The effect of altered awareness of hypoglycaemia on cognitive function during acute hypoglycaemia in patients with insulin-dependent diabetes. *Diabetic Medicine* 1993; **10 suppl 1**: S45.

Gold, A.E., MacLeod, K.M., Frier, B.M. Prospective assessment of the frequency of severe hypoglycaemia in insulin-dependent diabetic patients (IDDM) with altered awareness of hypoglycaemia. *Diabetic Medicine* 1993; **10 suppl 1**: S45.

MacLeod, K.M., Gold, A.E., Ebmeier, K., Hepburn, D.A., Deary, I.J., Dougall, N., Murray C., Goodwin, G., Frier, B.M. Preservation of the regional cerebral blood flow responses to hypoglycaemia in patients with Type 1 diabetes who have diminished awareness of hypoglycaemia. *Diabetic Medicine* 1993; **10 suppl 1**: S9.

Gold, A.E., MacLeod, K.M., Deary, I.J., Frier, B.M. Cognitive function during acute hypoglycaemia in patients with insulin-dependent diabetes and altered awareness of hypoglycemia. *Diabetes* 1993; **42 suppl 1**: 104A.

MacLeod, K.M., Gold, A.E., Frier, B.M. Frequency of severe hypoglycemia in insulin-dependent diabetic patients with altered awareness of hypoglycemia. *Diabetes* 1993; **42 suppl 1**: 26A.

Gold, A.E., MacLeod, K.M., Deary, I.J., Frier, B.M. Short-term cerebral adaptation to acute hypoglycemia does not occur in non-diabetic humans. *Diabetologia* 1993; **36 suppl 1**: A151.

**P125. The effect of altered awareness of hypoglycaemia on cognitive function during acute hypoglycaemia in patients with insulin-dependent diabetes.**

Gold A. E.,<sup>a</sup> MacLeod K. M.,<sup>a</sup> Deary I. J.,<sup>b</sup> Frier B. M.<sup>a</sup>  
<sup>a</sup>Department of Diabetes, Royal Infirmary, Edinburgh  
and <sup>b</sup>Department of Psychology, University of Edinburgh.

Cognitive function was examined in 20 IDD patients: 10 had normal awareness of hypoglycaemia (group 1) and 10 had altered awareness of hypoglycaemia (group 2). The groups were matched for age, intelligence, glycaemic control, age of onset and duration of diabetes. The following cognitive function tests: Rapid Visual Information Processing (RVIP), Trail Making B (TMB), Paced Auditory Serial Addition Test (PASAT) and Digit Symbol Substitution Test (DSST) were measured during: a) euglycaemia ( $4.5 \text{ mmol l}^{-1}$ ), b) hypoglycaemia ( $2.5 \text{ mmol l}^{-1}$ ) and c) restoration of euglycaemia ( $4.5 \text{ mmol l}^{-1}$ ) achieved with a hyperinsulinaemic glucose clamp. A normoglycaemic control study ( $4.5 \text{ mmol l}^{-1}$ ) was performed on another day. Multivariate analysis of variance demonstrated a significant difference in performance in all the tests in the hypoglycaemic study compared with the normoglycaemic control study ( $p < 0.01$ ). A trend towards an overall effect of awareness on performance was observed ( $p = 0.08$ ). Although performance at baseline did not differ between the groups, group 2 showed a tendency to perform more poorly during hypoglycaemia (RVIP correct responses  $p = 0.08$ ) and their performance remained significantly impaired following recovery from hypoglycaemia compared to group 1 (TMB  $p = 0.04$  and RVIP correct responses  $p = 0.02$ ). Thus, IDD patients with altered awareness of hypoglycaemia exhibited more profound and protracted cognitive dysfunction during and after acute hypoglycaemia.

*Diabetic Medicine* 1993; 10 suppl 1: S45.

**P126. Prospective assessment of the frequency of severe hypoglycaemia in insulin-dependent diabetic patients (IDDM) with altered awareness of hypoglycaemia.**

Gold A. E., MacLeod K. M., Frier B. M. Department of Diabetes, Royal Infirmary, Edinburgh.

Diabetic patients with altered awareness of hypoglycaemia are reported to have an increased frequency of severe hypoglycaemia, when assessed retrospectively. 57 IDDM patients were studied prospectively for 12 months: 29 having altered awareness of hypoglycaemia (group A) and 28 retaining normal awareness of hypoglycaemia (group B). The groups were matched for age, age of onset of diabetes, duration of diabetes and glycaemic control. Episodes of severe hypoglycaemia were recorded within 24 hours of the event. Nineteen (66%) of patients in group A had one or more episodes of severe hypoglycaemia in 12 months with an overall incidence of 2.8 episodes per patient per year. Group B had significantly fewer episodes of severe hypoglycaemia with 7 (25%) of the patients experiencing one or more episodes and an incidence of 0.4 episodes per patient per year ( $p < 0.001$ ). The time of day of severe hypoglycaemia differed between the two groups: group A patients experienced a greater proportion of episodes in the evening ( $p = 0.05$ ) and tended to have a smaller proportion of episodes overnight ( $p = 0.09$ ) than group B. This prospective evaluation confirmed that altered awareness of hypoglycaemia predisposes to more episodes of severe hypoglycaemia, many of which occur at home during waking hours.

*Diabetic Medicine* 1993; 10 suppl 1: S45.

**A32. Preservation of the regional cerebral blood flow responses to hypoglycaemia in patients with Type 1 diabetes who have diminished awareness of hypoglycaemia.**

MacLeod K.M.<sup>a</sup>, Gold A.E.<sup>a</sup>, Ebmeier K.<sup>c</sup>, Hepburn D.A.<sup>a</sup>, Deary I.J.<sup>b</sup>, Dougall N.<sup>c</sup>, Murray C.<sup>c</sup>, Goodwin G.<sup>c</sup>, Frier B.M.<sup>a</sup>. <sup>a</sup>Department of Diabetes, Edinburgh Royal Infirmary, <sup>b</sup>Department of Psychology, University of Edinburgh and <sup>c</sup>MRC Brain Metabolism Unit, Royal Edinburgh Hospital.

Regional cerebral blood flow (rCBF) was examined in 20 Type 1 diabetic patients, 10 of whom had normal awareness of hypoglycaemia (group 1) while 10 had diminished hypoglycaemia awareness (group 2). rCBF was determined sequentially during 1. normoglycaemia (blood glucose concentration 4.5 mmol l<sup>-1</sup>) and 2. hypoglycaemia (2.5 mmol l<sup>-1</sup>) induced with a hyperinsulinaemic glucose clamp technique. The distribution of the isotope <sup>99m</sup>Technetium-Exametazime was detected using a single slice multi-detector head scanner, employing a split-dose technique, with 250 MBq injected during steady-state normoglycaemia and 250 MBq during hypoglycaemia. rCBF was estimated in 30 regions of interest, derived from a standard neuroanatomical atlas, on two parallel slices at 35 and 55 mm above the orbito-meatal line. In comparison with the rCBF during euglycaemia, both patient groups demonstrated a significant alteration in the pattern of rCBF during hypoglycaemia with an increase in regional blood flow to both frontal cortices (weighted mean average blood flow 1.01 vs 1.07 and 1.00 vs 1.05 for the right and left cortex respectively; both  $p < 0.01$ ); an increased flow to the right thalamus (1.02 vs 1.11;  $p < 0.01$ ) and reduced flow to the right posterior cingulate cortex (1.03 vs 0.95;  $p = 0.05$ ) and the right putamen (1.06 vs 0.98;  $p = 0.03$ ). No between group differences in the pattern of regional cerebral blood flow were demonstrated during hypoglycaemia. In conclusion the pattern of changes in regional blood flow during hypoglycaemia is preserved in those with diminished hypoglycaemia awareness.

*Diabetic Medicine* 1993; 10 suppl 1: S9.

Cognitive Function During Acute Hypoglycemia in Patients with Insulin-Dependent Diabetes and Altered Awareness of Hypoglycemia, ANN E GOLD, KENNETH M MACLEOD, IAN J DEARY and BRIAN M FRIER\*, EDINBURGH, UK.

Cognitive function was examined in 20 Type 1 diabetic patients, 10 of whom had normal awareness of the onset of hypoglycemia (group 1) while 10 had altered awareness of hypoglycemia (group 2). A hyperinsulinemic glucose clamp was used to produce the following conditions: (a) euglycemia (blood glucose concentration 4.5 mmol/l), (b) hypoglycemia (2.5mmol/l) and (c) restoration of euglycemia (4.5mmol/l). On another day a normoglycemic control study was performed (4.5mmol/l). Cognitive function was assessed using Rapid Visual Information Processing (RVIP), Trail Making B (TMB), Paced Auditory Serial Addition Test (PASAT) and Digit Symbol Substitution Test (DSST). Multivariate analysis demonstrated a significant difference in performance in the hypoglycemic study compared with the normoglycemic control study ( $p < 0.01$ ). There was a trend towards an overall effect of awareness on performance ( $p = 0.08$ ). Further analysis of the individual tests demonstrated a significant effect of awareness on RVIP correct responses across time ( $p = 0.05$ ) and an interaction of awareness by study by TMB ( $p = 0.03$ ). Analysis of the simple effects revealed that on recovery from hypoglycemia, cognitive function remained impaired in group 2 compared to group 1 (TMB  $p = 0.04$  and RVIP correct responses  $p = 0.02$ ). In conclusion patients with altered awareness of hypoglycemia exhibited more profound cognitive dysfunction during acute hypoglycemia which persisted for longer following blood glucose recovery.

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Frequency of Severe Hypoglycemia in  
Insulin-Dependent Diabetic Patients with  
Altered Awareness of Hypoglycemia, KENNETH M  
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The frequency of severe hypoglycemia has been shown to be increased in patients with altered awareness of hypoglycemia, when measured retrospectively. 57 IDD patients, 29 of whom had altered awareness of hypoglycemia and 28 with normal awareness of hypoglycemia, were studied prospectively for one year. The groups were matched for age, age of onset of diabetes, duration of diabetes and glycemc control. The patients were asked to document all episodes of severe hypoglycemia within 24 hours of the event. Possible precipitating factors, activity, time of day, location, form of treatment and any resulting morbidity were documented. Nineteen (66%) of patients with altered awareness of hypoglycemia experienced one or more episodes of severe hypoglycemia in 12 months with a frequency of 2.8 episodes per patient per year. By comparison 7 (25%) of the patients with normal awareness of hypoglycaemia experienced an episode of severe hypoglycemia with a frequency of 0.4 episodes per patient per year ( $p < 0.001$ ). One unaware patient sustained significant injuries. The timing of hypoglycemia differed between the two groups: the patients with altered awareness experienced a greater proportion of episodes of severe hypoglycemia in the evening ( $p = 0.05$ ) and tended to have a smaller proportion of episodes overnight ( $p = 0.09$ ) compared with those with normal awareness of hypoglycemia. This prospective evaluation confirmed that patients with altered awareness experience more episodes of severe hypoglycemia, which may have a greater morbidity, and many of these occur during waking hours in the home environment.

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## SHORT-TERM CEREBRAL ADAPTATION TO ACUTE HYPOGLYCEMIA DOES NOT OCCUR IN NON-DIABETIC HUMANS

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Acute hypoglycaemia causes cognitive dysfunction both in diabetic and non-diabetic humans. Previous studies have suggested that cerebral adaptation may occur during acute hypoglycaemia. The aim of the present study was to examine changes in cognitive function and symptoms during hypoglycaemia. Hypoglycaemia was induced with a hyperinsulinaemic glucose clamp on 3 separate occasions in 12 non-diabetic subjects and cognitive function was assessed using a cognitive test battery: Paced Auditory Serial Addition Test (PASAT), Rapid Visual Information Processing (RVIP), Trail-Making B (TMB), Digit Symbol Substitution Test (DSST) and Four Choice Reaction Times (CRT). In Study A the blood glucose was maintained at 4.5mmol/l throughout. On two occasions (Study B and Study C) the blood glucose was stabilised at 4.5mmol/l for 30 minutes, lowered to 2.5mmol/l for 60 minutes and restored to 4.5mmol/l for 30 minutes. In each study the first cognitive test battery was performed immediately after stabilisation of blood glucose at 4.5 mmol/l, then was repeated as follows: Study A - after a further 40 minutes of normoglycaemia; Study B - after 40 minutes of hypoglycaemia; Study C - after 5 minutes of hypoglycaemia. Acute hypoglycaemia induced a significant deterioration in cognitive function in DSST ( $p < 0.01$ ), PASAT ( $p < 0.05$ ), Choice Reaction Time ( $p < 0.03$ ) and RVIP ( $p < 0.005$ ). No differences in performance were identified between Studies B and C. Symptoms assessed by a scaled symptom questionnaire increased significantly during hypoglycaemia ( $p = 0.002$ ) but the symptom scores after 30 minutes and after 60 minutes of hypoglycaemia did not differ. This study suggests that after exposure to 40 minutes of hypoglycaemia (2.5 mmol/l) neither cognitive function nor symptom score improve, thus excluding an adaptation effect.

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