HYPOGLYCAEMIA IN TYPE 1 DIABETES:

RISK FACTORS, SYMPTOMS AND RECOVERY

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DECLARATION

a. This thesis was composed by me.

b. I made a substantial contribution to all the studies described here:

Study 1: Serum Angiotensin-Converting Enzyme and frequency of severe hypoglycaemia in type 1 diabetes: does a relationship exist?

I designed this study. Data collection was contributed to by Dr Jacqueline Geddes of the Department of Diabetes, Royal Infirmary of Edinburgh. I analysed the data and wrote up the study. Dr Riccardo Marioni of the University of Edinburgh provided assistance with statistical analyses.

Study 2: Modelling the consistency of hypoglycaemic symptoms: high variability in diabetes

Data for this study were collected as part of a multi-centre epidemiological hypoglycaemia study, funded by the Department for Transport, which had no role in study design, data collection, data analysis, data interpretation or writing of the paper. I collated all the symptom data for this study and collected all the Edinburgh data. I would like to acknowledge the work of the UK Hypoglycaemia Study Group in collecting data from the 5 other participating centres which has been used for these analyses. I collaborated with Dr George Streftaris to develop the statistical model for assessing hypoglycaemia symptom consistency. I provided the data, diabetes input and initial ideas and the statistical analyses were designed by Dr George Streftaris and Prof Gavin Gibson of Heriot Watt University.

Study 3: Delayed recovery of cognitive function following hypoglycaemia in adults with type 1 diabetes and the effect of impaired awareness of hypoglycaemia

The protocol for this study and data collection were jointly undertaken by Dr Roderick Warren and myself. I analysed all the data and wrote up the study

c. This thesis has not been submitted for any other degree or professional qualification

Nicola Naomi Zammitt

Date: 16/05/2012

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I am deeply indebted to Professor Brian Frier, without whom this research would not have been possible. He fostered my interest in clinical research and continues to push me to seek new challenges and keep my research interests alive. I am also indebted to Professor Ian Deary, for his generosity with his time and advice. I will be eternally grateful for his ability to make the esoteric clear to those who lack his incredible mind and I have relied heavily on his input with cognitive testing and statistical analysis.

I shared a research office and good times with three research fellows: Kate Allen, Jacqueline Geddes and Roderick Warren. I am particularly grateful to Roderick, who taught me the ropes and encouraged me to question everything. I would certainly have floundered without him. I am also grateful to the research nurses at the Royal Infirmary of Edinburgh, particularly Margaret Boyd, who kept me sane. Dr Mark Strachan has guided my career at several crucial moments and I am grateful to him for pointing me in the direction of a research job with Professor Frier. Finally, I would like to thank my collaborators: Dr George Streftaris and Dr Ricardo Marioni.

DEDICATION

For dad, who is sadly missed. I wish he could have seen this in print.

For Fintan, who has been a constant support during my writing up and in all my endeavours.

ABSTRACT

Hypoglycaemia in type 1 diabetes: risk factors, symptoms and recovery

Hypoglycaemia is the commonest side-effect of insulin treatment for diabetes mellitus. Appreciation of the risk factors for hypoglycaemia and early recognition of its symptoms can help the affected individual with prompt self-treatment of hypoglycaemia, preventing progression to severe hypoglycaemia. The proposed MD project will consist of three major studies to investigate the risks for, symptoms of, and rate of recovery from, hypoglycaemia.

Study one

This study will examine the alleged association between severe hypoglycaemia and serum angiotensin converting enzyme (ACE) levels. While many patients rarely experience severe hypoglycaemia, a small subgroup experiences recurrent episodes. These are very disruptive to daily life and may be dangerous, for example if they occur when the individual is driving. It is therefore of clinical importance to identify risk factors for severe hypoglycaemia.

Scandinavian studies have reported an association between elevated serum ACE activity and an increased risk of severe hypoglycaemia in type 1 diabetes. A hypothetical explanation for these findings is that lower ACE activity confers increased ability for cerebral function to be maintained despite metabolic substrate deprivation. It is possible that in diabetes, this could manifest as greater impairment of mental ability during hypoglycaemia in people with high ACE activity. This would explain their increased risk of severe hypoglycaemia for a given level of blood glucose as they would be more incapacitated, and therefore less able to self-treat. However these studies have methodological limitations and these findings have not yet been reproduced outside Scandinavia.

In this study, it is proposed to examine the relationship between serum ACE levels and the incidence of severe hypoglycaemia. Blood will be sampled for serum ACE activity and the self-estimated frequency of severe hypoglycaemia will be recorded in 300 people with type 1 diabetes attending diabetes clinics at the Royal Infirmary of Edinburgh.

Study two

This study will examine the variability of hypoglycaemia symptom reporting. It is known that the symptoms of hypoglycaemia are idiosyncratic and age-specific. However, no studies have assessed the extent of any intra-individual variability in symptom reporting.

A cohort of 350 people with type 1 and type 2 diabetes, with different disease durations and varying treatment modalities, will be recruited and the symptoms associated with each hypoglycaemic episode will be recorded prospectively over a 12 month period. The reported symptom clusters will be analysed to assess the consistency of symptom reporting for each individual. Regression analysis will be used to assess whether an individual's consistency coefficient is related to any other factors such as disease duration or treatment modality. The ability to predict which individuals will report a consistent group of symptoms and which individuals will experience an erratic pattern of symptoms would assist patient education and allow clinicians to inform patients about how to anticipate and recognise hypoglycaemia.

Study three

This study will examine the time taken for full cognitive recovery from hypoglycaemia and the possible effect of the clinical syndrome of impaired awareness of hypoglycaemia on this process. The effects of acute insulin-induced hypoglycaemia on cognitive function have been investigated extensively but the recovery period after hypoglycaemia has not been rigorously assessed. Previous studies examining recovery have had multiple limitations.

The objective of this third study is to measure the recovery time for various domains of cognitive function in a large group of patients with type 1 diabetes who have either normal (n=20) or impaired (n=16) awareness of hypoglycaemia. A hyperinsulinaemic glucose clamp technique will be used to induce controlled hypoglycaemia and a battery of cognitive tests will be applied at baseline, at the beginning and end of a one hour period of hypoglycaemia, then at ten minute intervals during a 90 minute recovery period. Each subject will act as their own control by undergoing a euglycaemic clamp on a separate occasion. Test scores will be compared using general linear modelling with awareness of hypoglycaemia as a between-subjects factor. The findings of this study will have important clinical implications and help to advise patients how long to wait after restoration of euglycaemia before resuming activities such as driving.

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CHAPTER 1: CLINICAL AND PHYSIOLOGICAL EFFECTS OF HYPOGLYCAEMIA IN HUMANS

1.1 Introduction

The consequences of iatrogenic hypoglycaemia were recognised shortly after the discovery of insulin in 1922 (1) with a list of hypoglycaemic symptoms being published that same year (2). However, it was many years before interest developed in hypoglycaemia as a formal research area. It is now well established that hypoglycaemia is the commonest side-effect of insulin treatment (3) and that people with diabetes fear hypoglycaemia as much the vascular complications of advanced diabetes, such as renal failure or blindness (4), and that it therefore represents the principal barrier to good glycaemic control (5). Although the symptoms of hypoglycaemia had been described in the 1920's, it is only in the last 20 years that researchers have addressed clinically pertinent issues such as determining which symptoms are most commonly and most reliably associated with hypoglycaemia. Similarly, it was only in the 1990's that researchers formally grouped hypoglycaemic symptoms into categories using physiological and statistical techniques (6-9).

An understanding of hypoglycaemia requires an appreciation of the difficulties involved in defining this clinical entity as well as a knowledge of normal glucose metabolism, the various physiological defence mechanisms that have evolved to defend us from hypoglycaemia and the symptoms generated by low blood glucose levels, all of which will be reviewed. In some individuals, the symptomatic warnings of hypoglycaemia wane. These people with impaired awareness of hypoglycaemia are therefore at increased risk of hypoglycaemia and merit special consideration. Finally, in order to put the physiology into context, the epidemiology of hypoglycaemia in type 1 diabetes and the risk factors that contribute to hypoglycaemia will be considered. The ways in which hypoglycaemia can affect cognitive function will be discussed in chapter two.

1.2 Definitions

There is no universal consensus on the definition of hypoglycaemia (10). A set of purely biochemical criteria overlook the fact that blood glucose thresholds for the onset of symptoms and counterregulatory hormonal changes may vary according to factors such as recent antecedent hypoglycaemia (11-15) or the prevailing level of glycaemic control (16,17). Nonetheless, pragmatic biochemical cut-offs are often employed when offering advice to patients in order to ensure safety. For example, while this would not be regarded as a definition of hypoglycaemia, Diabetes UK recommends that people with diabetes "make four the floor" and avoid blood glucose levels below 4 mmol/l (18).

Whipple's triad, developed in the context of pancreatic surgery for insulinoma patients, requires the presence of biochemical evidence of hypoglycaemia, symptoms of hypoglycaemia and resolution of symptoms with rescue carbohydrate (19). In practice, individuals with diabetes frequently treat hypoglycaemia on the basis of symptoms without biochemical confirmation and it is therefore often accepted that two out of Whipple's three criteria are sufficient to confirm the presence of hypoglycaemia. However, in one prospective study, biochemical hypoglycaemia only accompanied apparently hypoglycaemic symptoms on 29% of occasions (20) so there are clearly inaccuracies inherent in any definition of hypoglycaemia that does not require biochemical corroboration.

For the purposes of clinical practice, perhaps the most useful definition is the one that was used in the Diabetes Control and Complications Trial (DCCT) (3) to distinguish between mild and severe hypoglycaemia. The former is self-treated while the latter requires external assistance. Severe hypoglycaemia is easier to quantify than mild hypoglycaemia, partly because the former is more likely to be memorable. In people with type 1 diabetes, recall of severe hypoglycaemia is relatively robust over a period of one year, while recall of mild hypoglycaemia is unreliable after an interval of one week (4,21).

1.3 Glucose metabolism and glucose sensing

In humans, glucose homeostasis is tightly regulated in order to protect the body from the vascular complications of chronic hyperglycaemia and the brain from the neuroglycopenic effects of hypoglycaemia. An understanding of normal glucose metabolism is necessary to appreciate the defence mechanisms that have evolved to protect against hypoglycaemia.

1.3.1 Normal glucose metabolism

The two principal hormones controlling glucose homeostasis are insulin and glucagon. Insulin is an anabolic hormone which reduces hepatic glucose output by increasing glycogenesis, proteogenesis and lipogenesis and decreasing gluconeogenesis and glycogenolysis. Glucagon opposes the hepatic effects of insulin. However, whereas glucagon has no significant extra-hepatic actions, insulin is also active peripherally. It increases the uptake of glucose by both adipose tissue and muscle and increases glycolysis and glycogenesis in muscle.

During the fasted state, the concentration of insulin decreases and glucagon increases, resulting in increased hepatic glucose output, availability of alternative fuels such as amino acids and lipids and decreased peripheral glucose utilisation. During short fasts, glycogen provides 60-80% of the glucose used, with the brain consuming up to 80% of this as it is unable to use alternative fuels to any significant extent. During more prolonged fasts, glycogen stores are depleted and glucose is primarily provided by gluconeogenesis. The reduced plasma concentrations of insulin and increased glucagon during the fasted state have a greater catabolic effect on fat than on muscle, favouring the relative preservation of muscle while ensuring adequate cerebral glucose supplies (22). Conversely, in the fed state, insulin secretion increases while glucagon secretion decreases. This favours an anabolic state with an increase in protein synthesis, inhibition of lipolysis, increased hepatic glycogenesis and decreased glycogenolysis and gluconeogenesis.

The brain is the most vulnerable organ to hypoglycaemia because it has a restricted capacity to synthesise or store glucose and relies on a constant supply of glucose for its energy supply. Transport of glucose across the blood-brain barrier acts as the rate-limiting step in this process. The brain does have the capacity to metabolise fuels such as amino acids, lactate,

lipids and ketones in certain situations. For example, during prolonged starvation, the brain metabolises ketones to provide up to 60% of the energy it requires (23).

Hypoglycaemic clamp studies in healthy volunteers have also demonstrated reductions in symptoms and counterregulation during hypoglycaemia with the infusion of intralipid and heparin, although these measures were unable to prevent changes in measures of cognitive function such as reaction time (24). More recently however, a clamp study in 11 adults with intensively treated type 1 diabetes demonstrated that the ingestion of medium chain fatty acids prevented hypoglycaemia-associated impairment on tests of immediate and delayed verbal memory, and verbal memory recognition. However, it did not protect all cognitive functions, with performance on the digit span backwards test deteriorating despite fatty acid ingestion (25). Studies in rats undertaken by the same authors to investigate the mechanism by which fatty acids are protective demonstrate that beta-hydroxybutyrate supports synaptic transmission in vitro (25).

Although alternative metabolic fuels may be utilised under experimental conditions, under physiological conditions the supply of these alternative substrates is insufficient to make their use viable and the brain's two main sources of energy are ATP and creatine phosphate. It is possible to detect changes in cerebral function once blood glucose levels fall to 3 mmol/l, although neither ATP nor creatine phosphate are depleted at these blood glucose concentrations. It is possible that some of these changes in cerebral function are linked to reductions in the production of phospholipids required for cell membrane synthesis and neurotransmitters such as acetylcholine and gaba-amino butyric acid.

1.3.2 Glucose sensing

The capacity to detect changes in blood glucose levels is widely distributed throughout the body and proteins called glucose transporters (GLUT) mediate the movement of glucose into cells down a concentration gradient by facilitated diffusion. The pancreatic beta cell is the classical glucose sensing cell, as it enables modulation of the secretion of insulin in the fed and fasted states as discussed above. However, glucose-sensing neurones have also been demonstrated in the intestine (26), the hepatic portal vein (27,28), the carotid body (29) and in multiple areas within the brain such as the septum (30), amygdala (31), striatum (32), motor cortex (33), hindbrain (34) and hypothalamus (35).

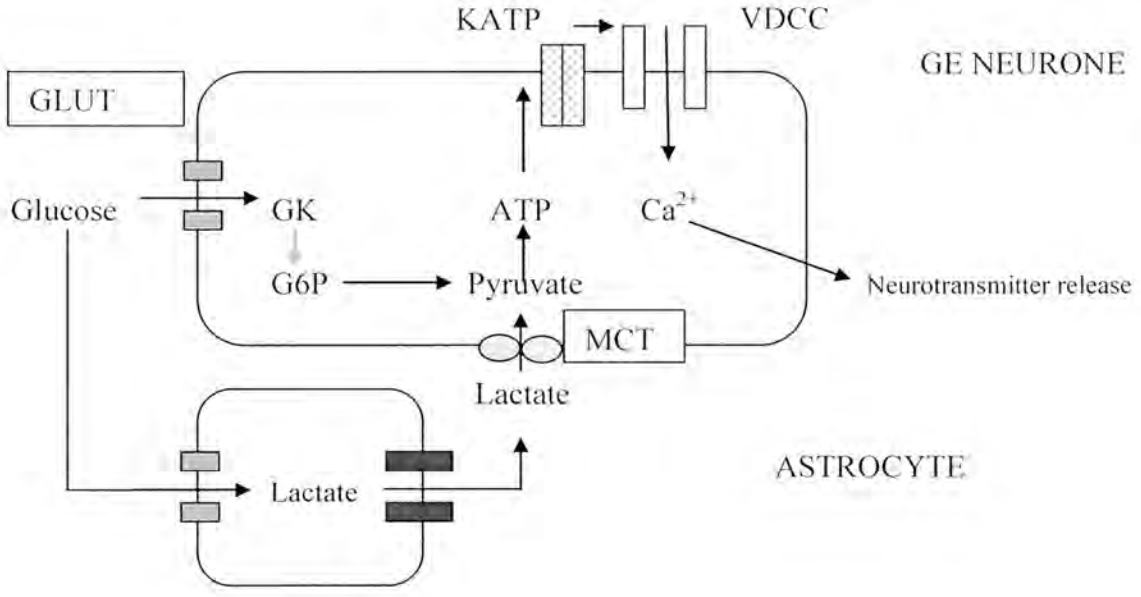
Glucose-sensing neurones in the hindbrain and hypothalamus are closely linked to glucose sensing during hypoglycaemia (36) and in 1953, the 'glucostatic hypothesis' was proposed, which suggested that hypothalamic 'glucoreceptors' could translate changes in ambient glucose into neural signals (37). Glucose sensing neurones can be divided into those that demonstrate an increase in activity in response to glucose (glucose-excited [GE] neurones) and those that show decreased activity (glucose-inhibited [GI] neurones) (38,39).

It is now known that K_{ATP} channels and glucokinase play a central role in the mechanisms governing GE cells (36,40-43) and that these mechanisms appear similar to those involved in glucose sensing in the pancreatic beta cell (Figure 1.1). Glucose is transported into the GE cell by GLUT2 or GLUT3 and is subsequently phosphorylated by glucokinase, which acts a gatekeeper by regulating the production of ATP. In turn, ATP closes K_{ATP} channels, resulting in depolarisation and subsequent influx of calcium ions through voltage dependent calcium channels (VDCC), stimulating neurotransmitter release. Lactate, produced by astrocytes, enters the neurone by monocarboxylate transporter-2 (MCT2) and is also metabolised to ATP, which contributes to neurotransmitter release as above.

Single cell RT-PCR studies have demonstrated that glucose-sensing neurones express mRNA for the Kir 6.2 and SUR-1 subunits of the sulphonylurea receptor (44). Injection of glibenclamide (a K_{ATP} blocker) into the ventromedial hypothalamus (VMH) has been shown to suppress the hormonal counterregulatory responses to systemic hypoglycaemia (45). Conversely, in vivo perfusion of the VMH with diazoxide (a K_{ATP} opener) augments the counterregulatory responses to hypoglycaemia (46).

Figure 1.1

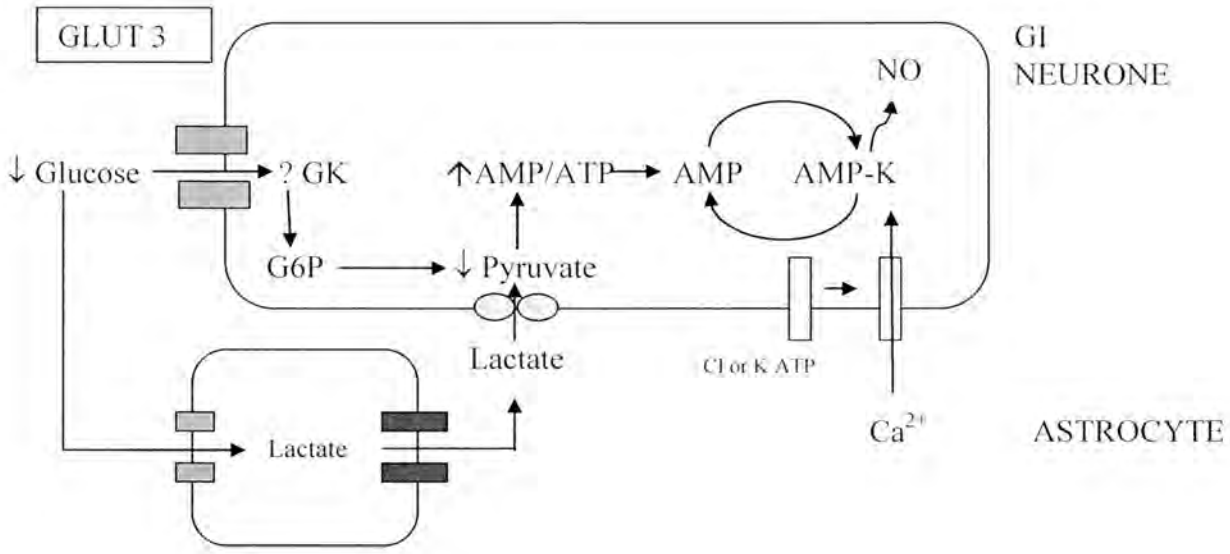
Hypothetical glucose sensing mechanism of GE neurones. Glucose enters the GE cell via GLUT2 or GLUT3 transporters and is phosphorylated by glucokinase. ATP closes K_{ATP} channels, resulting in influx of calcium ions through voltage dependent calcium channels (VDCC), stimulating neurotransmitter release. Lactate, produced by astrocytes, enters the neurone by monocarboxylate transporter-2 (MCT2) and is metabolised to ATP. Adapted from McCrimmon, 2009 (42).



GI neurones behave much more like pancreatic alpha-cells (36,42) and it is thought that AMP-activated protein kinase (AMPK) plays a role in their glucose-sensing mechanisms (42,47). A fall in glucose increases the AMP:ATP ratio. This in turn activates AMPK and stimulates the formation of nitric oxide, which may act as a neurotransmitter. AMPK may also act on chloride channels, leading to membrane depolarisation (figure 1.2).

Figure 1.2

Hypothetical glucose sensing mechanism of GI neurones. A fall in glucose increases the AMP:ATP ratio which activates AMPK and stimulates the formation of nitric oxide (NO), which may act as a neurotransmitter. AMPK may also act on chloride channels, leading to membrane depolarisation. Adapted from McCrimmon, 2009 (42).



Many of these GI neurones are glutaminergic and recent rodent studies show that absence of the VMH-specific glutamate transporter VGLuT2 is associated with an attenuated counterregulatory response to insulin-induced hypoglycaemia (48). Our knowledge of how GI neurones function is more limited than our understanding of GE neurones (36,40). However, it appears likely that both types of neurone are regulated by levels of intracellular ATP rather than glucose levels because their responses to alternative fuels such as lactate are similar to their responses to glucose (49-51).

Most of what we know about cerebral glucose sensing has been learnt from animal studies and it is clear that the studies that will subsequently be described could not be replicated in humans. The glucose-sensing neurones of the hypothalamus are located around the VMH, paraventricular nucleus (PVN) and dorsomedial hypothalamus (DMH) and in vivo studies in rat models have suggested that the VMH plays a central in the detection of hypoglycaemia.

In the VMH, 14-19% of neurones are GE and 3-14% are GI neurones (41,52). Pharmacological ablation of the VMH with ibotenic acid reduces counter-regulatory hormone release by approximately 75% during hypoglycaemia (53). The release of counter-regulatory hormones is markedly reduced during systemic hypoglycaemia by the infusion of glucose locally into the VMH (54). Conversely, local hypoglycaemia can be induced in the VMH by perfusion of 2-deoxyglucose, which is a non-metabolisable form of glucose. This excites a systemic counter-regulatory response, even in the face of systemic normoglycaemia (55).

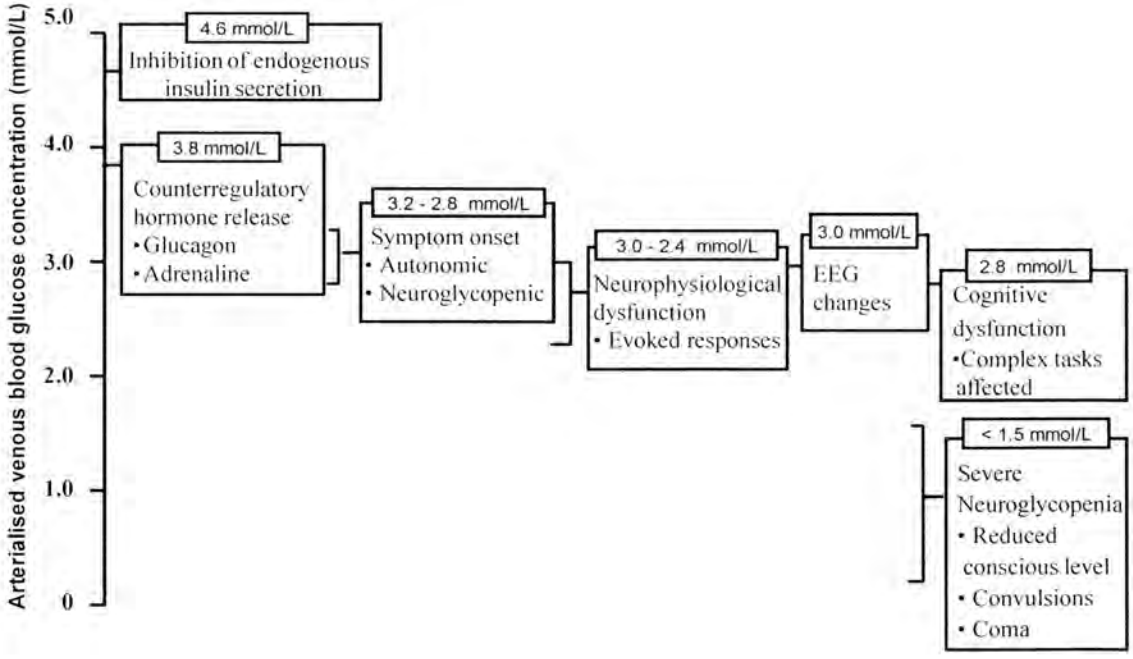
Brain glucose-sensing neurones are located in areas involved in controlling energy homeostasis and regulating autonomic and neuroendocrine function, thereby enabling them to respond appropriately to the detected blood glucose levels (36,42). A further significant feature of this cerebral glucose-sensing system is that glucose-sensing neurones are generally located in areas where the blood-brain barrier is permeable or absent, which allows them to better sense the ambient glucose levels. This is an important feature of the glucose-sensing system because extracellular brain glucose levels are approximately two thirds of the glucose levels found in blood and it takes 15-30 minutes for CSF glucose to equilibrate with blood glucose (56). In fact, animal studies suggest that basal glucose levels in the brain are even lower at around 1.4 mmol/l in the hypothalamus (57), 1.0 mmol/l in the hippocampus (58) and 0.5 mmol/l in the striatum (58). Thus, cerebral glucose levels are often only 10-30% of the levels seen in peripheral blood (36), which is well below the levels at which glucokinase usually acts. Although the concept of the GE neurone acting as a cerebral equivalent of the beta cell is attractive, it is inconsistencies such as this that mean that this hypotheses is not universally accepted

1.4 Counterregulation during hypoglycaemia

To protect cerebral function, several physiological mechanisms have evolved to counteract the effects of hypoglycaemia (22,59-61). When blood glucose falls in a non-diabetic adult, the secretion of counterregulatory hormones and the onset of cognitive, physiological and symptomatic changes occur at reproducible blood glucose thresholds (60,62) within a defined hierarchy (61) (Figure 1.3).

Figure 1.3

Hierarchy of counterregulatory responses. Reproduced from Zammitt and Frier, 2005 (63).



Glucagon, catecholamines and GH are secreted once blood glucose levels drop to 3.5-3.7 mmol/l while cortisol is only produced once blood glucose drops to 3mmol/l or less (61). Cognitive dysfunction develops at lower blood glucose levels than those required to initiate

the counterregulatory response, with changes such as impairment in reaction time occurring at blood glucose levels between 2.8-3.0mmol/l (64,65).

1.4.1 Methodological considerations

Early studies examining the hormonal responses to hypoglycaemia employed bolus injections of insulin or insulin infusions. These studies were hampered by the difficulties of conducting measurements of counterregulation when the timing of the hypoglycaemic episode and the depth of the glucose nadir were hard to control. It is now known that counterregulation can be affected by factors such as antecedent hypoglycaemia (12-15), the depth of the glucose nadir (66) and the duration of hypoglycaemia, with acute adaptation being observed during longer periods of hypoglycaemia (67,68). Studies examining whether counterregulation is affected by the rate at which blood glucose falls have produced mixed results. Most studies suggest that counterregulation is unaffected (69,70). However, in one study the adrenaline response to hypoglycaemia was blunted when blood glucose was rapidly lowered in the postprandial state (71).

The amount of insulin used to induce hypoglycaemia can also affect the counterregulatory response under certain circumstances. In nine healthy lean men who underwent two separate glucose clamps, high doses of insulin (3056 ± 236 versus 486 ± 33 pmol/l) resulted in significantly greater increases in catecholamine and cortisol secretion, hepatic glucose output, lipolysis, heart rate and systolic blood pressure, despite equivalent blood glucose nadirs (2.8 mmol/l) (72). However, when the same group repeated a similar experiment in seven lean subjects with poorly controlled type 1 diabetes (HbA1c 10.9%), plasma levels of epinephrine, norepinephrine, cortisol and growth hormone increased similarly during low (742 ± 212 pmol/l) and high (3360 ± 710 pmol/l) dose insulin infusions (73). However, the plasma concentration of insulin has now been standardised by the widespread use of the glucose clamp technique (74) where insulin is infused at a constant rate while blood glucose levels are altered by varying the rate of an infusion of glucose. Subjects are usually studied in the semi-recumbent position as standing can augment the autonomic symptoms associated with hypoglycaemia (75).

The type of blood sample used for glucose measurement also affects interpretation of results (10). In studies of the effects of hypoglycaemia on cognitive function, the blood glucose levels that are theoretically of most interest are cerebral arterial blood glucose levels but sampling is difficult, potentially dangerous and clearly impractical. By convention, arterialised venous blood glucose samples are used as a surrogate measurement. These are obtained by insertion of a retrograde cannula in a dorsal hand vein with warming of the hand using techniques such as a warm-air box. Measurement of arterialised venous blood is less invasive than sampling arterial blood and, given that the difference in whole blood glucose concentration between venous arterialised blood and arterial blood is only around 0.1 mmol/l (76), it is felt that the former provides a reasonable approximation of the arterial blood glucose level.

Furthermore, some studies have measured plasma glucose while others use whole blood glucose. Awareness of the method used is important when comparing results from different studies as analysis of plasma yields glucose results that are 10-20% higher than on an equivalent whole blood sample. When extrapolating results of hypoglycaemic clamp studies it is also important to remember that the plasma glucose concentration in arterialised venous blood is approximately 15% higher than the glucose concentration in a capillary sample (76).

Finally, factors such as recent (antecedent) hypoglycaemia can also modify the counterregulatory response. An episode of hypoglycaemia in the preceding 24-48 hours can blunt the symptomatic and counterregulatory responses to hypoglycaemia (11-15). Studies are therefore usually postponed if there has been an episode of hypoglycaemia within the preceding 24-48 hours. Moderators of hypoglycaemia outwith the setting of experimental hypoglycaemia are discussed in more detail below.

1.4.2 Counterregulatory hormones

Despite methodological limitations, early studies were able to infer the presence of a counterregulatory hormonal defence system. In non-diabetic individuals, the intravenous injection of 0.1 U/kg insulin provokes a fall in blood glucose within a few minutes with a

glucose nadir being achieved after 20-30 minutes (77). Blood glucose levels rise approximately 30 minutes after injection of insulin, even though blood insulin levels are still at 10 times their baseline value at this stage. By implication, other hormones must be involved in the process of counterregulation and the reversal of hypoglycaemia cannot simply be attributed to a fall in insulin levels. Although in healthy adults the first defence against falling blood glucose levels is suppression of endogenous insulin secretion, this defence mechanism is not available to individuals with diabetes who are reliant on either exogenous insulin or insulin secretagogues to control their blood glucose levels, as there is no feedback between ambient blood glucose levels and insulin secretion. However, other hormones have a role to play in counterregulation.

Subsequent studies were able to elucidate the role of the various counterregulatory hormones and demonstrate that glucagon is the most important hormone during acute hypoglycaemia, with the catecholamines providing a second-line defence (59). Growth hormone (GH) and cortisol become important counterregulatory hormones during prolonged hypoglycaemia but do not play a major role in the counterregulatory response to acute hypoglycaemia (59).

This hierarchy has been established by the sequential blockade of individual counterregulatory hormones. Somatostatin infusions block both glucagon and GH release and individual effects can be studied by replacing each hormone separately. Counterregulation is delayed in the case of isolated glucagon deficiency and combined glucagon and GH deficiency but not in the case of isolated GH deficiency, confirming the precedence of glucagon as an acute counterregulatory hormone (59). Phentolamine and propranolol can be infused to achieve complete adrenoceptor blockade. An isolated adrenoceptor deficiency does not impair the acute counterregulatory response whereas the combination of glucagon and adrenergic blockade or glucagon deficiency alone does impair the counterregulatory response (59). All four counter-regulatory hormones increase gluconeogenesis. Both glucagon and the catecholamines increase glycogenolysis, while the catecholamines, cortisol and growth hormone also decrease peripheral glucose utilisation (22). Although counterregulatory hormones exert the bulk of their effect on glucose metabolism, they also affect fatty acid utilisation. Increased epinephrine levels stimulate release of fatty acids via lipolysis, which can be used as an alternative fuel to glucose.

However, counterregulatory deficiencies are associated with increasing duration of type 1 diabetes. Table 1.1 summarises the proportion of individuals with type 1 diabetes demonstrating deficient responses in the various counterregulatory hormones over time. Each counterregulatory hormone will then be discussed in turn.

Table 1.1

Percentage of individuals with type 1 diabetes with deficiencies in counterregulatory hormones with increasing duration of diabetes. Adapted from Mokan et al. 1994 (78).

Duration of diabetes (years)	Glucagon (%)	Adrenaline (%)	Cortisol (%)	Growth hormone (%)
<1	27	9	0	0
1-5	75	25	0	0
5-10	100	44	11	11
>10	92	66	25	25

The secretory response of glucagon to hypoglycaemia can be lost within 5 years of diagnosis of type 1 diabetes (78-80). The glucagon response to other stimuli, such as a protein load, remains largely intact (81), suggesting that the defective glucagon response to hypoglycaemia is stimulus-specific and may result from defective alpha cell signalling rather than irreversible structural damage. In a study of 14 non-diabetic individuals, hypoglycaemia was induced on two separate occasions with an insulin infusion being administered from 0 to 120 minutes. Between -60 and +60 minutes, subjects also received an infusion of either somatostatin or saline placebo, with the aim of suppressing endogenous intraislet insulin secretion in the somatostatin arm and thereby reducing the decrement in intraislet insulin during the second hour of hypoglycaemia. During the somatostatin session, the lack of a decrement in intraislet insulin secretion was associated with 30% lower plasma glucagon levels in response to hypoglycaemia, suggesting that the fall in intraislet (as opposed to systemic or exogenous) insulin is necessary for the release of glucagon in response to hypoglycaemia (82). This finding is consistent with the observation that defects in glucagon

secretion develop in parallel to the loss of endogenous insulin secretion (83,84) and it therefore seems plausible that the loss of reciprocal signalling between alpha and beta cells with advancing diabetes underlies the loss of the glucagon secretory response (82,85-88).

The catecholamine response compensates for the defects in glucagon secretion for several years but it too declines with time (89). The lipolytic effects of epinephrine can outweigh the anabolic effects of insulin on insulin-resistant adipose tissue, resulting in a rise in plasma free fatty acids in response to hypoglycaemia in type 2 diabetes (90-92) but not in type 1 diabetes (93). After 10 years duration of type 1 diabetes, the glucagon response to hypoglycaemia is almost universally lost while around two thirds of individuals will have lost their epinephrine response (94). If individuals with type 1 diabetes who have lost both epinephrine and glucagon responses are exposed to intensive insulin treatment, they are at 25-fold greater risk of severe hypoglycaemic events than those who retain an intact epinephrine response (95,96).

The release of counterregulatory hormones contributes to the physiological changes evident during hypoglycaemia. Direct recordings from sympathetic nerves demonstrate that injection of insulin provokes an increase in the amplitude and frequency of muscle sympathetic activity within 8 minutes, with the peak in sympathetic activity coinciding with the glucose nadir (97). An increase is also seen in skin sympathetic activity which coincides with the onset of sweating (98). It has been demonstrated that sweating occurs within 10 minutes of blood glucose falling to 2.5 mmol/l or below (99). The haemodynamic changes during hypoglycaemia are also largely mediated via epinephrine secretion and activation of the sympathetic nervous system. The increased cardiac output and vasodilatation associated with hypoglycaemia, combined with epinephrine-mediated beta-adrenoceptor stimulation, are responsible for the tremor seen during hypoglycaemia (100).

1.4.3 Moderators of counterregulation

1.4.3.1 Antecedent hypoglycaemia

Several factors may affect the process of counterregulation including antecedent hypoglycaemia. Early suggestions that antecedent hypoglycaemia affected counterregulation came from small studies in non-diabetic adults. In a clamp study in 1992 of 9 non-diabetic adults, blood glucose was maintained at 3 mmol/l for 2 hours. Subjects underwent a second clamp with a blood glucose nadir of 2.8 mmol/l 18 hours later, at which time symptoms and counterregulatory responses were reduced (101).

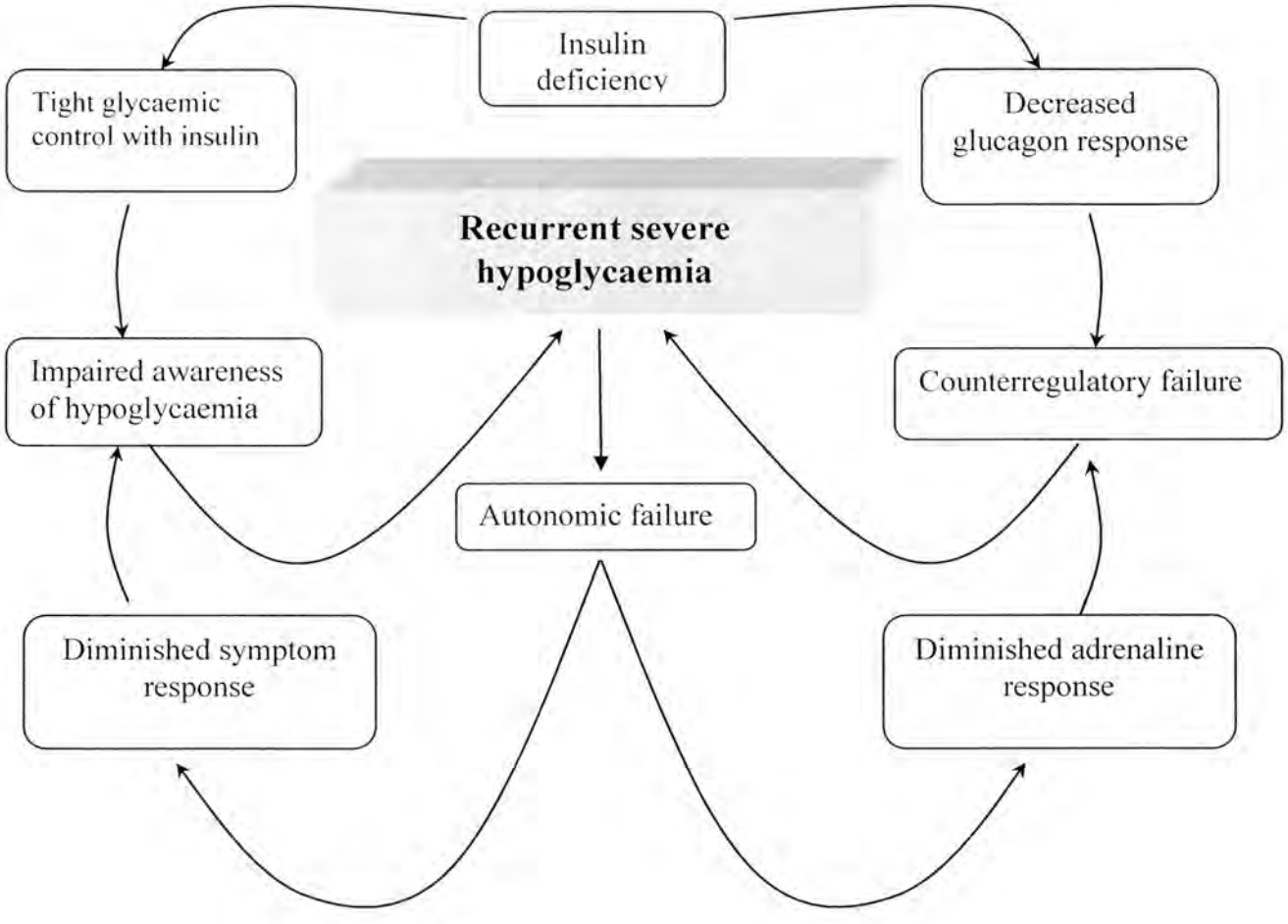
These findings have been confirmed in subsequent clamp studies of diabetic volunteers. In these studies, subjects underwent two stepped glucose clamps where thresholds for symptoms, counterregulatory responses and cognitive function were compared between the two sessions. Participant numbers ranged from 8 (13) to 38 (14) and the interval between the first and second test clamps has ranged from one hour (13) to 2 days (12,15). In one study, subjects underwent twice weekly glucose clamps to a nadir of 2.8 mmol/l for one month prior to a stepped clamp 48h later. Regardless of differences in methodology, these studies have consistently demonstrated a reduction in counterregulatory responses (12-15), symptoms (12,14) and cognitive impairment (11,12) during the second period of hypoglycaemia, confirming that antecedent hypoglycaemia affects responses to subsequent hypoglycaemia.

1.4.3.2 Hypoglycaemia Associated Autonomic Failure

In type 1 diabetes, individuals who experience frequent hypoglycaemia may develop a condition that has been termed *Hypoglycaemia Associated Autonomic Failure* (HAAF) (102,103). The underlying premise is that recurrent hypoglycaemia leads to failure of the centrally mediated counterregulatory response to hypoglycaemia, resulting in impaired awareness of hypoglycaemia. Impaired hypoglycaemia awareness has been associated primarily with type 1 diabetes but people with insulin-deficient type 2 diabetes are also at risk of developing HAAF (83). HAAF is discussed in more detail in the section on impaired awareness of hypoglycaemia (section 1.6.3).

Figure 1.4

Diagram illustrating the concept of Hypoglycaemia Associated Autonomic Failure, based on Cryer, 1992 (102).



1.4.3.3 Gender

Interestingly, the effects of antecedent hypoglycaemia differ between men and women. This was studied in a group of healthy volunteers consisting of 8 men and 7 women who underwent 4 separate 2-day protocols in random order (104). On day 1 of each protocol, subjects underwent a glucose clamp for 2h in the morning and again in the afternoon. The hypoglycaemic nadir differed depending on which of the four experimental protocols was

employed that day, with glucose nadirs of 5.1 (euglycaemia), 3.9, 3.3 and 2.9 mmol/l. Day 2 on all four protocols involved a 2 hour hypoglycaemic clamp with a glucose nadir of 2.9 mmol/l. Following the day 1 euglycaemia protocol (5.1 mmol/l), day 2 counterregulatory responses were greater in men compared to women. However, following the day 1 hypoglycaemic protocols (3.9, 3.3 and 2.9 mmol/l), counterregulatory responses were blunted in men on day 2 while women appeared to be resistant to the blunting effects of antecedent hypoglycaemia on their counterregulatory responses. In the female group, the diminished counterregulatory response on day 2 was only evident following the lowest glucose nadir of 2.9 mmol/l on day 1 (104).

This study demonstrates gender-related differences in the counterregulatory responses to antecedent hypoglycaemia. However, it also demonstrates a more general effect of gender on counterregulation because females were shown to have a lower counterregulatory response compared to men following antecedent euglycaemia (104). However, the first indications of sexual dimorphism in hypoglycaemic counterregulation date from the 1970's, when it was observed that blood glucose levels during moderate fasting fell to lower levels in women than in men (105).

It is now well established that in both non-diabetic and type 1 diabetic subjects, women have attenuated counterregulatory responses to hypoglycaemia compared to men (106-108), with observed reductions in glucagon and epinephrine and decreased endogenous glucose production in response to hypoglycaemia (106,107,109,110). In healthy individuals who underwent a single-step hypoglycaemic clamp, females had adrenaline responses that were 44% lower and noradrenaline responses that were 17% lower than those of their male counterparts, with greater prolongation of endogenous glucose output in women (106).

Glucose counterregulation does not appear to alter between the follicular and luteal phases of the menstrual cycle (111). Some researchers have suggested that the diminished counterregulatory response in women is accounted for by a gender difference in the glucose thresholds required for hormone release (110) while other studies report no gender difference in glycaemic thresholds (112). Subsequent glucose clamp studies have demonstrated that the

thresholds for release of glucagon, epinephrine, cortisol and growth hormone occur between glucose levels of 3.9 and 4.4 mmol/l in both men and women and that it is actually differences in central nervous system efferent output, measured by microneurography, which underlie the observed sexual dimorphism in hypoglycaemic counterregulation (113).

It has been difficult for researchers to reconcile the fact that women have a lesser counterregulatory response with the fact that the prevalence of hypoglycaemia appears to be no higher in women with diabetes than in men (3), as it is to be expected that counterregulatory deficiencies will lead to a vicious cycle of further hypoglycaemia (102). However, it may be that women receive some protection from the fact that they are more resistant to the down-regulation of counterregulation that is observed following antecedent hypoglycaemia in men (104).

1.4.3.4 Exercise

One further observation regarding the sexual dimorphism of the counterregulatory response is that female counterregulatory responses are less affected by exercise than in men (114). However, in both males and females, moderate intensity exercise prior to hypoglycaemia has been shown to blunt the counterregulatory response to hypoglycaemia in both healthy individuals (115,116) and subjects with type 1 diabetes (117).

In non-diabetic subjects, counterregulatory responses to hypoglycaemia were blunted by 2 exercise sessions the preceding day for 90 minutes at 50%VO_{2max} (115) or for 60 minutes at 70%VO_{2max} (116). In a study in type 1 diabetes, 27 individuals underwent a hypoglycaemic clamp one day after either no exercise or after two 90-minute exercise sessions 3 hours apart at either low (30%VO_{2max}) or moderate intensity (50%VO_{2max}). These repeated episodes of both low and moderate intensity exercise were found to reduce muscle sympathetic nerve activity and attenuate the responses of epinephrine and pancreatic polypeptide (117).

One study found no effect of antecedent exercise (60 minutes at 60%VO_{2max}) on hypoglycaemic counterregulation 90 minutes later (118) but methodological inconsistencies such as differences in the intensity, frequency and duration of exercise along with differences in the timing between exercise and hypoglycaemia hamper direct comparisons between this latter study and previously discussed studies in non-diabetic individuals. Interestingly, hypoglycaemia can itself affect the physiological responses to exercise. Ninety minutes of exercise with a work level of 50% VO_{2max} has been shown to reduce the neuroendocrine and metabolic responses to exercise by around 50% (119).

1.4.3.5 Alcohol

Although alcohol can theoretically contribute to hypoglycaemia by causing a direct fall in blood glucose, this is probably only relevant in a state of glycogen depletion such as malnourishment or following prolonged exercise. In one glucose clamp study, 7 subjects with type 1 diabetes and 8 healthy volunteers consumed either ethanol or placebo prior to undergoing clamped hypoglycaemia. Counterregulatory hormone secretion following ethanol did not differ from counterregulatory changes following placebo (120).

However, another research group found that the ingestion of alcohol 1 hour before clamped hypoglycaemia does impair glucose counterregulation in individuals with type 1 diabetes, possibly by suppressing lipolysis (121). It is therefore possible that the effects of alcohol on blood glucose will only be relevant at certain times such as during the night, when lipolysis increases to promote gluconeogenesis (122). Although one study found a reduction in the glucagon response to hypoglycaemia, this has not been replicated in other studies (120).

A number of studies show that ingestion of alcohol attenuates the growth hormone response to hypoglycaemia (120,123,124). Unpublished data described by Kerr and colleagues suggests blunting of the epinephrine response to clamped hypoglycaemia following ingestion of alcohol 12 hours earlier (125). The effects of alcohol on blood glucose can persist for many hours with delayed hypoglycaemia following evening alcohol demonstrated in both laboratory studies (126) and field studies employing continuous glucose monitoring (127).

These findings are all consistent with the observation that glucagon is important or counterregulation the setting of acute hypoglycaemia while growth hormone is more relevant in prolonged hypoglycaemia (59).

1.4.3.6 Age

Early studies assessing the effects of age on counterregulation yielded conflicting results, partly because data interpretation was confounded by the presence of co-morbidities in elderly participants. However, subsequent studies point to the presence of age-related alterations in counterregulation. One study used an intravenous infusion of insulin to compare the counterregulatory responses to hypoglycaemia in non-diabetic elderly and young adults and suggested that with advancing age, the secretion of glucagon, growth hormone and cortisol are diminished, the rise in plasma epinephrine is slowed, the rate of insulin clearance is reduced and blood glucose recovery is modestly attenuated (128). A reduced rate of clearance of insulin has also been noted in several other studies (129-131). These counterregulatory changes do not appear to be affected by preceding physical training, suggesting that they are not simply a consequence of a more sedentary lifestyle associated with ageing (128).

The glycaemic thresholds for the secretion of glucagon and epinephrine in response to hypoglycaemia also occur at a lower blood glucose level than in younger subjects. In young non-diabetic adults, these hormones are released at a blood glucose level of 3.3mmol/l, compared to approximately 2.8mmol/l in older adults (132).

With increasing age, the depth of the blood glucose nadir appears to influence the magnitude of the counterregulatory response. In clamp studies comparing elderly and young non-diabetic subjects, the magnitude of the glucagon and epinephrine responses was lower in the elderly group during mild hypoglycaemia (blood glucose 3.3 mmol/l) but identical in the two groups at a lower blood glucose of 2.8 mmol/l, indicating that in the elderly, counterregulatory responses are preserved during more profound hypoglycaemia (66).

Other similar studies in non-diabetic elderly subjects have not demonstrated any significant age-related impairment of the counterregulatory hormonal responses to hypoglycaemia (133,134). Furthermore, although the symptomatic and counterregulatory responses to hypoglycaemia may be modified by advancing age, it is not known at what age these changes become apparent nor whether these effects are influenced by gender or the menopause in women.

1.4.3.7 Type of diabetes

An additional difficulty lies in the fact that most of the work on glucose counterregulation has been conducted in type 1 diabetes or young healthy non-diabetic individuals. Most elderly patients with diabetes will have type 2 diabetes so it is relevant to ask whether type 2 diabetes *per se* has effects on the counterregulatory process.

Early studies of the counterregulatory responses to hypoglycaemia in type 2 diabetes were limited by methodological factors such as differences in blood glucose nadir between the diabetic and control groups, poorly matched or absent control groups and the inconsistent methods used to induce hypoglycaemia with techniques such as intravenous or subcutaneous bolus injections of insulin (135).

However, three later studies that examined counterregulatory responses to hypoglycaemia in people with type 2 diabetes, receiving treatment either with diet alone or with oral medication, are methodologically more robust. These studies demonstrated that the secretion of counterregulatory hormones occurs at higher blood glucose levels in individuals with type 2 diabetes than in non-diabetic subjects (136,137) and in people with type 1 diabetes (138) which may confer greater protection against hypoglycaemia on individuals with type 2 diabetes than those with type 1 diabetes. However, when HbA1c is lowered with intensive therapy in type 1 diabetes, the thresholds for the counterregulatory responses are shifted to a lower glycaemic level (89,139) and the same phenomenon appears to occur in type 2 diabetes (136,138).

Similarly, some of the counterregulatory deficiencies seen in long-standing type 1 diabetes are also evident in insulin-deficient patients with type 2 diabetes. The counterregulatory responses to hypoglycaemia were examined in 15 non-diabetic controls and in 13 people with type 2 diabetes, six of whom were treated with insulin, and who were demonstrated to be insulin-deficient with low plasma C-peptide, while the remaining seven were being treated with oral anti-diabetic agents (83). The glucagon response to hypoglycaemia was preserved in the patients on oral agents and in the non-diabetic controls, but was virtually absent in the insulin-deficient patients, demonstrating an association of acquired counterregulatory abnormalities and insulin deficiency in type 2 diabetes. Deficient counterregulation was also observed in a group of patients with type 2 diabetes with moderate beta cell failure, in whom the reduction in endogenous insulin secretion that normally occurs during hypoglycaemia was delayed and reduced, and responses of glucagon and growth hormone were impaired (86).

1.4.3.8 Obesity

One potential confounding factor in studies of individuals with type 2 diabetes is obesity, as this can also affect counterregulation in its own right. Morbidly obese non-diabetic subjects underwent hypoglycaemic clamps (glucose nadir 3.4 mmol/l) before and after bariatric surgery (average weight loss 40kg over 12 months). This study suggested excess activation of the glucagon, epinephrine, norepinephrine and pancreatic polypeptide responses prior to surgery with normalisation of these responses after surgery. Growth hormone results showed an opposite pattern with an increased response after weight loss (140).

1.4.3.9 Sleep

Finally, the sleeping state has a direct effect on counterregulation. Studies in children with type 1 diabetes conducted in their own homes demonstrated an attenuated epinephrine response to spontaneous nocturnal hypoglycaemia (141). This finding has been replicated in adolescents both with and without type 1 diabetes when counterregulation was compared during daytime and night-time glucose clamps with a glucose nadir or 2.8 mmol/l (142). In adults with type 1 diabetes, similar findings have been observed although non-diabetic adults appear to have preserved counterregulation during sleep (143).

1.5 Symptoms of hypoglycaemia

The study of hypoglycaemic symptoms is clinically relevant because prompt recognition of hypoglycaemic symptoms is required to allow the instigation of early corrective treatment (144,145). The symptoms of hypoglycaemia were first described in 1922 (2) and these are listed in table 1.2. Although more recent studies have categorised and refined these symptoms, contemporary symptom lists do not differ substantially from this original one.

Symptoms are generated at arterialised blood glucose concentrations around 2.8-3.2 mmol/l and field studies in which young adults with insulin-treated diabetes have reported symptoms during episodes of hypoglycaemia have allowed the most common symptoms to be identified (145) and subdivided into autonomic, neuroglycopenic and general malaise groups (6). These are summarised in table 1.2 alongside Fletcher and Campbell's original classification.

The concept that symptoms can be divided into different groups is supported by evidence from two different but complementary experimental approaches: statistical analyses of symptom reports and physiological/pharmacological studies. The main statistical technique employed in the classification of hypoglycaemic symptoms is that of Principal Axis Factor Analysis. Reports of hypoglycaemic symptoms have been collected from people with (146,147) and without (147) insulin-treated diabetes in both laboratory (147) and field (146) studies. These have been analysed by principal components analysis, also known as factor analysis, which confirmed that symptoms segregate into three distinct groups: neuroglycopenic, autonomic and general malaise (6,146,147). The eleven common hypoglycaemic symptoms contained within this 'three factor' validated model make up the 'Edinburgh Hypoglycaemia Scale' (6) which allows each symptom to be given an intensity score from 1 to 7 on a visual analogue scale, allowing for comparison of symptoms between different episodes of hypoglycaemia.

Table 1.2

Comparison of different classifications of hypoglycaemic symptoms. Compiled from data in *Hypoglycaemia in clinical diabetes* 2nd edition (2007) (eds Frier BM, Fisher BM) (144).

Hypoglycaemic symptoms described by Fletcher and Campbell (2)	List of symptoms described by Deary (6)	Symptom categories (6)
Sweating	Sweating	Autonomic
Tremulousness	Shaking	
Feeling of hot or cold	Warmth	
Change in pulse rate	Palpitations	
Excessive hunger	Hunger	
Dysarthria, sensory and motor aphasia	Speech difficulty	Neuroglycopenic
Incoordination	Incoordination	
Nervousness, anxiety, excitement, emotional upset	Odd behaviour	
Confusion, disorientation	Confusion, difficulty concentrating	
Weakness	Drowsiness, weakness	
Diplopia	Blurred vision	
Vertigo, faintness, syncope		Malaise
Emotional instability		
Pallor		

Statistical studies demonstrate that symptoms cluster into groups and allow researchers to develop hypotheses as to how these symptoms are generated. For example, it seems intuitive to label a group of symptoms that includes confusion, decreased concentration and drowsiness as neuroglycopenic while a symptom that comprises sweating, anxiety and pounding heart would seem to represent an autonomic group.

However, statistical techniques can only demonstrate the association between symptoms but cannot absolutely confirm their physiological origin. In order to demonstrate that these symptoms do indeed have an either an autonomic or neuroglycopenic origin, differing pharmacological blockades have been employed in healthy non-diabetic individuals. Subjects were clamped on 4 different occasions in random sequence during euglycaemia, hypoglycaemia (2.5 mmol/l), hypoglycaemia and combined alpha- and beta-adrenergic blockade and hypoglycaemia with full autonomic blockade. Phentolamine and propranolol were used to achieve alpha- and beta-blockade respectively while atropine was added to achieve full-autonomic blockade. This study demonstrated that tremulousness, anxiety and racing heart are all mediated by the adrenergic system (7), while sweating and hunger are generated by sympathetic cholinergic stimulation (7,8). Those symptoms that were provoked by hypoglycaemia but not reduced by complete autonomic blockade were designated as neuroglycopenic in nature and included confusion, drowsiness and weakness (7). Evidence that adrenalectomised individuals exhibit typical autonomic symptoms suggests that these largely arise from sympathetic neural activation rather than release of epinephrine from the adrenal medulla (9).

When individuals with diabetes have previously been asked to indicate which symptoms they most associate with hypoglycaemia, the commonest symptoms were sweating, difficulty concentrating, decreased coordination and weakness (80%, 80%, 75% and 70% of respondents respectively) (148). The earliest symptoms to develop when blood glucose falls into the hypoglycaemic range are trembling, sweating, tiredness, decreased concentration and hunger (149). The symptoms which correlate most accurately with blood glucose levels are hunger, trembling and weakness (53%, 33% and 27% of people respectively) (150).

Surprisingly, the relationship between symptomatic and biochemical hypoglycaemia is not as robust as might be expected. During stepped clamps, blinded individuals with type 1 diabetes were asked to rate their symptoms at blood glucose levels of 8.9, 5.6 and 2.2 mmol/l on a hypoglycaemic clamp and at 8.8, 14.4 and 21.1 mmol/l during a hyperglycaemic clamp (151). They were also asked to estimate their blood glucose levels at each step of the clamp. During the hypoglycaemic clamp, 34% of subjects had no awareness of autonomic symptoms while

15% had no symptoms at all. Hypoglycaemia and hyperglycaemia were frequently confused, with potentially serious errors made by 66% patients at blood glucose levels of 21.1 mmol/l and 17% of subjects at 2.2 mmol/l (151). Earlier field studies examining the relationship between symptomatic and biochemical hypoglycaemia also found that they were unreliably linked, with biochemical hypoglycaemia (defined as < 3mmol/l) present in only 29% of symptomatic episodes and symptoms present in only 16% of episodes of biochemical hypoglycaemia (20).

Even when symptoms are present at the time of biochemical hypoglycaemia, there is no guarantee that an individual will translate their symptoms into appropriate actions such as the measurement of capillary glucose and the ingestion of carbohydrate to correct the hypoglycaemia. This is because multiple factors such as patient knowledge, distractors and symptom beliefs can affect an individual's response to hypoglycaemic symptoms (152). For example, a standing position augments the autonomic but not the neuroglycopenic symptoms associated with hypoglycaemia (75).

Lack of knowledge can also be an important factor influencing the ability to respond appropriately to hypoglycaemia, particularly amongst elderly people with type 2 diabetes (153,154) and their relatives and carers (155). However, even in young adults, knowledge of diabetes and its treatment declines with time (156) and regular educational reinforcement on interpretation of hypoglycaemic symptoms is seldom undertaken in clinical practice.

It has been suggested that in daily life, people with diabetes tend to rely predominantly on autonomic rather than neuroglycopenic symptoms to warn them of the onset of hypoglycaemia (7). However, neuroglycopenic symptoms are as closely related to blood glucose concentrations as autonomic symptoms (148) and, at the onset of hypoglycaemia, people with insulin-treated diabetes report symptoms from both groups with equal frequency (147). Thus, both autonomic and neuroglycopenic symptoms are of equal value in warning people with type 1 diabetes of the onset of hypoglycaemia, provided that the symptoms peculiar to the individual are identified and interpreted correctly. This reinforces the

importance of educating individuals to be aware of their own individual constellation of hypoglycaemic symptoms.

When educating patients about the recognition of hypoglycaemia, it is also important to be aware of factors that may cause variation in the symptom profile. Hypoglycaemic symptoms are idiosyncratic and age-specific (145). For example, young children have difficulty recognising hypoglycaemia and distinguishing between autonomic and neuroglycopenic symptoms (157) and they often exhibit behavioural changes as part of their symptom profile (158,159). In elderly patients, neurological symptoms, such as visual disturbance and decreased coordination, are prominent and general malaise symptoms are less frequently reported (160). The neurological symptoms and signs generated in response to hypoglycaemia in elderly people may masquerade as other conditions, such as a transient ischaemic attacks or vaso-vagal episodes.

In a study comparing hypoglycaemia generated by tolbutamide and insulin (161), the symptoms reported were unaffected by the causative agent. In an analysis of multiple studies by the same group, the symptoms of hypoglycaemia did not appear to be affected by the method of induction of hypoglycaemia (insulin infusion versus hypoglycaemic clamp) (162).

However, while the symptoms generated by insulin and sulphonylureas may not differ in nature, a stepped glucose clamp study suggested that in patients treated with sulphonylureas, hypoglycaemic symptoms are more intense and occur at higher blood glucose concentrations that in patients with insulin treated type 2 diabetes, even when the two groups are matched for glycaemic control and duration of diabetes (163). While this may offer some additional protection from severe hypoglycaemia for those treated with sulphonylureas, it may also act as a barrier to the achievement of tight glycaemic control in this group. Retrospective recall of symptoms in field studies (164,165) and symptom measurement during experimental hypoglycaemia (162,166) suggest that the symptom profile does not differ between type 1 diabetes and insulin-treated type 2 diabetes.

Although there are gender differences in the extent of the symptomatic and counterregulatory responses to hypoglycaemia (104,106,108,114), symptoms of hypoglycaemia develop at similar blood glucose thresholds in men and women with type 1 diabetes (62). A study reviewing symptoms recorded during experimentally-induced hypoglycaemia in 160 adults (with and without diabetes) did not find any evidence of a gender effect on the nature of the reported symptoms (167).

Prospective field studies have demonstrated that some hypoglycaemia-related symptoms may be more reliably associated with a patient's blood concentration than others, but a given symptom is not equally predictive of hypoglycaemia in everyone (150). This points to the existence of between-subject variability in the reporting pattern of symptoms and it is widely accepted that each individual's symptom complex is idiosyncratic (145). However, an additional important issue is the degree to which individuals report similar patterns of hypoglycaemia-related symptoms across episodes. The reliability with which hypoglycaemic symptoms occur will influence an individual's ability to detect the onset of hypoglycaemia. People who have at least one reliable symptom of hypoglycaemia detect blood glucose levels below 3.9mmol/l correctly on 50% of occasions, whereas individuals with four or more reliable symptoms recognize similar blood glucose levels on 75% of occasions (148).

The symptoms reported by children exhibit marked within-subject (or intra-individual) variability between episodes of hypoglycaemia (157) but it is not known whether the same is true of adults. This is a relevant issue because many of the studies which have informed us about the nature and classification of hypoglycaemic symptoms have relied on patients documenting their 'typical' hypoglycaemic symptoms. These studies partly rest on the assumption that each individual will have a group of hypoglycaemic symptoms which is reasonably constant at an intra-individual level. While the idiosyncrasy of an individual's hypoglycaemic symptoms is widely accepted (144), the intra-individual consistency of symptom reporting has not been formally studied so far.

The effects of alcohol on symptom generation have been studied. In one clamp study, seven subjects with type 1 diabetes and eight healthy volunteers consumed either ethanol or placebo prior to undergoing clamped hypoglycaemia. At euglycaemia, ethanol caused a transient

increase in systolic blood pressure, a sustained increase in heart rate and a slowing in reaction time. During hypoglycaemia, ethanol was associated with a more marked slowing of reaction time, and a greater increase in sweating and finger tremor than on the placebo study (120). Furthermore, following ethanol, only 2 out of the 15 subjects were aware of symptoms during hypoglycaemia as compared to 11 out of 15 subjects following placebo (120).

Antecedent exercise also affects symptomatic responses to hypoglycaemia. In a study involving 27 individuals with type 1 diabetes, subjects were clamped on three occasions, 1 day after either no exercise or after two 90-minute exercise sessions at 30%VO_{2max} or two-90 minute sessions at 50%VO_{2max}. These repeated episodes of both low and moderate intensity exercise were found to the blunt hypoglycaemic symptoms on both days compared to the control clamp with no antecedent exercise (117).

In conclusion therefore, hypoglycaemic symptoms can be grouped into autonomic, neuroglycopenic and general malaise categories and they are usually initiated at blood glucose levels of approximately 2.8-3.2 mmol/l. However, multiple factors can affect an individual's perception of symptoms and their ability to act appropriately in response to them. Although it is accepted that each individual has an idiosyncratic set of hypoglycaemia symptoms, the degree to which these vary from one episode of hypoglycaemia to the next is unknown.

1.6 Awareness of hypoglycaemia

1.6.1 Definition

Awareness of hypoglycaemia can be defined as "the initial perception of *any* symptom of hypoglycaemia, irrespective of whether this is autonomic, neuroglycopenic or simply a vague sensation of apprehension or loss of well-being" (168). The clinical syndrome of impaired awareness of hypoglycaemia has long been recognised and was clearly described by Lawrence in 1941 (169). There is no universally accepted definition of impaired awareness of hypoglycaemia but it is clear that with increasing duration of type 1 diabetes, many individuals experience a change in symptom profile and/or a reduction in symptom intensity such that they are less aware of the onset of hypoglycaemia. An increase in the frequency of asymptomatic biochemical hypoglycaemia during routine blood glucose monitoring (170,171) or continuous glucose monitoring (172) can be suggestive of impaired awareness. Despite the lack of consensus over a precise definition, the term "impaired awareness" of hypoglycaemia is more useful than "hypoglycaemia unawareness" because loss of awareness is not an all or none phenomenon. Total absence of all warning symptoms is rare (170,171).

1.6.2 Classification and prevalence of impaired awareness

Several systems of classifying impaired awareness are in use (21,170,171,173). The method developed by Clarke and colleagues consists of 8 questions to document the individual's exposure to moderate and severe hypoglycaemia as well as their threshold for developing hypoglycaemic symptoms and the nature of these symptoms, with a score of 4 or above suggesting impaired awareness of hypoglycaemia (171). The method by Gold and colleagues poses the question "do you know when your hypos are commencing?" The subject gives their answer on a 7-point Likert scale where 1 represents "always aware" and 7 represents "never aware". A score of 4 or above suggests impaired awareness of hypoglycaemia (170). The method by Pedersen-Bjergaard and colleagues asks the question "can you feel when you are low?" The patient can reply either "always", "usually", "sometimes" or "never". Patients answering anything other than "always" are considered to have impaired awareness.

These three methods have been directly compared in one study where 80 participants with type 1 diabetes completed all 3 methods of assessment in random order and then complete 4-point daily blood glucose monitoring for a 4 week period (174). Any documented blood

glucose reading below 3 mmol/l was accompanied by an assessment of symptoms using the Edinburgh Hypoglycaemia Score (6). The prevalence of impaired awareness of hypoglycaemia was 26%, 24% and 62.5% using the Clarke, Gold and Pedersen-Bjergaard methods respectively (174). There was a strong correlation between the results using the Clarke and Gold methods ($r=0.868$, $p=0.001$) with the Pedersen-Bjergaard method appearing to overestimate the prevalence of impaired awareness of hypoglycaemia (174). The prevalence in this study using the Clarke and Gold methods is similar to figures in previous population studies, suggesting a prevalence of 20-27% in unselected individuals with insulin-treated diabetes (4,175-177). The clinical history is of paramount importance in assessing awareness as individuals who feel that they have impaired hypoglycaemic warnings are usually correct (171).

The prevalence of impaired awareness of hypoglycaemia has been re-examined more recently. Earlier prevalence data was based on retrospective cohorts prior to the introduction of insulin analogues and it was therefore postulated that the rates of hypoglycaemia and impaired awareness of hypoglycaemia might have fallen with more modern treatment regimens. In a cohort of 518 people with type 1 diabetes recruited randomly from a hospital clinic over a two year period, the prevalence of impaired awareness using the Gold method (170) was 19.5% (178), compared to prevalence figures of 20-27% in earlier studies (4,175-177). Those with impaired awareness were older with a longer duration of diabetes and had a six-fold greater incidence of severe hypoglycaemia in the preceding year (178). Older studies have also shown that impaired awareness of hypoglycaemia is associated with a six-fold increase in the incidence of severe hypoglycaemia (170,171) and studies employing CGMS confirm that those with impaired awareness have a much higher rate of undetected daytime hypoglycaemia than those with intact awareness (172). Impaired awareness of hypoglycaemia becomes more common with increasing duration of insulin therapy (175) and by the time individuals have been treated for 25 years, up to 50% will have impaired awareness (4). 0.48, 2.83

It is important to identify impaired awareness of hypoglycaemia in view of its association with increased risk of severe hypoglycaemia. Retrospective studies suggest that the prevalence of severe hypoglycaemia in this group is 90%, compared to 18% in individuals with normal awareness (175). Prospective studies suggest that individuals with impaired awareness have a six-fold increased frequency of severe hypoglycaemia (170).

1.6.3 Pathogenesis of impaired awareness of hypoglycaemia

Impaired awareness predisposes to severe hypoglycaemia for a number of reasons, including changes in the blood glucose thresholds required to trigger symptoms and counterregulatory responses. A study that compared the thresholds for the onset of symptoms and counterregulation during hypoglycaemia in non-diabetic subjects and in people with type 1 diabetes who had either normal or impaired awareness of hypoglycaemia found that those with impaired awareness developed neuroglycopenic symptoms and counterregulatory hormone secretion at lower blood glucose levels than in those with normal awareness and non-diabetic control subjects respectively (78).

There is also evidence for functional cerebral changes in response to hypoglycaemia. Adaptation in brain glucose uptake following recurrent or prolonged hypoglycaemia has been demonstrated in non-diabetic people and in subjects with type 1 diabetes (17,179) and neuroimaging studies have demonstrated permanent alterations in regional cerebral blood flow in those with a history of severe hypoglycaemia, with increased perfusion of the frontal cortex and a decrease to caudal regions (180).

Central nervous system adaptation and exposure to recurrent hypoglycaemia are not the only potential factors implicated in the pathogenesis of impaired awareness. It has been observed that impaired awareness of hypoglycaemia often co-exists with counterregulatory deficiencies in individuals with long-standing type 1 diabetes (181). It has therefore been suggested that there may be a common aetiology for counterregulatory failure and impaired awareness of hypoglycaemia. Individuals who experience frequent hypoglycaemia can develop a condition that has been termed *Hypoglycaemia Associated Autonomic Failure* (HAAF) (14,102,103). The underlying premise is that antecedent hypoglycaemia results in attenuation of the epinephrine response to hypoglycaemia in individuals who have already lost their glucagon response. This results in defective counterregulation and reduced warning symptoms. Supporting evidence for this suggestion comes from evidence that both awareness of hypoglycaemia and the epinephrine response can be partially restored by avoidance of hypoglycaemia for 2 or more weeks (11,62,182,183). While antecedent hypoglycaemia

underlies the counterregulatory defects and impaired awareness observed in HAAF, both of these problems themselves predispose to recurrent hypoglycaemia, thus setting up a vicious self-perpetuating cycle.

Although it is clear that antecedent hypoglycaemia moves the thresholds for sympathoadrenal responses to lower blood glucose levels, the mechanisms underlying these threshold shifts are less well understood. Several hypotheses exist to explain the potential central nervous system alterations that might underlie the altered glucose thresholds observed in HAAF, antecedent hypoglycaemia and impaired awareness of hypoglycaemia (184).

The first of these is the systemic mediator hypothesis, which postulates that antecedent hypoglycaemia increases levels of a systemic factor, such as cortisol, which acts on the brain to reduce the sympathoadrenal responses to hypoglycaemia (185). There are data from animal studies to support this theory. Corticotrophin-releasing hormone (CRH) acts at key sites in the brain involved in autonomic activation. CRH delivered to the VMH and acting via the CRH-1 receptor amplifies the counterregulatory response to hypoglycaemia (186) while local delivery of urocortin, acting via the CRH-2 receptor, suppresses counterregulation (187).

The potential for cortisol to be implicated in the development of impaired awareness of hypoglycaemia is also supported by human data. In healthy subjects, cortisol infusions during euglycaemia reduce epinephrine and muscle sympathetic responses to hypoglycaemia the following day, in a manner analogous to that of antecedent hypoglycaemia (185). Infusions of ACTH resulting in supraphysiological cortisol levels similarly led to diminished symptom responses to hypoglycaemia the following day (116). It has therefore been hypothesised that elevations of cortisol in the context of hypoglycaemic counterregulation blunt subsequent responses to hypoglycaemia. In a clamp study of individuals with primary adrenocortical failure (who cannot mount a cortisol response during hypoglycaemia), participants' usual glucocorticoid therapy was replaced by a continuous cortisol infusion designed to mimic the normal circadian variation. When the adrenocortical failure group and healthy controls underwent hyperinsulinemic clamps on two consecutive days, the control

group demonstrated blunted counterregulation on the second day while the adrenocortical failure group had preserved counterregulation. The authors suggest that the lack of a rise in cortisol during the first clamp resulted in preservation of counterregulation during the second clamp (188). However, these findings have been challenged by Phil Cryer;s group, who demonstrated that low dose cortisol infusions, at a level similar to that present during systemic hypoglycaemia, do not reduce the symptom response to hypoglycaemia the following day (189). Furthermore, CRH knockout mice, who are unable to mount a cortisol response, still develop counterregulatory changes in response to recent antecedent hypoglycaemia (190). These latter studies therefore question the role of cortisol in the pathogenesis of impaired awareness and HAAF.

The brain fuel transport hypothesis suggests that the brain up-regulates blood-brain barrier transporters, such as GLUT-1, in response to recent antecedent hypoglycaemia. This allows increased transport of glucose and other metabolic fuels into the brain, which in turn reduces the sympathoadrenal responses to subsequent hypoglycaemia. Studies in rodents undergoing 3 days of hypoglycaemia demonstrate increases in brain GLUT-1 and protein and glucose uptake (191-194). Similarly, humans undergoing 2 days of hypoglycaemia demonstrate preserved glucose uptake during subsequent hypoglycaemia (17,179). However, in healthy subjects undergoing 24h of experimental hypoglycaemia, sympathoadrenal and autonomic responses were attenuated, as in HAAF, but brain glucose transport, as measured by [1-^{11}C]glucose positron emission tomography, was not altered (195). Furthermore, the features of HAAF can begin to become evident after just a few hours of hypoglycaemia (101,196,197) so the relevance of studies of prolonged hypoglycaemia to our understanding HAAF and impaired awareness is unclear.

According to the brain metabolism hypothesis, recent antecedent hypoglycaemia alters brain metabolism in a way which reduces sympathoadrenal responses to subsequent hypoglycaemia. The difficulty with verifying this hypothesis is that, although certain areas such as the VMH are key to cerebral glucose-sensing (45,51,53-55), counterregulation involves widespread brain activation (198), making this a difficult area to study. A number of possible alterations in metabolism have been examined, including glucokinase activity. Glucokinase is thought to mediate glucose-sensing in the VMH (41), where its expression has

been demonstrated (199). In one study, the infusion of fructose, given to modulate glucokinase activity, resulted in near-normalisation of the epinephrine response to hypoglycaemia in patients with type 1 diabetes (200).

Finally, the brain glycogen supercompensation hypothesis posits that reduced sympathoadrenal responses are caused by a rebound increase in astrocyte glycogen levels following antecedent hypoglycaemia (201). However, there is little concrete evidence for a hypothesis which relies heavily on the concept of supercompensation, given that brain glycogen turnover is a small fraction of total glucose consumption measured in rats (202) and brain glycogen stores are orders of magnitude lower than stores in muscle and liver.

Regardless of whether antecedent hypoglycaemia alters glucose thresholds via a systemic mediator or by affecting brain metabolism, delivery of brain fuel or storage of brain glycogen, the final effects may well be mediated by changes in the cross-talk between GI and GE neurones (42). Both sets of neurones function over a range of glucose values with considerable overlap but hypoglycaemia favours activity of GI neurones (which act to promote counterregulation) while hyperglycaemia favours activity of GE neurones (which suppress counterregulation). Recurrent hypoglycaemia is associated with reduced AMPK activity in the VMH and AMPK is implicated in the functioning of GI neurones (203). Thus, glucose counterregulation would be expected to commence at a lower blood glucose level due to reduced activation of GI neurones.

1.6.4 Conclusions on impaired awareness of hypoglycaemia

Although there is a clear appreciation of impaired awareness of hypoglycaemia as a clinical problem, current understanding of its pathogenesis and our ability to treat and correct it is limited. Hypoglycaemia will activate a number of defence systems and, while individual studies may help elucidate fragments of this system, our understanding of the cross-talk between these various defence systems remains restricted. Clearly, ongoing work is needed in this field in view of the fact that patients with impaired awareness represent one of the groups who are most vulnerable to the potentially devastating effects of hypoglycaemia.

1.7 Epidemiology of hypoglycaemia in type 1 and type 2 diabetes mellitus

The studies in this thesis all relate to hypoglycaemia in type 1 diabetes so this section will focus on the frequency of hypoglycaemia in type 1 diabetes. However, the frequency of hypoglycaemia in type 2 diabetes will also be mentioned because some studies consider insulin-treated patients with both types of diabetes and other studies in type 2 diabetes contribute to the debate on the safety of tight glycaemic control and the dangers of hypoglycaemia in general.

In people with type 1 diabetes, several studies have recorded that, mild hypoglycaemia occurs on average around twice weekly (4,173,204). However, calculating the frequency of mild hypoglycaemia can be difficult for a variety of reasons including the fact that recall of mild hypoglycaemia is unreliable after an interval of one week (4,21). Furthermore, mild episodes may be unnoticed or the symptoms may be misinterpreted without this leading to any obvious consequences. Definitions of mild hypoglycaemia differ between studies, hampering comparison. Finally, inclusion and exclusion criteria in some studies may limit the generalisability of their data on hypoglycaemia. For example, one study reporting an extremely low rate of mild hypoglycaemia of eight episodes per person per year (205) included patients with insulin-treated type 2 diabetes and examined a cohort with poor glycaemic control (mean HbA1c 9.1%), factors which would be expected to reduce the frequency of observed hypoglycaemia. Similarly, participants in the Diabetes Control and Complications Trial (DCCT) (3,206) were excluded if they had experienced more than one episode of severe hypoglycaemia in the last two years, which would probably result in a lower rate of hypoglycaemia than would be observed in an unselected population (207).

By contrast, recall of severe hypoglycaemia is relatively robust over a period of one year and severe hypoglycaemia is therefore easier to record accurately. In studies in northern Europe of unselected populations with type 1 diabetes, the estimated incidence of severe hypoglycaemia ranges from 1.0 to 1.7 episodes/patient/year (4,173,204,208-210). The annual prevalence is around 30% (206,208,211) but can be as high as 40.5% (209). However, the frequency of severe hypoglycaemia is skewed with many people with type 1 diabetes never experiencing severe hypoglycaemia and a small minority experiencing repeated episodes. In

the UK Hypoglycaemia Study, individuals were stratified according to type and duration of diabetes. In subjects with less than 5 years duration of type 1 diabetes, the incidence of severe hypoglycaemia was 1.1 episodes per person per year, with a prevalence of 22%. However, in subjects with greater than 15 years duration of type 1 diabetes, the incidence was 3.2 episodes per patient per year with a prevalence of 46% (212). Thus, the incidence of severe hypoglycaemia is higher in certain groups, such as those with a longer duration of diabetes (212) or those with impaired awareness of hypoglycaemia (170,171). By comparison, those with insulin-treated type 2 diabetes of greater than 5 years duration had a much lower mean incidence of severe hypoglycaemia of 0.7 episodes per person per year with a prevalence of 25% (212).

One of the strengths of the UK Hypoglycaemia Study is the subdivision of subjects according to treatment modality and disease duration. The United Kingdom Prospective Diabetes Study (UKPDS) (213), which reported the prevalence of hypoglycaemia in different treatment groups of people with type 2 diabetes, is frequently cited in discussions of the frequency of hypoglycaemia in type 2 diabetes. A higher frequency of hypoglycaemia was associated with intensive compared to conventional treatment, with either sulphonylureas or insulin. With intensive insulin treatment, the prevalence of severe hypoglycaemia was 2.3%. One of this study's main strengths is its duration. However, although it subdivides subjects by treatment modality, the oral agents used (glibenclamide and chlorpropamide) are no longer in mainstream use in the UK and there is no stratification by disease duration. Furthermore, it lacks accurate incidence data because only the most severe episode of hypoglycaemia was documented at each four monthly review.

Similarly, hypoglycaemia data from the Diabetes Control and Complications Trial (DCCT) (3,206) are frequently cited in the context of type 1 diabetes. However, these figures must be interpreted with caution for a number of reasons. Firstly, this was an interventional trial so the figures are not a true reflection of the frequencies of hypoglycaemia observed in routine clinical practice. Secondly, patients at high risk of hypoglycaemia were excluded from the DCCT (207), which explains the lower incidence of severe hypoglycaemia observed (0.19 to 0.62 episodes/patient/year) (3). The risk of severe hypoglycaemia was higher in the intensively managed arm of the trial, which serves as a reminder that the ambient level of glycaemic control will affect hypoglycaemic risk. This fact further limits the generalisability

of the DCCT hypoglycaemia data to a contemporary patient cohort because of the anachronistic definition of intensive management in the DCCT. This trial shaped the way that diabetes is currently managed such that the level of glycaemic control that was formerly considered to be strict would now be viewed as desired management. The DCCT therefore does not give any indication of the risk of severe hypoglycaemia for unselected patients who are treated intensively by modern standards, and the same criticism could be applied to the UKPDS for data in type 2 diabetes.

More recently, two large randomised controlled trials were published in the same issue of the New England Journal of Medicine comparing modern-day intensive management with conventional management of blood glucose in type 2 diabetes. Although their reported frequencies of hypoglycaemia are not directly relevant to individuals with type 1 diabetes, these trials sparked extensive debate over the safety of tight glycaemic targets and their effect on overall mortality as well as on the frequency of hypoglycaemia. They therefore merit brief discussion here.

The ACCORD trial (Action to Control Cardiovascular Risk in Diabetes) (214) included intensive and conventional blood pressure, lipid and glycaemic control arms. The intensive glucose control arm, which aimed for an HbA1c <6%, was terminated early because of an increase in cardiovascular and all-cause mortality. In contrast, there was no increase in mortality in the intensive glycaemic control arm in the ADVANCE trial (Action in Diabetes and Vascular Disease: Preterax and Diamicron Modfied Release Controlled Evaluation) (215), which aimed for HbA1c ≤6.5%. Although both trials achieved a median HbA1c of 6.4%, baseline HbA1c values differed so that the intensive group in ACCORD had an absolute HbA1c reduction of 1.4% within 4 months, while participants in the intensive arm of ADVANCE had an absolute decrease in HbA1c of 0.6% at 12 months. Furthermore, the use of thiazolidinediones, alone or in combination with insulin, was much higher in ACCORD. The prevalence of severe hypoglycaemia was 3.1% in the intensive arm of ACCORD compared to 0.7% in the intensive arm of ADVANCE. Nineteen of the 41 excess deaths in ACCORD were attributed to "unexpected or presumed cardiovascular disease. It has been suggested that the excess mortality may have been precipitated by severe hypoglycaemia, although this cannot be confirmed.

Although the frequency of hypoglycaemia in these studies is much lower than that quoted in studies of unselected patients with type 1 diabetes, these patients with type 2 diabetes were older with more extensive comorbidities and they may therefore have been less able to tolerate hypoglycaemia. Consideration of the other potential contributors to the differences in mortality, such as the use of particular oral agents, is beyond the scope of this discussion. However in the wake of these studies, it seems sensible to avoid lowering HbA1c levels below 6% (with the exception of pregnancy) as this may increase mortality, possibly by increasing the risk of severe hypoglycaemia.

The true frequency of hypoglycaemia is difficult to estimate for the reasons discussed above. This is particularly true in type 2 diabetes where both the disease and treatment modalities are highly heterogeneous (63). However, even within type 1 diabetes there is extensive evidence that the risk of hypoglycaemia is not uniform. For example in a cross-sectional survey of 1076 consecutive adults with type 1 diabetes, the self-reported incidence of severe hypoglycaemia was estimated at 1.3 episodes per patient per year with a prevalence of 36.7% (204). However, 54% of all episodes of severe hypoglycaemia were accounted for by 5% of patients (204). Clearly, certain patient sub-groups are more vulnerable to frequent and debilitating hypoglycaemia. It is therefore useful to consider the factors which moderate risk of hypoglycaemia, particularly severe episodes requiring third party assistance.

1.8 Risk factors for hypoglycaemia

There are a number of established moderators of risk of hypoglycaemia, listed in table 1.3, which will be discussed briefly. Subsequently, more detailed consideration will be given to a recently proposed moderator of hypoglycaemia risk, serum ACE level, which is the subject of one of the studies in this thesis.

Table 1.3

Established risk factors for hypoglycaemia.

Moderator of hypoglycaemia	References
Low HbA1c, intensive glycaemic control	(3,206)
Impaired awareness of hypoglycaemia	(170,171,175)
Duration of diabetes	(3,173,205,212,216).
Antecedent hypoglycaemia	(11-15)
Hypoglycaemia Associated Autonomic Failure	(14,102,103)
Extremes of age	(3,216)
Negative C peptide levels	(3,173)
Sleep	(217)
Pregnancy	(218-220)

1.8.1 Intensive glycaemic control

Studies such as the DCCT demonstrate that low HbA1c and intensive treatment increase the risk of hypoglycaemia in type 1 diabetes (3,206). In the DCCT, 77% of the episodes of severe hypoglycaemia occurred in intensively treated subjects, with a recorded incidence of severe hypoglycaemia that was two to six times higher in the intensive compared to the conventional arm (221) .

1.8.2 Impaired awareness of hypoglycaemia

In a one-year prospective study, the frequency of hypoglycaemia in individuals with type 1 diabetes was compared between 29 patients with impaired awareness and 31 patients with normal awareness of hypoglycaemia, matched for age, duration of diabetes, age at onset of diabetes and level of glycaemic control (170). In the impaired awareness group, the incidence of severe hypoglycaemia was 2.8 episodes per person per year with a prevalence of 66%. In contrast, the incidence in the normal awareness group was only 0.5 episodes per person per year with a prevalence of 26%.

In a 6 month prospective study by a different group, the subjects with impaired awareness of hypoglycaemia had an incidence of severe hypoglycaemia of 2.6 episodes per patient per year while the group with normal awareness only experienced 0.87 episodes of severe hypoglycaemia per person per year (171). A retrospective survey of the prevalence of severe hypoglycaemia found that 90% of individuals with impaired awareness experienced an episode of severe hypoglycaemia the previous year, compared to just 18% or individuals with normal awareness (175).

1.8.3 Duration of diabetes

Impaired awareness of hypoglycaemia is associated with increased duration of diabetes, so it is not surprising that increased disease duration also increases hypoglycaemia risk (3,173,205,212,216).

1.8.4 Antecedent hypoglycaemia

As previously discussed (sections 1.4.3.2 and 1.6.3), one episode of severe hypoglycaemia can cause blunting of warning symptoms and therefore increase the likelihood of further episodes (11-15). This cycle of hypoglycaemia begetting hypoglycaemia is thought to underlie HAAF (14,102,103).

1.8.5 Age

The extremes of age are associated with an increased risk of hypoglycaemia (3,216), with under-reporting occurring frequently in both the very young and the very old. A number of

large prospective studies examining the frequency of severe hypoglycaemia in children have indicated an increased risk of hypoglycaemia in younger children (35,222-224).

In older patients, hypoglycaemia can produce neurological symptoms (160,225) which may masquerade as alternative conditions such as transient ischaemic attacks or vaso-vagal episodes (63), complicating the recognition of hypoglycaemia. In addition, the elderly report lower symptom scores than younger patients (133) with some studies suggesting that the diminished symptom response is a result of diminished autonomic activation, which is a feature of increasing age, independent of any effects of diabetes (132,226). In addition, the blood glucose thresholds for symptom generation and cognitive dysfunction occur almost simultaneously in the elderly (134), which limits the time available for self-treatment before progression to severe hypoglycaemia. Thus, an altered symptom profile, lower symptom intensity and altered glycaemic thresholds combine to increase the risk of progression to severe hypoglycaemia in the elderly.

1.8.6 C peptide levels

Individuals who are C peptide negative have no endogenous insulin secretion and this confers a two to four times increased risk of severe hypoglycaemia (3,173). For this reason, severe hypoglycaemia is uncommon within the first year of type 1 diabetes (227).

1.8.7 Sleep

Nocturnal hypoglycaemia is common (217), with 55% of severe hypoglycaemic episodes occurring while individuals were asleep (221). This is because during sleep, both counterregulatory and symptoms are diminished (143).

1.8.8 Pregnancy.

During pregnancy, the peak incidence of hypoglycaemia occurs during the first and second trimesters . In one study, 84% of severe hypoglycaemic episodes resulting in loss of consciousness occurred within the first 20 weeks of pregnancy (220). In a large study comparing the incidence of severe hypoglycaemia in different stages of pregnancy, there

were almost 2.5 times as many episodes in the first trimester as compared to the third (218,219).

1.8.9 Serum ACE levels

More recently, a number of studies have examined serum angiotensin-converting enzyme (ACE) as a potential marker for risk of severe hypoglycaemia. Although three recent papers from Scandinavia suggest that increased serum ACE levels are associated with an increased risk of hypoglycaemia, this has not been validated in a non-Scandinavian population and is therefore an area that requires exploration. The first study in this thesis examines the potential role of serum ACE as a marker of risk of severe hypoglycaemia and the background to this study will now be discussed.

The controversy surrounding ACE dates back to the early 1990s when case reports suggested improvements in insulin sensitivity in patients on ACE inhibitors (228,229). Studies formally examining the relationship between ACE inhibitors and insulin sensitivity produced mixed results, with some suggesting an increase in sensitivity (230) while others did not (231). Further observational studies have examined the relationship between ACE and hypoglycaemia. A nested case control study employing the Dutch PHARMO database included 300,000 residents of 6 Dutch cities and examined hospital admissions and drug dispensing for this group between 1986 and 1992 (232). There were 94 patients with diabetes admitted to hospitals with hypoglycaemia over this period. Following adjustment for potential confounders and after 654 controls were assigned, ACE inhibitors were the only drugs found to be associated with an increased risk of hypoglycaemia with an odds ratio of 2.8 (95% confidence interval 1.4-5.7). However, it should be noted that this study was a retrospective analysis and as such it can only demonstrate an association, not causation. In addition, the study did not adjust for renal impairment, which is both a risk for hypoglycaemia and an indication for ACE inhibition in patients with diabetes. There were also problems with the matching of cases and controls and factors such as duration of treatment were poorly matched. Control patients were selected from amongst the diabetic population admitted to hospital for other reasons, but the fact that many of these admissions may have related to hyperglycaemic emergencies such as diabetic ketoacidosis would suggest that this group may be been inherently less likely to experience hypoglycaemia. Finally,

most cases of severe hypoglycaemia do not require hospital admission so this study only examines a subset of those with significant hypoglycaemia.

A more recent nested case control study in Scotland examined the same question using the DARTS database (233). They matched 440 controls for sex and age to 64 patients admitted to hospital with severe hypoglycaemia. This study also found an association between ACE inhibition and severe hypoglycaemia with an odds ratio of 3.2 (95% confidence interval 1.2-8.3, p=0.023). Although there were differences between cases and controls in terms of diabetes duration, treatment type and comorbidities such as the presence of congestive cardiac failure, adjusting for these potential confounders only strengthened the observed association with an adjusted odds ratio of 4.3 (95% confidence interval 1.2-16.0). However, only 7 out of the 64 patients admitted with severe hypoglycaemia were taking ACE inhibitors, which probably accounts for the wide confidence intervals reported. Creatinine measurements were only available for 49% of patients and after adjusting for creatinine, the odds ratio linking ACE inhibitors with severe hypoglycaemia was no longer statistically significant.

Other studies have not supported the proposal that ACE inhibitor drugs are associated with hypoglycaemia. For example, the EUCLID study was a randomised controlled trial comparing lisinopril with placebo on albumin excretion in 530 individuals with type 1 diabetes (234). No difference in hypoglycaemia was observed in the treatment and control arms, although it should be noted that clinical trials often select patients at low risk of ill-health or complications and they are not the optimal way to seek unintended effects of a drug.

More recently, a new avenue of research opened up with studies exploring the role of serum ACE in exercise physiology. The ACE gene is located in chromosome 17q23. Individual variation in serum ACE levels is partly mediated by a gene polymorphism via I (insertion) and D (deletion) alleles. The insertion polymorphism consists of a 287 base pair Alu element in intron 16, with an insertion frequency of 50% in European populations. The II genotype is associated with low serum ACE activity while the DD genotype is associated with high serum ACE activity (235,236). Studies in elite athletes demonstrated an association between low serum ACE and the II genotype with enhanced athletic performance in events requiring stamina (237-240). Further studies examined body composition in 123 army recruits before

and after a 10 week training programme. When participants with the II genotype were compared to participants with ID or DD genotypes, the former group displayed a greater anabolic response in both fat and non-fat mass with relative sparing of fat stores during structured physical training (241). This suggests that ACE may have effects on energy balance and on the efficiency with which oxidative fuel is used for metabolism.

On the basis of these studies, it has been postulated that a lower ACE activity confers a greater ability to function effectively during periods of metabolic substrate deprivation. If this theory was extrapolated to the arena of hypoglycaemia, it could be hypothesised that those with high ACE activity and the DD genotype might have a more limited functional capacity when challenged by glucose deficiency. In people with type 1 diabetes with high ACE activity, this may be manifest by greater cognitive impairment during hypoglycaemia than in those with low ACE activity. This might explain the variable risk of developing SH within a population with type 1 diabetes. Those who are unable to maintain reasonable cognitive function during mild to moderate hypoglycaemia will be less likely to treat low blood glucose in a timely fashion which in turn makes it more likely that third party assistance will be required. Interestingly, if this hypothesis were correct, it would point to the potential to use ACE inhibitors to lower ACE levels and reduce the frequency of severe hypoglycaemia, despite the earlier concerns that ACE inhibitors might increase the risk of hypoglycaemia.

Two Danish studies in adults and one Swedish study in children and adolescents, all with type 1 diabetes, have examined the relationship between serum ACE and risk of severe hypoglycaemia. In a retrospective study of 207 consecutive adults with type 1 diabetes, participants were asked to record the number of episodes of mild hypoglycaemia experienced in the preceding week and the number of episodes of severe hypoglycaemia experienced each year for the last 2 years (173). Individuals treated with ACE inhibitors or angiotensin 2 receptor blockers (ARBs) were excluded. Awareness of hypoglycaemia was graded on a 4 point visual analogue scale and blood was sampled for C peptide, HbA1c, serum ACE and ACE genotype. Patients with the DD genotype had a relative risk of severe hypoglycaemia of 3.2 (95% confidence interval 1.4-7.4) compared to the II group (173). Other significant determinants of severe hypoglycaemia risk were impaired awareness of hypoglycaemia, degree of residual beta cell function as reflected by C peptide levels and duration of diabetes

above 20 years. The same group followed this study with a one year observational prospective study of 171 adults with type 1 diabetes, which demonstrated a relative risk of severe hypoglycaemia of 2.9 in individuals in the top quartile of ACE activity when compared to those in the bottom quartile (210).

A Swedish group examined this relationship in children and adolescents. In this prospective study of 86 patients aged 7-19 years old (median 12.8), incidence and prevalence of severe hypoglycaemia were calculated and serum ACE was measured. The incidence of severe hypoglycaemia in those with serum ACE levels below the median was 0.5 episodes per patient per year compared to 3 episodes per patient per year in those with ACE levels above the median ($p=0.008$) (242). The high ACE group had a prevalence of severe hypoglycaemia of 61% compared to a prevalence of 40% in the low ACE group ($p=0.053$) (242).

Although these results appear to demonstrate a strong association between serum ACE and risk of severe hypoglycaemia, these results have not been validated in a non-Scandinavian population. It is possible that the relationship between serum ACE levels and severe hypoglycaemia may differ between ethnically different groups of people with diabetes. There are other potential difficulties in interpreting the Danish data. For example, previous work has suggested that the predictive value of serum ACE is strongest in patients whose defence against SH is compromised, such as those with impaired hypoglycaemia awareness (173). The Danish method of assessing hypoglycaemia awareness probably over-estimates the prevalence of impaired awareness of hypoglycaemia as being 60% (21) compared to a prevalence of around 25% in other population studies (168) and this may hamper comparison of their study population with other cohorts of patients with diabetes. It is therefore still premature to add serum ACE to the list of conventional moderators of hypoglycaemia risk such as impaired awareness of hypoglycaemia, duration of diabetes and antecedent severe hypoglycaemia. This association should be validated in a non-Scandinavian population using a method of assessment of hypoglycaemia awareness which correlates well with other validated methods (174). This forms the basis of the first original study in this thesis and is discussed in chapters 3 and 4.

1.9 Summary on the clinical and physiological effects of hypoglycaemia

Hypoglycaemia is a common and much-feared iatrogenic complication of diabetes therapy. There is a lack of consensus over a precise definition of mild hypoglycaemia but agreement that severe hypoglycaemia constitutes any episode requiring third party assistance. Several factors, such as disease duration and the presence of impaired awareness of hypoglycaemia, are known to increase the risk of hypoglycaemic episodes. The recent emergence of serum ACE as an additional putative factor requires further exploration and this forms the basis of the first study in this thesis, which is discussed in chapters 3 and 4.

Although we have a reasonably good understanding of the physiology of hormonal counterregulation, our understanding of brain glucose sensing remains incomplete. Similarly, although the symptoms of diabetes have been thoroughly classified and grouped into autonomic, neuroglycopenic and general malaise categories, we have next to no knowledge of the extent of intra-individual variation in hypoglycaemic symptoms. The second study in this thesis, discussed in chapters 3 and 5, explores the within-subject variability of hypoglycaemic symptoms.

CHAPTER 2: EFFECTS OF HYPOGLYCAEMIA ON COGNITIVE FUNCTION

2.1 Testing cognitive function

The symptoms of hypoglycaemia include some, such as confusion and difficulty with speech, which indicate an effect on cognitive function. Any decrement in cognitive function can affect an individual's ability to self-treat an episode of hypoglycaemia and the greater the degree of neuroglycopenia, the greater the risk that severe hypoglycaemia will ensue. The effects of hypoglycaemia on cognition have therefore been studied in an attempt to characterise the cognitive domains affected by hypoglycaemia and the glycaemic thresholds at which cognitive function is disrupted.

2.1.1 Methodological issues

Comparison of different studies is hampered by methodological and statistical inconsistencies, the effects of which have been reviewed previously (243,244). The inaccuracies inherent in the method of inducing and measuring hypoglycaemia have been discussed in chapter 1. Inconsistencies in the method of cognitive function testing will now be considered.

Some issues, such as variability in performance by study volunteers, must be considered regardless of the study methodology. Hypoglycaemic clamp studies suggest that *intra*-individual variability is not a major issue, with diabetic individuals exhibiting reasonably reproducible glycaemic thresholds for impaired performance on psychomotor tests when re-assessed after a 4-6 week interval (245). However, a significant degree of *inter*-individual variation exists with respect to performance on cognitive tasks (243,245,246). It is therefore important to ensure that studies are adequately powered to minimise the possibility of the performance of one or two individuals skewing the overall results. Practice effects must also be considered as performance on most tests improves with familiarity (245,247). A robust study design should therefore allow sufficient practice sessions on the day of testing such that a stable plateau in performance has been achieved at baseline and subsequent testing during

hypoglycaemia is assessing the effects of low blood glucose without practice effects confounding the results.

Some methodological problems can be minimised by attention to subject selection and study design. Previous studies have suggested that factors such as higher IQ (246) and male gender (248) can be associated with a greater decrement in cognitive function. Studies should therefore ensure an equal gender spread and always include a euglycaemic control arm, with participants being blinded to the experimental condition (hypoglycaemia versus euglycaemia).

The order in which subjects undergo each arm of the study should be randomised and counterbalanced. In other words, half the participants should undergo the euglycaemic arm while the other half undergo hypoglycaemia on their first study session. This study design allows each participant to act as their own control. This minimises the effect of potentially confounding effects such as boredom and tiredness as it can be assumed that each individual will become fatigued at around the same point during each experimental condition. There should be an opportunity to practise the tests first to avoid practice effects and tests should be administered in the same order on both study sessions. In addition, the choice of cognitive test is also important.

2.1.2 Choice of cognitive test

Some researchers favour testing individual cognitive domains while others prefer the use of a broad battery of tests to assess cognitive function as a whole. When a comprehensive test battery is employed, researchers sometimes present their findings as z scores (also known as a standard score). This score indicates how many standard deviations an observation is above or below the mean. The score is obtained by subtracting the population mean from an individual score and dividing the difference by the population standard deviation. The unit of the z score is therefore the standard deviation, which allows scores on different tests to be standardised and amalgamated into a composite score.

Clearly, if a battery of tests is employed, it is important to report results of individual tests rather than a z score alone as performance on one test unaffected by hypoglycaemia could mask the decrement in a different test score when results are combined. For example, in one

study by Mitrakou and colleagues (61), a battery of cognitive tests was applied repeatedly during a stepped glucose clamp. The overall Z score implied that hypoglycaemia affected performance at a glucose threshold of 2.4 mmol/l. However, the published raw scores suggest that some of the tests were affected at much higher blood glucose levels (choice reaction time affected at a threshold of approximately 3.7 mmol/l; Stroop test affected at an approximate threshold of 3.1 mmol/l).

The number of tests employed is also a relevant consideration. It could be argued that increasing the number of tests will increase the sensitivity of the test battery but it will also increase the chance that boredom and fatigue may affect performance. Furthermore, increasing the number of tests also increases the risk of introducing a type 1 statistical error. In other words, the larger the number of tests employed in any given study, the greater the likelihood of performance on one test being affected simply by chance.

One of the criticisms levelled against cognitive function tests is that they assess isolated domains in an abstract fashion and may not represent cognitive function with respect to everyday activities. For example, the p300 brain evoked potential is taken to be a sensitive indicator of cognitive dysfunction (249) but there is no intuitive way to correlate changes in p300 potentials to the ability to perform daily tasks. By contrast, one of the tests frequently used in clamp studies is the choice reaction time test (182,250-253), which is simple and quick to administer and reflects skills used in daily life such as psychomotor function and reaction time.

Tests which are specifically designed to reflect "real-life" situations are described as having "ecological validity" (254). For example, the prospective memory test designed by Titov and Knight (255) and adapted by Warren and colleagues (256) requires subjects to memorise a list of tasks that have to be conducted at specific establishments on a shopping thoroughfare. Later, they are asked to recall the list of tasks while viewing a video of the same shopping street. Compared to traditional memory tests which involved rote learning of lists, this is probably a more accurate reflection of the use of memory in routine life, where circumstances and surroundings can act as prompts and reminders and other cognitive processes, such as planning and vigilance, become relevant to the task in hand. Perhaps the best example of an ecologically valid test is the driving simulator, which will be discussed later.

2.2 Effects of hypoglycaemia on cognitive function

Nowithstanding the methodological challenges involved in cognitive function testing during hypoglycaemia, there is a broad literature in this area which has attempted to answer a number of pertinent questions. For example, which cognitive domains are affected by hypoglycaemia? Are they all equally affected? At what blood glucose thresholds are different cognitive functions impaired? How long does it take for cognitive function to recover following hypoglycaemia? Can the brain adapt to recurrent hypoglycaemia? How can results of laboratory studies be extrapolated to routine activities such as driving? These questions will be considered in turn.

2.2.1 Cognitive domains affected by hypoglycaemia

Specific aspects of cognitive function are essential for various everyday tasks including work and leisure activities. It has been demonstrated that acute hypoglycaemia affects multiple aspects of cognitive function including simple auditory processing (257,258), attention (259), concentration (260), visual information processing (261,262), multiple aspects of memory (256,263,264), higher level cognitive function (265),psychomotor function (174) and spatial awareness (266). Unpublished data from our group also suggests that language processing is affected by hypoglycaemia (personal communication, Dr Kate Allen). Table 2.1 summarises the effects of hypoglycaemia on various domains of cognitive function, with test scores given for hypoglycaemia and euglycaemia. Studies in this table are limited to those published by our group. This is because methodological differences (such as the use of whole blood versus plasma, discussed in section 2.1.1) hamper comparison between studies and our group has employed consistent methodology throughout all the studies cited here. The extent of the effect of hypoglycaemia on each cognitive domain (effect size) is given by Cohen's d, which is the difference between two means divided by the standard deviation of the data. Comparison of the mean scores during hypoglycaemia and euglycaemia in this way conveys the extent of the effect of hypoglycaemia in a more meaningful way than the simple reporting of a p value. The greater the Cohen's d value, the greater the effect of hypoglycaemia on the domain being tested. The CRT and TMB tests have negative Cohen's d values because the results are completion times where a better (quicker) result is represented by a lower number. For these tests, the lower the Cohen's d, the greater the effect of hypoglycaemia.

Table 2.1

Summary of mean (SD) test scores during hypoglycaemia and euglycaemia from previous studies. Where multiple studies have employed the same cognitive test, weighted means are given (*). Effect sizes are given as Cohen's d. TMB = Trail-Making B; DSST = Digit Symbol Substitution Test, 4CRT = Four choice reaction time, TBAC = Test of Basic Auditory Capabilities

Test		N	Domain tested	EU	HYPO	Cohen's d
4 term order (267)		32	Memory: working memory	12.4 (4.3)	3.7 (2.3)	2.52
Validation span (264)		16		20.7 (2.3)	14.9 (2.2)	2.58
Letter-Number Sequencing (264)		16		11.8 (1.9)	9.7 (1.6)	1.20
Logical memory (264)	Score	16	Memory: delayed memory	13.6 (2.1)	6.1 (3.7)	2.49
	% retained			78.4 (6.0)	47.0 (21.3)	2.01
Map search* (259,268)		36	Attention: visual selective attention	72.8 (5.6)	69.1 (9.7)	0.47
Visual elevator timing score* (259,268)		36	Attention: attentional switching	3.0 (0.6)	3.5 (0.7)	0.95
Auditory elevator with distraction* (259,268)		36	Attention: auditory selective attention	9.1 (1.4)	7.6 (2.0)	0.87
TMB* (secs) (250,257,258,262,264-267)		155	Attention, visual search, motor function, mental flexibility	35.3 (10.4)	46.8 (14.4)	-0.92
DSST* (250,257,258,262,264-267)		155	Sustained attention, speed of response, visual scanning	72.7 (12.6)	61.8 (13.6)	0.83
4CRT* (ms) (250,251)		56	Response time, speed of decision-making	608 (52)	655 (53)	-0.90
Raven's advanced progressive matrices (265)		16	Higher level cognitive skills: abstract problem solving	18.9 (3.1)	16.5 (3.5)	0.73
TBAC loudness* (257,258)		35	Auditory: single tone loudness	63.7 (5.3)	60.8 (6.3)	0.50

While it is possible to document all the individual areas of cognitive function affected by hypoglycaemia, it is perhaps more useful to draw some general conclusions from published studies. In laboratory studies, controlled mild hypoglycaemia causes rapidly reversible cognitive dysfunction, both in diabetic and non-diabetic humans, with no deterioration in level of consciousness (144). Although the laboratory studies confirming these findings have been undertaken only in the last 20 years, this fact was apparent to observers long before researchers began formally studying hypoglycaemia (2). Simple motor and cognitive tasks tend to be relatively well preserved during moderate hypoglycaemia in comparison to more complex, attention demanding tasks (144), such as those involving working memory (267). Speed is often sacrificed in order to preserve accuracy during hypoglycaemia, as demonstrated in studies where subjects achieved the same proportion of correct answers but completed fewer Stroop tasks and mathematical calculations during hypoglycaemia (269,270).

To date, studies have tended to assess specific and discrete areas of cognitive function. However, it is known that all mental tests show a universal positive covariation such that performance during one task can predict performance during another (271). Spearman noted a positive correlation in children's test scores across apparently unrelated subjects and proposed the presence of an underlying dominant general intelligence factor, termed "*g*" (271,272). It is unknown whether hypoglycaemia causes a deterioration in mental performance by affecting this general factor shared by all tests or through specific effects on different aspects of cognitive function.

2.2.2 Thresholds for cognitive dysfunction

Various studies have employed a "stepped clamp" methodology to lower blood glucose in defined decrements and establish the thresholds at which various changes occur. These studies have examined the effects of hypoglycaemia on cognitive function in diabetic and non-diabetic individuals and found that the glycaemic threshold for developing cognitive dysfunction ranges between a blood glucose of 2.3 and 3.3 mmol/l (61,62,112,252,253,273-276). The thresholds for different cognitive tests are summarised in table 2.2. As discussed in chapter 1, in people who have normal hypoglycaemia awareness, symptom generation and counterregulatory hormone release generally occur at higher blood glucose levels than the development

of neuroglycopenia (60,61,112). These thresholds are not fixed and in the subsequent sections on recovery of cognitive function and cerebral adaptation to hypoglycaemia, it is discussed how these thresholds can be modified.

Table 2.2

Blood glucose thresholds for deterioration of performance in cognitive function tests during acute hypoglycaemia. All studies in this table employed a "stepped clamp"

Test (reference)	Blood glucose(mmol/l)	Subjects
Four choice reaction time (253)	3.3	Type 1 diabetes with normal awareness
Stroop test (179)	3.3	Healthy volunteers
Four choice reaction time (252)	3.2	Type 1 diabetes with normal awareness and healthy volunteers
Four choice reaction time (253)	3.1	Healthy volunteers
Four choice reaction time (252)	3.0	Type 1 diabetes and impaired awareness
Four choice reaction time (182)	2.8	Type 1 diabetes
Z score for cognitive battery	2.8 (62)	Type 1 diabetes and impaired awareness: hypoglycaemia avoided 3 months- 1 year
	2.7 (62)	Healthy volunteers
	2.6 (273)	Healthy volunteers
	2.6 (62)	Type 1 diabetes and impaired awareness: hypoglycaemia avoided for 2 weeks
	2.4 (62)	Type 1 diabetes and impaired awareness
	2.3 (61,274)	Healthy volunteers
Cognitive battery of 12 tests (112)	2.9 (7 out of 12 tests*)	Healthy volunteers
	2.5 (all tests*)	Healthy volunteers
Immediate recall (274)	2.5	Healthy volunteers
Late recall (274)	2.5	Healthy volunteers

* Test battery consisted of trail-making A and B, verbal fluency, Stroop test (interference subtest, word and colour subtest), simple and choice reaction time, digit vigilance test, verbal memory test and forward and backward digit span. Details not given as to which of these tests deteriorated at the higher blood glucose level.

2.2.3 Recovery of cognitive function

Recovery of cognitive function following hypoglycaemia has only been partially explored. Anecdotal accounts from individuals with diabetes suggest that the recovery of cognitive function can lag behind the restoration of biochemical euglycaemia. Some of the early studies on hypoglycaemia and cognitive function also supported this suggestion (277) although these findings were not universally replicated (278). However, this early work is limited by methodological constraints, such as the use of an insulin infusion rather than a glucose clamp technique to induce hypoglycaemia (278) or the use of tests such as simple reaction time (277,279,280) and finger tapping (278), which are not consistently affected by hypoglycaemia.

More recently, further attempts have been made to quantify the time taken for cognitive recovery but these studies have also had limitations. For example, in some studies, non-diabetic volunteers were recruited rather than subjects with type 1 diabetes (279,281,282), or a small sample of subjects was studied (282), or the study lacked a euglycaemic control arm in its design (281,283) or neurophysiological measurements were used rather than direct tests of cognitive function (279-281,284). Furthermore, precise measurement of the time taken for recovery requires repeated testing, but many studies have restricted cognitive testing to just one or two time points (282-285). Finally, the interval between the restoration of euglycaemia and the testing of cognitive function has not been clearly defined in several studies and it is therefore difficult to be certain whether the timing of cognitive testing between different participants was consistent (279,280,283,284).

Some of the limitations with earlier studies may relate to the fact that several were not specifically designed to assess recovery. For example, the study by Herold *et al* was designed to study cognitive function *during* hypoglycaemia rather than in the recovery phase. In four of the subjects, reaction time was still prolonged at the end of the study after 40 minutes of euglycaemia but no further measurements were made (277). The choice of cognitive tests was also flawed as testing was restricted to measurements of simple reaction time in response to a red light visual stimulus; a test which is now known not to be consistently affected by hypoglycaemia (277).

Some studies did set out specifically to examine recovery time. Evans *et al* (282) clamped 8 healthy volunteers and assessed cognitive function serially with 4 choice reaction time (4CRT), Stroop word and colour word tests and Trail Making B (TMB). The 4CRT test remained impaired up to 20 minutes after restoration of euglycaemia and TMB did not show consistent deterioration during hypoglycaemia so recovery could not be assessed. Stroop tests showed no impairment at 20 minutes after euglycaemia was restored. However, recovery was only assessed immediately after restoration of euglycaemia and just once more after 20 minutes of euglycaemia. It is therefore impossible to ascertain whether there was a brief lag in the recovery of the Stroop tests or whether 4CRT remained impaired beyond 20 minutes. Similarly, a more recent study in subjects with type 2 diabetes assessed hormonal, symptomatic and cognitive responses (reaction time) to 30 minutes of hypoglycaemia and found a prolonged reaction time 30 minutes after euglycaemia was restored (286). However, as no subsequent measurements were taken it is not possible to conclude how long it took for recovery to be complete.

Investigators in Sweden also looked specifically at restoration of cognitive function, in non-diabetic men alone (281) and in diabetic versus non-diabetic men (284). However, these investigators employed purely neuropsychological tests (EEGs, P300 latency and somatosensory evoked potentials in the median nerve) and the ecological relevance of these measurements is unclear. Furthermore, these investigators employed a 0.9% sodium chloride infusion for the control arm whereas it would have been more appropriate to use a euglycaemic clamp. Finally, the diabetic subjects also underwent a longer period of hypoglycaemia than the control subjects.

Similarly, Blackman *et al* clamped healthy volunteers (279) and people with poorly controlled type 1 diabetes (280) in two separate studies and measured simple reaction time, which is not reliably affected by hypoglycaemia, and P300 latencies, which are considered to be an electrophysiological marker of decision-making processes but do not clearly relate to the ability to perform routine daily tasks. Like the Swedish investigators, Blackman and colleagues found that there was a delay in normalisation

of P300 potentials ranging from 45 to 75 minutes after restoration of euglycaemia but the timing of the recovery period was not clearly defined.

A previous study from our centre comparing the effects of hypoglycaemia on cognitive function in 20 people with type 1 diabetes and either normal or impaired awareness of hypoglycaemia applied a cognitive battery of 20 minutes duration during hypoglycaemia and then during the recovery period at a single time point 10 minutes after euglycaemia was restored (285). Performance during the recovery period remained impaired on the trail-making B and rapid visual information processing tests, although there was no persistent impairment in the paced auditory serial addition and digit symbol substitution (DSST) tests (285). However, as no further testing was conducted after 10minutes into the recovery period, this study could not fully define the duration of cognitive impairment in the recovery period after hypoglycaemia.

So far, all of the studies discussed have examined immediate recovery. Some investigators have examined longer term recovery after hypoglycaemia but have not found strong evidence of persistent cognitive impairment. For example, one hour of nocturnal hypoglycaemia (2.3-2.7mmol/l) does not affect cerebral function the following morning in subjects with type 1 diabetes (287,288).

Most studies assessing the recovery period have examined mild hypoglycaemia but one study from our centre examined cognitive function and mood prospectively in 20 people with insulin-treated diabetes who had recently experienced severe hypoglycaemia (SH) and 20 matched controls with insulin-treated diabetes and no recent episodes of SH (289). One subject in each group had type 2 diabetes and the others had type 1 diabetes. An extensive cognitive battery was administered 1.5, 9 and 30 days after SH but recovery was already complete at the first time point so it is not possible to conclude precisely when cognition returned to normal after SH, or even to conclude whether or not there was any lag in recovery of cognition (289).

Thus, it is apparent that there is a delay in the recovery of cognitive function following hypoglycaemia but this has not been well defined and there is no evidence of any long-term impairment.

2.2.4 Cerebral adaptation to hypoglycaemia

It has been demonstrated previously that glycaemic thresholds for cognitive dysfunction differ depending on the state of hypoglycaemia awareness. A study that compared the thresholds for the onset of cognitive impairment during hypoglycaemia in non-diabetic subjects and in people with type 1 diabetes who had either normal or impaired awareness of hypoglycaemia found that those with impaired awareness had a threshold for cognitive impairment of (mean±SE) 2.39±0.07 mmol/l compared to thresholds of 2.69±0.06 mmol/l and 2.65±0.06 mmol/l in those with normal awareness and non-diabetic control subjects respectively (78). The thresholds for neuroglycopenic symptoms and for counterregulatory hormone secretion were also set at lower blood glucose levels in the impaired awareness group compared to the other two groups. (78). Clamp studies by a different group also confirmed that cognitive dysfunction is milder and begins at lower blood glucose levels in those with impaired awareness of hypoglycaemia compared to non-diabetic individuals (62,274).

Previous exposure to hypoglycaemia in individuals with type 1 diabetes and normal awareness can also cause the thresholds for cognitive dysfunction to shift to lower blood glucose levels, regardless of whether the antecedent hypoglycaemic episodes occur by day (12,290) or night (11,291). Neurophysiological changes, such as alterations in p300 event-related potentials, are also shifted to lower blood glucose levels by antecedent hypoglycaemia (249). Glucose clamp studies in non-diabetic individuals have shown that 90-150 minutes of hypoglycaemia the day before cognitive testing attenuates the deterioration in short term memory, reaction time and auditory-evoked brain potentials (292-294), although performance on some parameters such as DSST and some elements of event-evoked brain potentials did not show evidence of adaptation (293,294). In a small study of adults with type 1 diabetes, twice weekly episodes of experimentally-induced hypoglycaemia over one

month resulted in preservation of cognitive function across a range of cognitive tasks (12).

Prolonged hypoglycaemia has also been shown to affect thresholds for cognitive dysfunction in a series of studies by Boyle and colleagues. Non-diabetic volunteers underwent cognitive testing during two hypoglycaemic clamps, separated by a 56h period of controlled hypoglycaemia where mean blood glucose levels were maintained at 2.9 mmol/l, including during post-prandial periods. Following prolonged hypoglycaemia, the glucose level at which performance on the Stroop test deteriorated shifted from 3.3 mmol/l to 2.5 mmol/l (179). Details of functional cerebral changes in response to hypoglycaemia give insights into possible mechanisms for these observed alterations in the thresholds for cognitive dysfunction. Total brain glucose utilisation can be estimated from measurements of cerebral blood flow and blood glucose concentrations in jugular venous and arterial blood. In this study by Boyle and colleagues, brain glucose uptake was augmented following prolonged hypoglycaemia (179).

In a different study by the same research group, brain glucose uptake was observed to fall during hypoglycaemia by around 20% in healthy volunteers and individuals with poorly-controlled diabetes whereas it was preserved in those with strict glycaemic control (17), raising the possibility of adaptation to repeated exposure to hypoglycaemia. Although this study cannot confirm that increased exposure to antecedent hypoglycaemia was the mechanism of preserved brain glucose metabolism, it is certainly a plausible explanation.

Neuroimaging studies also demonstrate relevant alterations in regional cerebral blood flow, with increased perfusion of the frontal cortex and decreased blood flow to caudal regions observed during hypoglycaemia (295). These changes become permanent in those with a history of severe hypoglycaemia (180) or impaired awareness of hypoglycaemia (296). Neuropathological studies suggest that the brain's sensitivity to hypoglycaemia decreases in a rostro-caudal direction, (297) so

preservation of cerebral blood glucose delivery to more sensitive anterior areas of the brain can be viewed as an adaptive response to hypoglycaemia.

It is unclear whether adaptation of cerebral function can occur acutely. In one study by Kerr and colleagues, symptoms and choice reaction time values reverted towards baseline by the end of a 2hour period of clamped hypoglycaemia, although this study was limited by its lack of a euglycaemia control arm (298). The apparent improvement could therefore have been caused by a practice effect. A glucose clamp study by Gold and colleagues did not report any difference in performance on a battery of tests at the start and end of an hour of clamped hypoglycaemia (299), although it is possible that an improvement in performance may have been detected if the period of hypoglycaemia had lasted longer as in the study by Kerr. A single episode of antecedent hyperglycaemia does not affect physiological responses to subsequent hypoglycaemia (300).

It has been postulated that repeated episodes of hypoglycaemia in those with impaired awareness may lead to a degree of cerebral adaptation by allowing the body to "acclimatise" to low blood glucose, with counterregulation, symptoms and cognitive impairment being initiated at successively lower blood glucose levels. This hypothesis is partly suggested by the fact that individuals with impaired awareness have an increased incidence of both asymptomatic (170,172) and severe hypoglycaemia compared to those with normal awareness (170,171).

It may also seem that people with impaired awareness are in some way protected from hypoglycaemia in that they can function cognitively at blood glucose levels that would affect people with normal awareness of hypoglycaemia. However, several studies have demonstrated that the alteration of symptom thresholds can result in the development of significant neuroglycopenia before autonomic symptoms develop (78,301-303). For example, in a clamp study of 19 individuals with type 1 diabetes, in those with stricter glycaemic control and diminished awareness of hypoglycaemia, the blood glucose level associated with impaired four choice reaction time was

0.5mmol/l lower than the corresponding threshold in individuals with poorer glycaemic control, better hypoglycaemia awareness and a presumed lower exposure to repeated hypoglycaemia. In contrast, the blood glucose level at which autonomic and neuroglycopenic symptoms were initiated and epinephrine (adrenaline) was released was 0.6-0.8 mmol/l lower in those with better glycaemic control and poorer hypoglycaemia awareness (304). Therefore, although all thresholds shift in response to hypoglycaemia, it appears that that symptom and counterregulatory thresholds shift to a greater extent than the thresholds for cognitive dysfunction. Any protective effects on cognitive function are insufficient to compensate for the fact that changes in symptom thresholds result in a much narrower window of opportunity to treat clinically apparent hypoglycaemia before severe neuroglycopenia develops. In fact, on occasions, neuroglycopenia can ensue before symptoms and counterregulation are fully generated. Far from being protective, these threshold shifts should be viewed as maladaptive, given that they increase the risk of developing severe hypoglycaemia. It is therefore reassuring that avoidance of hypoglycaemia can restore symptoms (183) and glucose thresholds for cognitive dysfunction to higher levels in individuals with type 1 diabetes (62,274) and in non-diabetic individuals following treatment of an insulinoma (273).

Despite the large body of literature supporting the concept of adaptation to repeated episodes of hypoglycaemia, this finding is not universal. For example, one study found no change in the threshold for cognitive dysfunction (4 choice reaction time test) after avoidance of hypoglycaemia for 4 months, despite the fact that the thresholds for hormone and symptom responses did shift to higher blood glucose levels (182). In two studies, one hour of nocturnal hypoglycaemia (2.3-2.7mmol/l) did not affect cognitive function the following morning in subjects with type 1 diabetes, despite the fact that mood, well-being and the ability to cope with subsequent exercise were adversely affected (287,288).

A different clamp study has also shown that although individuals with type 1 diabetes and impaired awareness experience symptoms of hypoglycaemia at blood glucose levels of 2.3 mmol/l while those with normal awareness become symptomatic at

blood glucose levels of 3.0 mmol/l, both groups experience a deterioration in choice reaction time at similar blood glucose levels (3.2 mmol/l) (252).

In another clamp study, individuals with type 1 diabetes were grouped together on the basis of either good (HbA1c 8% ±0.2%, n=8) or poor (HbA1c 11.8% ±0.4%, n=9) glycaemic control and assessed with a small battery of cognitive tests. There was no significant difference in performance between the two groups despite the fact that the well-controlled group developed counterregulatory changes at a lower blood glucose level than those with poor glycaemic control (275). However, this study could be criticised for its small size as there were only 8-9 individuals in each group.

Finally, in a study of 20 men and 22 women with insulin-treated diabetes, the effects of glycaemic control and gender on cognitive function during hypoglycaemia (2.2 mmol/l) were examined. HbA1c values ranged from 5.8% to 18%. The observed cognitive impairment was not correlated to level of glycaemic control (248). However, no information was given on the state of awareness of the individuals with good glycaemic control and it is possible that preservation of cognitive function might have been observed if a group with well-categorised impaired awareness of hypoglycaemia had been examined.

Thus, no consensus exists as to whether either impaired awareness of hypoglycaemia or recurrent antecedent hypoglycaemia are associated with the relative preservation of cognitive function (11,62,78,273,274,290,292,304) or an exacerbation of the decrement in cognitive performance associated with hypoglycaemia (182,252,275,285,287,288). Although different studies have produced apparently discrepant results, several explanations can be offered for this. For example, inter-individual variation in the degree to which hypoglycaemia affects cognitive function is very wide (243) and this may explain why cognitive function appears preserved in those with impaired awareness in some studies and not in others. Furthermore, impaired awareness is not an all or none phenomenon so subjects in different studies may have differed in the extent to which awareness was impaired. Different studies have also employed different tests and it is accepted that not all cognitive functions are equally affected by hypoglycaemia. Different tests will assess different parts of

the brain and it is possible that some areas of the brain may be more capable of adaptation to recurrent hypoglycaemia than others. It is also possible that adaptation may occur more rapidly in some individuals than in others and that a more prolonged hypoglycaemic stimulus in some of these studies might have produced evidence of cerebral adaptation to hypoglycaemia.

Therefore, these various studies need not necessarily contradict each other and their discordant results may be explained by differences in degree of awareness, depth and duration of hypoglycaemic nadir, choice of cognitive function tests and inter-individual variability between subjects.

2.2.5 Driving and related skills

One recurring criticism of studies of cognitive function during hypoglycaemia is that they lack ecological validity and that their results may therefore not reflect how individuals cope with hypoglycaemia in real life. For instance, most daily tasks involve the use of multiple cognitive domains and may therefore be more complex than cognitive tests in a research laboratory. However, there will also be a degree of automaticity to certain daily tasks with familiar surroundings and possessions providing cues to the individual undertaking the task.

Driving is one area of particular interest because hypoglycaemia in a driver may have an impact on their driving licence, insurance, safety and, potentially, their livelihood, as well as having broader issues regarding public safety if an accident occurs as a consequence of low blood glucose levels. A survey undertaken across 11 diabetes centres in the US and Europe highlighted increased driving mishap rates in drivers with type 1 diabetes, compared to those with type 2 diabetes and non-diabetic spouses (305). More drivers with type 1 diabetes reported episodes of hypoglycaemia while driving than those with type 2 diabetes (305), raising the possibility that the increased rate of driving mishaps observed in type 1 diabetes relates to hypoglycaemia. In a survey of 202 insulin-treated diabetic drivers in Edinburgh, 25% did not consider that a blood glucose of above 4 mmol/l was prerequisite for safe driving, 60% would not

routinely test blood glucose before driving and only 14% of participants would wait longer than 30 minutes after correction of hypoglycaemia before resuming driving (306).

Some studies have examined cognitive functions relevant to driving, such as visual perception. It is known that hypoglycaemia affects contrast sensitivity, inspection time, visual change detection and visual movement detection (261), which could affect vision in relatively poor light or when visual discriminations must be made rapidly. However, colour discrimination does not appear to be affected (307). Attention (259,268) and volume discrimination (257,258) are also affected and all of these skills are required for driving. Nonetheless, none of these aforementioned studies examines the totality of skills required for driving.

Several studies, most notably from the group led by Dan Cox in Virginia, USA, have employed complex driving simulators during clamped hypoglycaemia to ensure greater ecological relevance than studies of isolated cognitive domains. Although most simulator studies have examined the effects of hypoglycaemia on driving, one glucose clamp study assessed the effects of driving on the development of hypoglycaemia (308). Heart rate, epinephrine and dextrose infusion rates were compared on two occasions in individuals with type 1 diabetes who either watched a driving video or drove a simulator while undergoing clamped hypoglycaemia. Higher dextrose infusion rates were needed while using the simulator, suggesting that driving makes significant metabolic demands which may in themselves contribute to hypoglycaemia (308).

Driving simulator studies suggest that driving ability is not significantly affected by mild hypoglycaemia (3.6 mmol/l) while moderate hypoglycaemia (2.6 mmol/l) is associated with disrupted steering, increased swerving, increased spinning and increased time spent over the midline of the road as well as time spent completely off the road (309). Clearly, the problem here is that neuroglycopenia does not simply impair driving ability but also affects the ability to judge whether it is safe to drive.

Field studies where hand-held computers were used to record information over 3-4 weeks, including details of symptoms, blood glucose levels and decision to drive, suggest approximately 50% of drivers with type 1 diabetes will drive at least 50% of the time when blood glucose is below 3.9 mmol/l (310). Even more worryingly, subjects made a decision to drive on at least 38% of occasions when blood glucose levels were below 2.2 mmol/l (310).

Awareness of hypoglycaemia also affects the decision to drive. For example those with diminished warning symptoms are less likely to self-treat an episode of hypoglycaemia while driving on a simulator (311). Regardless of the state of awareness, individuals with type 1 diabetes being tested on a driving simulator under hypoglycaemic conditions will be aware of impaired driving at moderate levels of hypoglycaemia (2.8-4 mmol/l) but will often not treat hypoglycaemia till blood glucose falls below 2.8 mmol/l (312). When individuals with type 1 diabetes and either normal or impaired awareness of hypoglycaemia were asked whether they felt hypoglycaemic and whether they would drive during clamped euglycaemia (5.0 mmol/l) and hypoglycaemia (2.7 mmol/l), 43% of subjects in the impaired awareness group decided to drive during hypoglycaemia as compared to just 4.2% of those with normal awareness (313). Perhaps surprisingly, in one study middle-aged men with type 1 diabetes were more likely to deem themselves safe to drive during a stepped hypoglycaemic clamp than middle-aged women or subjects under 25 years of age with type 1 diabetes, reinforcing the importance of education on driving safety for all patients with diabetes and not just those in the stereotyped high risk groups such as young males (314).

2.3 Conclusions on cognitive function

Although statistical and methodological considerations complicate comparison between different studies, it is clear that acute hypoglycaemia can affect cerebral function without impairing consciousness at blood glucose levels below 3.3 mmol/l (144). Complex tasks and those requiring rapid responses are more significantly affected than simple tasks (144) and speed is often sacrificed at the expense of preserving accuracy during hypoglycaemia (269,270). It is not clear whether hypoglycaemia affects multiple cognitive domains individually or via its effects on a general intelligence factor. The recovery of cognitive function is delayed following restoration of biochemical euglycaemia but the timing of the recovery period has previously been ill-defined. Although previous studies have produced mixed results, a significant body of evidence suggests that repeated hypoglycaemia leads to a degree of cerebral adaptation. However, this is insufficient to protect from severe hypoglycaemia and simply serves to narrow the window of opportunity for intervention between the onset of symptoms and the development of severe neuroglycopenia. Both the recovery period after hypoglycaemia and the potential for cerebral adaptation to recurrent hypoglycaemia merit further study.

CHAPTER 3: HYPOTHESES FOR STUDIES

Despite advances made in the last three decades, there are still gaps in our knowledge of both the physiological and cognitive consequences of hypoglycaemia. Over the last 25 years, our research group has made a substantial contribution to the existing literature on hypoglycaemia and the studies presented here pick up on unanswered questions from previous research studies.

This thesis consists of three studies which investigate in turn the risk factors for developing severe hypoglycaemia, the variability of symptoms reported during hypoglycaemia and the rate of cognitive recovery from hypoglycaemia.

3.1 Serum Angiotensin Converting Enzyme and frequency of severe hypoglycaemia in type 1 diabetes: does a relationship exist? (Chapter 4)

While many patients rarely experience severe hypoglycaemia, a small subgroup experiences recurrent episodes (170,171,212). Recovery from severe hypoglycaemia is usually complete, but it is very disruptive to daily life and may be dangerous, for example if it occurs when the individual is driving. It is therefore of direct clinical relevance to identify risk factors for severe hypoglycaemia. Established risk factors include intensive treatment (3,206), impaired awareness of hypoglycaemia (170,171,175), increased disease duration (3,173,205,212,216), antecedent hypoglycaemia (11,12,14,15,315), extremes of age (3,216), negative C peptide levels (3,173), sleep (217), renal insufficiency and pregnancy.

Studies in the last 8 years have suggested that serum angiotensin converting enzyme (ACE) activity, which is largely influenced by ACE genotype, may also influence the risk of hypoglycaemia. Scandinavian studies have reported a direct association between elevated serum ACE activity and an increased risk of severe hypoglycaemia

in both adults and children with type 1 diabetes (173,210,242). However these studies have methodological limitations and had not been reproduced outside Scandinavia at the time that the present study was designed. Study one (chapter four) examines the putative association between severe hypoglycaemia and serum ACE levels in a population of 300 Scottish adults with type 1 diabetes.

3.2 Consistency of symptom reporting during hypoglycaemia (Chapter 5)

It is known that the symptoms of hypoglycaemia are idiosyncratic and age-specific (145). Statistical techniques have previously been used to show that these symptoms cluster into three categories in young adults: autonomic, neuroglycopenic and general malaise (6,146,147). While it is accepted that each individual will experience a different range of symptoms during hypoglycaemia, no studies have assessed the extent of any intra-individual variability in adult symptom reporting. The ability to predict which individuals will report a consistent group of symptoms and which individuals will experience a more variable pattern of symptoms would assist patient education and allow clinicians to better inform patients about how to anticipate and recognise hypoglycaemia.

Study two (chapter five) prospectively examines the symptoms reported by a cohort of 350 people with type 1 and type 2 diabetes with different disease durations and varying treatment modalities over a period of nine to twelve months. In those with a substantial number of recorded hypoglycaemic episodes, reported symptom clusters were analysed using a novel statistical model to assess the consistency of symptom reporting for each individual.

3.3 Recovery of cognitive function following hypoglycaemia in adults with type 1 diabetes and the effect of impaired awareness of hypoglycaemia (Chapter 6)

This study examines the time taken for full cognitive recovery from hypoglycaemia and the possible effect of the clinical syndrome of impaired awareness of hypoglycaemia on this process. The effects of acute insulin-induced hypoglycaemia on cognitive function have been investigated extensively but the recovery period after hypoglycaemia has not been rigorously assessed due to methodological limitations of previous studies. It is also unclear whether impaired awareness of hypoglycaemia is associated with the relative preservation of cognitive function during hypoglycaemia (62,78,273,290,292,316,317) or an exacerbation of the decrement in cognitive performance associated with hypoglycaemia (65,285,318). The objective of this third study (chapter 6) is to measure the recovery time for various domains of cognitive function in a large group of patients with type 1 diabetes who have either normal or impaired awareness of hypoglycaemia. The findings of this study will have important clinical implications and help to advise patients how long to wait after restoration of a normal blood glucose before resuming activities such as driving.

CHAPTER 4: SERUM ANGIOTENSIN CONVERTING ENZYME AND FREQUENCY OF SEVERE HYPOGLYCAEMIA IN TYPE 1 DIABETES: DOES A RELATIONSHIP EXIST?

The data in this chapter have been published as a multi-author paper in a peer-reviewed journal (See appendix). The first draft of this chapter was written entirely by me and I coordinated all subsequent editing. Dr Riccardo Marioni did the statistical analysis involving the negative binomial model but I did the remaining statistical work and wrote up the results. Co-authors on this paper provided editorial input, corrections and comments but did not write any individual sections of this paper.

4.1 Introduction

Hypoglycaemia is a common side-effect of insulin therapy. In type 1 diabetes most events are mild (self-treated) with an average frequency of 2.0 episodes per week (4,204). In northern European studies of unselected individuals with Type 1 diabetes, the estimated incidence of severe hypoglycaemia (defined by the need for assistance for recovery) ranges from 1.0 to 1.7 episodes/patient/year (204,205,208,209), with an annual prevalence between 30% (208,211) and 40.5% (205), similar to the Diabetes Control and Complications Trial (DCCT) (3). The frequency of hypoglycaemia varies considerably, with most people never or rarely developing severe hypoglycaemia, while a small subgroup frequently experience severe hypoglycaemia (204).

Several risk factors for severe hypoglycaemia have been identified (3,319), including strict glycaemic control and impaired awareness of hypoglycaemia (320,321). More recently, serum angiotensin-converting enzyme (ACE) activity has emerged as a possible marker for risk assessment. Individual variation in serum ACE levels is mediated in part by gene polymorphism, via I (insertion) and D (deletion) alleles. The

II genotype is associated with low serum ACE activity (235) and in type 1 diabetes has been linked to a lower frequency and risk of severe hypoglycaemia; the DD genotype is associated with higher serum ACE activity and an increased risk of severe hypoglycaemia (173,210). Low serum ACE and the II genotype are associated with enhanced athletic performance in events requiring stamina (237-239). It has therefore been postulated that a lower ACE activity confers greater ability to function efficiently during periods of metabolic substrate deprivation. Conversely, those who have a high ACE activity have more limited functional capacity when challenged by glucose deficiency.

In people with type 1 diabetes with high ACE activity, this may be manifest by greater cognitive impairment during hypoglycaemia than in those with low ACE activity. This might explain the variable risk of developing severe hypoglycaemia within a population with type 1 diabetes. Two Danish studies in adults, and one Swedish study in children and adolescents, all with type 1 diabetes, have suggested that a high serum ACE activity is associated with an increased risk of severe hypoglycaemia (173,210,242). However this observation has not been replicated in non-Scandinavian countries. The present study therefore examined the relationship between serum ACE levels and frequency of severe hypoglycaemia in a cohort with type 1 diabetes in Scotland.

4.2 Methods

4.2.1 Subjects

Three hundred adults with type 1 diabetes attending a hospital outpatient clinic were selected at random. Inclusion criteria consisted of type 1 diabetes of at least two years duration and being over 16 years of age. Exclusion criteria consisted of pregnancy, sarcoidosis or treatment with drugs affecting the renin-angiotensin system (RAS), such as ACE inhibitors or angiotensin 2 receptor antagonists. The local medical ethics committee approved the study, and informed consent was obtained from all participants.

4.2.2 Methods

Each participant completed a questionnaire quantifying the frequency of mild hypoglycaemia (self-treated) and severe hypoglycaemia (requiring external assistance). Participants were asked to estimate the total number of episodes of severe hypoglycaemia in their lifetime (using the following categories: 0, 1-2, 3-5 or >5 episodes of severe hypoglycaemia) and the specific number of episodes during each of the previous two years. Awareness of hypoglycaemia was assessed using a validated seven point visual analogue scale (170).

Information regarding microvascular complications was obtained from medical records. Screening for retinopathy was performed by non-mydriatic digital retinal photography in line with the standards demanded by the national retinal screening programme and was classified as absent, background, pre-proliferative, or retinopathy that had required laser treatment. The standard practice in our centre is to identify peripheral neuropathy as being present or absent based on clinical assessment with a 10-gram monofilament, while autonomic neuropathy is confirmed by autonomic function tests (322). Nephropathy is identified by the presence of microalbuminuria (urinary albumin: creatinine ratio >3.5mg/mmol) or frank proteinuria on two separate early morning urine samples or raised serum creatinine. It should be emphasised that

none of these assessments were repeated as part of the study and information in the medical records was accepted as accurate.

Serum ACE activity was measured using a continuous monitoring spectrophotometric assay (Sigma Diagnostics, St Louis, MO USA) (323). HbA_{1c} was measured by ion exchange high performance liquid chromatography via the Bio-Rad Variant II Haemoglobin testing system. The results are DCCT-aligned and the local non-diabetic range for HbA1c is 5.0-6.5%

4.2.3 Statistical analyses

Primary end points were the number of events of severe hypoglycaemia reported retrospectively over the previous two years and the proportion of participants reporting such events. Frequency of severe hypoglycaemia was compared between the top and bottom quartiles of ACE activity using Mann-Whitney U-tests (assuming non-normal distribution). Serum ACE levels were compared between those with a high number of severe hypoglycaemia events in the previous year (four or more) and those with no severe hypoglycaemia in the previous year. Spearman rank correlations were calculated for the associations between serum ACE activity and both frequency of severe hypoglycaemia and awareness of hypoglycaemia.

The association between severe hypoglycaemia and serum ACE was also examined with a negative binomial model using the statistical package R 2.4.1 (324). This model takes into account the large number of zero values in the data (325). Other analyses were performed using SPSS version 12.0 for Windows.

A p value of less than 0.05 was considered to be statistically significant. A formal power calculation could not be conducted as there are no data on the distribution of serum ACE levels within a Scottish population. However, the present study is larger than previous published studies on this subject.

4.3 Results

The clinical characteristics of the 300 participants are shown in table 4.1, alongside those of the participants of the three previous relevant studies. In the present study, the mean (SD) incidence of severe hypoglycaemia in the previous year was 0.93 (2.86) episodes per patient per year. However, the frequency of severe hypoglycaemia was markedly skewed (figure 4.1), with 207 subjects experiencing no severe hypoglycaemia, while only 44 individuals had experienced two or more episodes of severe hypoglycaemia in the previous year.

Figure 4.1

Frequency distribution of severe hypoglycaemia occurring in the previous year in 300 people with type 1 diabetes.

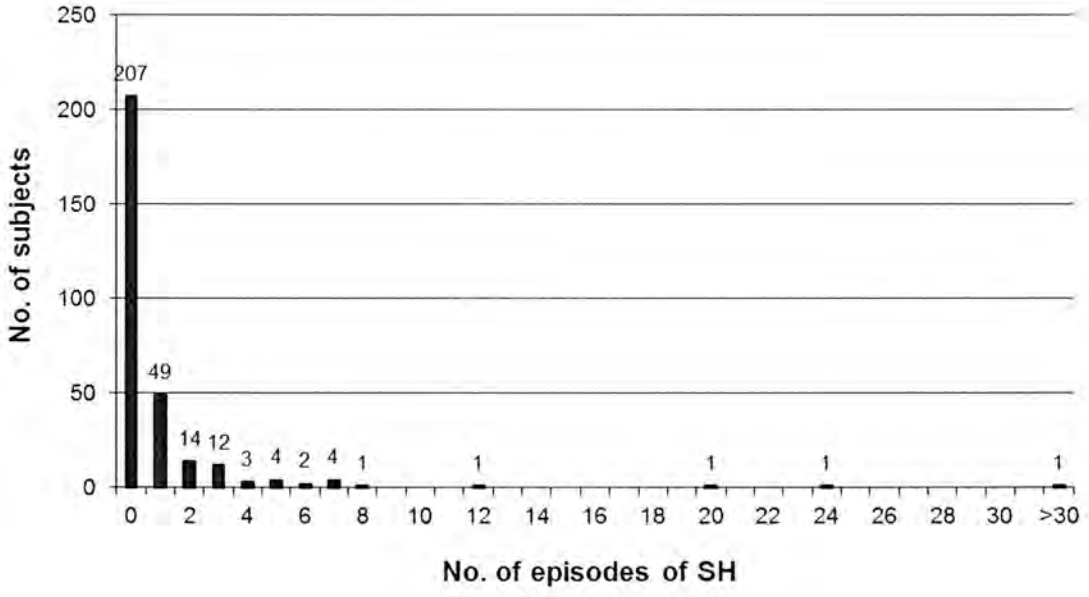


Table 4.1

Clinical characteristics of participants in the present study and in earlier Scandinavian studies examining an association between serum ACE and severe hypoglycaemia (SH).

Characteristics		Present study	Pedersen-Bjergaard 2001 (173)	Pedersen-Bjergaard, 2003 (210)	Nordfeldt, 2003 (242)
Location and type of study		Scotland, retrospective, adults	Denmark, retrospective, adults	Denmark, prospective, adults	Sweden, prospective, paeds/adolescent
Number of subjects		300	207*	171	86
Incidence of SH (episodes/patient/year)		0.93	1.1	1.1	1.8
Prevalence of SH	31%	31%	39%	39%	51%
Age (years)	Mean	38.1	43.1	44	13.0
	SD	13.0	Not reported	Not reported	3.1
	Median	36	Not reported	Not reported	12.8
	Range	16-88	12.8	12	7.1-18.5
HbA1c (%)	Mean	8.4	8.6	8.4	6.9
	SD	1.4	1.3	1.0	1.0
	Median	8.2	Not reported	Not reported	6.8
	Range	5.2-12.8	Not reported	Not reported	4.7-10.2
Diabetes duration (years)	Mean	16.4	18.4	19	5.5
	SD	10.4	10.9	11	3.3
	Median	14.5	Not reported	Not reported	5.3
	Range	2-49	Not reported	Not reported	1.2-14.7
Male/Female (%/%)		53% / 47%	54% / 46%	54% / 46%	Not reported
Retinopathy	Number (%)	95 (32%)	92 (46%)	Not reported (45%)	Not assessed
Peripheral Neuropathy	Number (%)	17 (6%)	52 (26%)	Not reported (26%)	Not assessed
Autonomic Neuropathy	Number (%)	10 (3%)	12 (9%)	Not reported (7%)	Not assessed
Nephropathy	Number (%)	9 (3%)	19 (10%)	Not reported (6%)	Not assessed
Awareness of hypoglycaemia**	Normal: impaired	196:104 (65:35%)	92:115 (44:56%)	70:101 (41:59%)	Not assessed
No. (%) with ≥ 1 SH in previous year		93 (31%)	Not reported	66 (39%)	44 (51%)

* 55/256 patients in this study were taking ACE inhibitors or angiotensin-II receptor antagonists. Their data are excluded from this table.

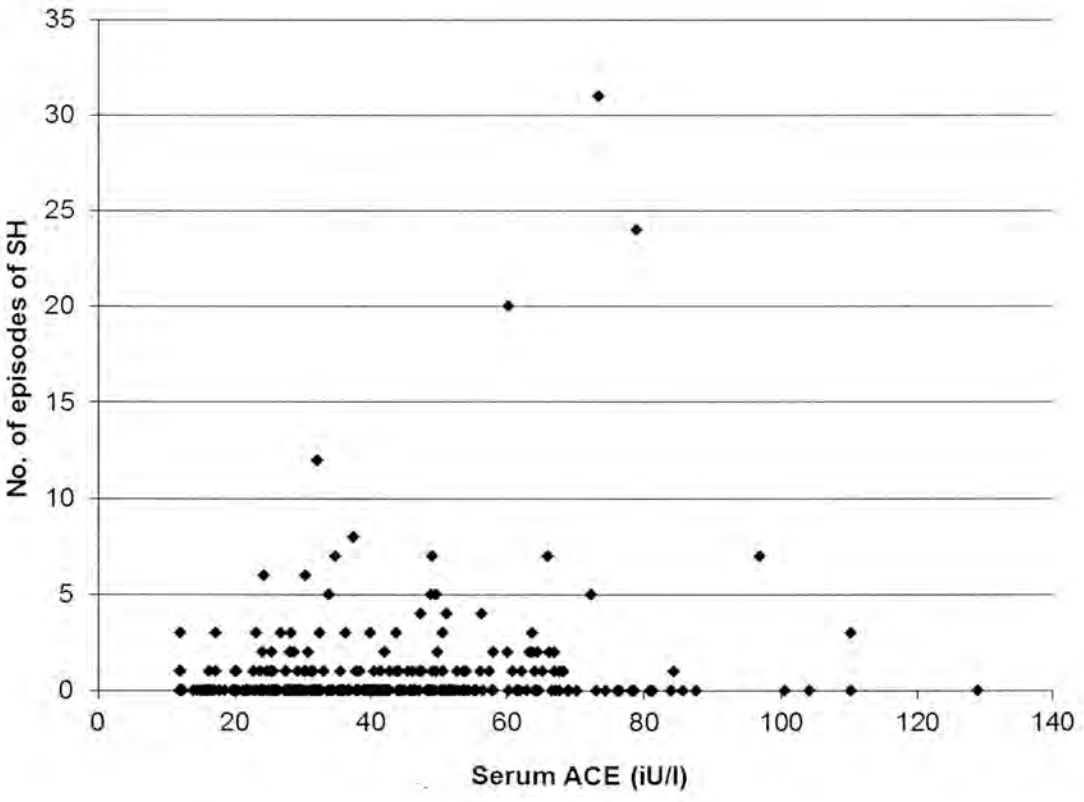
** Different methods were used to estimate awareness of hypoglycaemia in the Scottish and Danish studies

ACE = Angiotensin Converting Enzyme SH = Severe Hypoglycaemia

The relationship between serum ACE activity and frequency of severe hypoglycaemia over the previous year is shown in Figure 4.2. Data on incidence of hypoglycaemia for the previous year and lifetime frequency of hypoglycaemia are available for all 300 subjects but the two year data on severe hypoglycaemia frequency was available in only 257 subjects as several individuals felt that their recall was unreliable. One subject claimed to have experienced 175 episodes of severe hypoglycaemia during the preceding year and his data (which could not be verified) is reported as >30 episodes of severe hypoglycaemia. The median (range) serum ACE level was 39.4 iU/L (<12-129 iU/l).

Figure 4.2

The relationship between number of episodes of severe hypoglycaemia (SH) experienced by individual participants during the previous year and their serum Angiotensin Converting Enzyme (ACE) levels.



The correlation between serum ACE levels and frequency of severe hypoglycaemia was examined using Spearman's test (table 4.2). There was a small (in effect size)

but statistically significant correlation between serum ACE activity and the number of episodes of severe hypoglycaemia in the previous year ($p=0.047$, $\rho=0.115$). The correlations between serum ACE activity and all other estimates of frequency of hypoglycaemia all failed to reach statistical significance (table 4.2). No significant association was observed between serum ACE level and the hypoglycaemia awareness score ($p=0.701$).

Table 4.2

Correlations between serum Angiotensin Converting Enzyme (ACE) activity and various measures of frequency of severe hypoglycaemia (SH).

	Serum ACE	
	Correlation coefficent (r)	P
SH in previous year	**0.115**	**0.047**
SH in penultimate year	0.022	0.725
Mean annual incidence of SH (over 2 years)	0.079	0.175
Lifetime frequency of SH	0.013	0.816
Hypoglycaemia awareness score	-0.022	0.701

The association between serum ACE levels and frequency of severe hypoglycaemia was further examined using a negative binomial model. The subject with 175 episodes of severe hypoglycaemia was treated as an outlier and omitted from the analysis, but the association remained statistically significant ($p=0.002$). However, the frequency of severe hypoglycaemia is very skewed, as illustrated in figure 4.1. In order to assess the effect of the few individuals who experienced a high frequency of severe hypoglycaemia, the data were reanalysed using a negative binomial model but excluding two further subjects who had reported 20 and 24 episodes of severe hypoglycaemia respectively over the previous year. When the subject with 24 episodes was excluded, the association remained significant ($p=0.039$), but when the

subject with 20 episodes was also excluded, the result no longer achieved significance ($p=0.141$). Adjustments were made to the model to consider stratification by age and gender. However, neither had a significant impact upon the relationship between serum ACE and frequency of severe hypoglycaemia.

The incidence of severe hypoglycaemia was determined for each quartile of ACE activity (table 4.3) and compared between top and bottom quartiles using the Mann Whitney U test. The frequency of severe hypoglycaemia did not differ significantly between these two groups ($p=0.075$). The median serum ACE levels were compared between the subset of people who had experienced no severe hypoglycaemia ($n=207$) over the previous year and the small group who had experienced four or more episodes of severe hypoglycaemia ($n=18$). The serum ACE levels were significantly different between the two groups, ($p=0.009$) with median (range) ACE levels of 40.5 (12.0-129.0) iU/l and 49.3 (56.4-96.9) iU/l in the groups with low and high frequency of severe hypoglycaemia respectively ($p=0.008$).

Table 4.3

Number of episodes of severe hypoglycaemia (SH) in previous year for each quartile of serum ACE activity.

		Quartile 1	Quartile 2	Quartile 3	Quartile 4
Number of subjects		77	75	73	75
ACE	Minimum	12	28.0	39.5	51.5
	Maximum	27.9	39.4	51.4	129.0
SH	Number (%) with ≥1 SH	21 (27%)	21 (28%)	22 (30%)	29 (39%)
	Mean (SD) episodes of SH	0.5 (1.0)	0.8 (2.1)	0.7 (1.4)	1.7 (5.0)
	Median (range) episodes of SH	0 (0-6)	0 (0-12)	0 (0-7)	0 (0-30)

4.4 Discussion

Previous studies have reported that high serum ACE activity is *strongly* associated with an increased risk of severe hypoglycaemia, as demonstrated in adult cohorts with type 1 diabetes in retrospective (173) and prospective (210) studies in Denmark and in a prospective study of children and adolescents in Sweden (242). In the present study, a statistically significant relationship was observed between serum ACE activity and the incidence of severe hypoglycaemia, but this association was *weak*, with a low correlation coefficient.

When the data were analysed using a negative binomial model, the statistical significance of the relationship was determined by three individuals who reported a very high frequency of severe hypoglycaemia. If the data from these three subjects is omitted from analysis, this relationship is not statistically significant. The serial removal of outliers is not a recommended statistical technique. However, it illustrates how the relationship between ACE and severe hypoglycaemia may be disproportionately affected by a small minority who have a very high incidence of hypoglycaemia.

The incidence of severe hypoglycaemia did not differ significantly between subjects in the top and bottom quartiles of ACE activity, but when the 18 subjects who reported four or more episodes of severe hypoglycaemia over one year were compared with those who had no severe hypoglycaemia, the serum ACE levels of these two subgroups did differ significantly. The present study examined more people than any of the Scandinavian studies and excluded those receiving treatment with RAS-blocking drugs, as did two of the Scandinavian studies (210,242). A significant number of individuals with type 1 diabetes are treated with such drugs and their exclusion from this study may limit the generalisability of these findings. However, we believe that the exclusion of these individuals is necessary to avoid confounding of serum ACE data.

Various possibilities can be proposed to reconcile the much weaker association between serum ACE and severe hypoglycaemia observed in the present study with the results of the three studies originating from Scandinavia. Retrospectively collected data may be subject to recall bias, although recall of severe hypoglycaemia over a period of one year has been shown to be robust and reproducible (4,21). It is possible that the relationship of ACE to hypoglycaemia risk differs fundamentally between Danish and Scottish populations, although they share similar cultural, ethnic and genetic backgrounds and both countries have a similar prevalence of type 1 diabetes. Even if this is a genuine difference, it does not appear to alter the rates of severe hypoglycaemia observed in these national populations, possibly because the aetiology of severe hypoglycaemia is multifactorial and a subtle difference in one factor might be insufficient to alter the overall frequencies of hypoglycaemia. Previous studies in Denmark (4), Scotland (205,208), England (204) and the Netherlands (209) have reported very similar frequencies and distributions of severe hypoglycaemia within populations of people with type 1 diabetes.

The discrepant results could relate to the processes of selection and assessment rather than differences between the background populations from which subjects are recruited. Differences between the three Scandinavian studies and the present study are summarised in Table 4.1 and will now be discussed in more detail.

For example, although the present study differs from the others in the ages of the subjects, the two Danish studies included participants who were older than those in the present study (173,210), and the Swedish study examined adolescents and children (242), which suggests that age was not contributory. While the much younger Swedish patients had a shorter duration of diabetes and better glycaemic control (242), no consistent differences were observed between the adult participants of the present study and those in the Danish (173,210) studies, either in duration of diabetes or HbA1c. However marked differences were present in the frequencies of microvascular complications in the Danish groups compared with the present study cohort. Information about microvascular complications was not provided in the Swedish study, and these are rare in a paediatric age-group.

Previous work has suggested that the predictive value of serum ACE is strongest in patients whose defence against severe hypoglycaemia is compromised, such as those with impaired hypoglycaemia awareness (173). The Danish method of assessing hypoglycaemia awareness probably over-estimates the prevalence of impaired awareness of hypoglycaemia as being 60% (21) compared to a prevalence of around 25% in other population studies (321). If the patients in the Danish studies (173,210) had a higher frequency of impaired hypoglycaemia awareness than the present group they would certainly have a greater vulnerability to developing severe hypoglycaemia (170).

However, a study from our centre has compared three different methods of assessing impaired awareness of hypoglycaemia. The Danish method (173) requires the patient to answer the question "can you always feel when you are low?" using the responses "always", "usually", "sometimes" or "never" and they are classified as having impaired awareness if they answer anything other than "always". The Gold method uses a 7 point likert scale to grade awareness, with 1 representing "always aware of hypoglycaemia" and 7 representing "never aware". Prospective validation of this method was undertaken by recruiting 2 groups of patients: one with awareness scores of 1-2 (normal awareness, n=31) and one group with awareness scores of greater than 4 (impaired awareness, n=29). The impaired awareness group had an almost 6-fold increase in the incidence of hypoglycaemia over a 12 month follow-up period compared to those with normal awareness (2.83 episodes per person per year compared to 0.48 episodes per person per year, $p<0.001$) (170). The Clarke method includes questions to characterise previous exposure to hypoglycaemia and an assessment of the glycaemic threshold for and symptomatic responses to hypoglycaemia (171). Comparison of these three methods in a cohort of 80 patients with type 1 diabetes demonstrated good concordance between the Gold and Clarke methods (correlation coefficient $r_s = 0.868$, $p = 0.001$), which estimated prevalences of impaired awareness of 24 and 26% respectively, compared to a prevalence of 62.5% using the Danish method (174). It therefore appears that the Danish method of assessing impaired awareness may not be sufficiently specific or discriminatory.

While it could be suggested that the difference in complication rates relates to the exclusion from the current study of individuals treated with RAS-blocking drugs, only one of the previous studies included individuals treated with these medications (173). It could also be argued that exclusion of these individuals excludes those at higher risk of severe hypoglycaemia, but as both the Danish studies reported a frequency of hypoglycaemia of 1.1 episodes per patient per year, despite the fact that one study excluded (210) while the other included (173) people treated with these agents, this does not seem to be a likely possibility. In the latter study, the characteristics and risk of severe hypoglycaemia in those treated with RAS-blocking drugs did not differ from those of the other study participants so the investigators concluded that ACE inhibition exerted no overall significant effect (173). However, patients in the present study were often taking other antihypertensives including beta-blockers and thiazide diuretics, which are known to exert an effect on the renin-angiotensin system. For example, beta-blockers lower renin while thiazides, like RAS-blocking drugs, increase renin. Calcium channel blockers reduce aldosterone. We did not control for the presence of these other antihypertensives in our study and given that they all have the potential to modulate components of the renin-angiotensin system, they may have affected the measured serum ACE activity.

An alternative possibility is that there are differences in ACE genotype between the different study populations, as the participants in the current study were not genotyped. It has been noted previously that there is a close correlation between ACE genotype and phenotype (173,235) and that the effect of the genotype is mediated by the serum ACE levels (173), so when this study was designed a decision was made to measure serum ACE alone. There would have been some potential advantages to an assessment of ACE genotype in that it would have given a constant assessment of each individual's renin-angiotensin system unaffected by factors such as concomitant antihypertensive medication. It would also have simplified analysis in that we would have been assessing the effect of a dichotomous variable (high risk versus low risk genotype) rather than a continuous measurement (serum ACE level). Had patients been genotyped, it would have been possible to study them in a case-control manner by comparing the frequency of severe hypoglycaemia between those with high and low risk genotypes rather than the rather more arbitrary subdivisions of ACE activity into quartiles.

However, there would also have been some drawbacks to this approach. It has been accepted that if there is an increased risk of hypoglycaemia associated with the ACE DD genotype that this is mediated by an increase in serum ACE levels. It was therefore hoped that agents such as ACE inhibitors would reduce that risk by lowering serum ACE levels. On that basis, it seemed rational to assess the phenotype rather than the genotype because having a high risk genotype would not necessarily translate to increased risk if the patient was taking a medication which lowered the ACE levels. However, these are assumptions which cannot be proven and measurement of ACE genotype would also have allowed a potentially interesting 3 way stratification of patients into high risk genotype plus phenotype (ie DD genotype and high ACE activity), high risk genotype and low risk phenotype (DD genotype and low ACE activity) and low risk genotype plus low risk phenotype. This type of information would have been a valuable addition to the present study as it would have provided further data on the relationship between genotype and phenotype so it must be acknowledged that the lack of genetic data is a limitation of this study.

Two other studies outside Scandinavia have addressed the relationship between serum ACE levels and severe hypoglycaemia. The first of these is a study of 308 people with type 2 diabetes in the UK, 124 of whom were treated with insulin while the remaining 124 were treated with a combination of oral agents that included a sulphonylurea (326). ACE genotype was checked in all subjects, who were divided into two groups depending on whether or not they had ever experienced severe hypoglycaemia. A total of 12% of subjects had previously experienced at least one episode of severe hypoglycaemia and this proportion did not differ between ACE genotype subgroups. The group carrying the D allele (including DD homozygotes and DI heterozygotes) had an odds ratio of experiencing severe hypoglycaemia of 0.79 (95% confidence interval .035-1.78) relative to the II homozygotes. The authors therefore found no evidence for a relationship between carriage of the ACE D allele and an increased risk of severe hypoglycaemia in type 2 diabetes (326).

Similarly, a large prospective study in Western Australia concluded that the DD genotype (which is associated with higher serum ACE activity) did not predict a

significantly higher risk of severe hypoglycaemia in children and adolescents with type 1 diabetes (327). A total of 585 children and adolescents with type 1 diabetes were included in the study and the frequency of severe hypoglycaemia was assessed prospectively over a 13 year period from 1992 to 2004. However, in this study, the definition of severe hypoglycaemia was restricted to episodes resulting in loss of consciousness. Children were seen with their parents every three months during the study period and all subjects had their ACE genotype analysed. Of the 32% of children who experienced at least one severe hypoglycaemic event, 28% had the II genotype, 49% had the ID genotype and 23% had the DD genotype. The overall incidence of severe hypoglycaemia was 14 episodes per 100 patient-years with no significant increase in risk for those in the DD genotype group (incidence rate ratio relative to II genotype = 0.97, 95% confidence interval 0.61-1.55)

In the present study the incidence of severe hypoglycaemia did not differ significantly between subjects in the top and bottom quartiles of serum ACE activity, suggesting that serum ACE is not sufficiently specific as a marker to allow hypoglycaemia risk stratification of people with type 1 diabetes. Although serum ACE levels differed significantly between people who had no history of severe hypoglycaemia and those who had experienced four or more episodes, this has limited clinical applicability with respect to screening for risk of severe hypoglycaemia. A previous history of severe hypoglycaemia is a recognised risk factor for further severe hypoglycaemia. A retrospective finding of high serum ACE levels in people who have already been identified as having a high risk of severe hypoglycaemia based on their previous history, has no prognostic value.

Thus, in the present study, the association between serum ACE and severe hypoglycaemia in type 1 diabetes (173,210,242) was influenced disproportionately by a few individuals who reported a high frequency of severe hypoglycaemia, raising doubt as to the clinical significance of this finding. The present study suggests that serum ACE is not sufficiently specific to serve as a prognostic indicator of increased risk of severe hypoglycaemia. Further work is required to establish whether the association is present in ethnically different (non-Caucasian) populations.

CHAPTER 5: MODELLING THE CONSISTENCY OF HYPOGLYCAEMIC SYMPTOMS: HIGH VARIABILITY IN DIABETES

The data in this chapter have been published as a multi-author paper in a peer-reviewed journal (See appendix). The first draft of this chapter was written by me, with the exception of section 5.2.2 (Model for intra-individual consistency), which was drafted by Dr Streftaris and edited by me. The other co-authors on this paper provided editorial input, corrections and comments but did not write any individual sections of this paper. Dr Streftaris designed the statistical model and undertook the statistical analyses. The idea to devise a model to quantify between episode intra-individual variability in hypoglycaemia symptom reporting was mine.

5.1 Introduction

Hypoglycaemia is a common side effect of insulin treatment which can have a substantial morbidity. Rapid perception of the symptoms of hypoglycaemia is essential to permit early corrective action. Field studies in which adults with insulin-treated diabetes have reported symptoms experienced during hypoglycaemia have allowed the most common symptoms to be identified (145) and subdivided into autonomic, neuroglycopenic and general malaise groups (6).

When educating patients about the recognition of hypoglycaemia, it is important to consider factors that may cause variation in their symptoms. The symptoms of hypoglycaemia are age-specific, in that young children have difficulty recognising hypoglycaemia (157) and often exhibit behavioural changes (157-159). In the elderly, neurological symptoms are prominent and the signs of hypoglycaemia may mimic those of a transient ischaemic attack, a stroke or a vasovagal episode (160). The causative agent does not appear to influence hypoglycaemic symptoms, as demonstrated in a study comparing hypoglycaemia generated by tolbutamide and

insulin (161), where the symptoms reported were similar, irrespective of the hypoglycaemic trigger. Retrospective field studies (164,165) and studies of experimental hypoglycaemia (166) suggest that the symptom profile does not differ between type 1 diabetes and insulin-treated type 2 diabetes. Gender does not influence the nature of the symptoms experienced during hypoglycaemia (167).

Some hypoglycaemia-related symptoms may be more reliably associated with blood glucose levels than others and a given symptom is not equally predictive of hypoglycaemia in everybody (150). These observations suggest a degree of between-subject variability in the reporting of symptoms. It is accepted that each individual's symptom complex is idiosyncratic (145). However, an additional important issue is the degree to which individuals report similar patterns of hypoglycaemia-related symptoms across episodes. The reliability with which particular hypoglycaemic symptoms occur in an individual's experience of hypoglycaemia influences the person's ability to detect the onset of hypoglycaemia. People who have at least one reliable symptom of hypoglycaemia correctly detect blood glucose levels below 3.9 mmol/l on 50% of occasions, whereas individuals with four or more reliable symptoms recognise similar blood glucose levels on 75% of occasions (148). In a study where 100 children with type 1 diabetes and their parents completed a questionnaire on their experiences of hypoglycaemia, the symptoms reported by children exhibited marked variability between episodes of hypoglycaemia (157). It is not known whether adults exhibit similar intra-individual variability.

The aim of the present study was to examine the symptoms of hypoglycaemia recorded prospectively over 9-12 months by adults with type 1 and type 2 diabetes, to develop a model for quantifying the consistency of the symptom complex recorded on each occasion by every individual and to examine what factors might produce inter-individual differences in the consistency of symptom reporting.

5.2 Methods

Data for this study were collected during an epidemiological study examining the effects of type of diabetes and treatment modality on the frequency of hypoglycaemia (212). A total of 381 patients were followed for 9-12 months in six secondary care diabetes centres in the UK. Participants aged 17-75 years were recruited into five groups:

1. Type 2 diabetes treated with oral agents (which had to include a sulphonylurea)
2. Type 2 diabetes treated with insulin for <2 years
3. Type 2 diabetes treated with insulin for >5years
4. Type 1 diabetes with <5 years duration
5. Type 1 diabetes with >15 years duration

The clinical diagnosis of type 1 and type 2 diabetes was corroborated by ELISA measurements of glucagon-stimulated C-peptide. HbA1c was assessed in a central laboratory by a DCCT-aligned method. The presence of retinopathy was assessed using digital retinal photography. Serum Angiotensin Converting Enzyme (ACE), considered to be a putative marker for increased risk of severe hypoglycaemia at the time that the study was designed (173,210,242), was also measured in a central laboratory.

Subjects treated with insulin had to be taking at least two injections a day. Exclusion criteria were: HbA1c >9%, severe diabetic complications, history of seizures, malignant disease, severe systemic disease or pregnancy. The protocol received multi-centre ethics approval. Subjects gave informed consent.

Subjects performed regular capillary glucose monitoring using a Medisense G glucose meter (Abbott Laboratories). Subjects were asked to record every episode of hypoglycaemia on standard forms, noting the date, time, duration, symptoms, treatment received and concurrent blood glucose. Biochemical criteria for hypoglycaemia were not stipulated and it was left to subjects' discretion to report any episodes that they perceived to represent hypoglycaemia. However, subjects were

specifically asked to record any episodes associated with a capillary glucose <3.0 mmol/l regardless of whether or not these were associated with symptoms. Subjects were encouraged to measure blood glucose for every report form completed but episodes were accepted as valid, even if no blood glucose measurement was available, provided that symptoms resolved with ingestion of carbohydrate. All episodes associated with a blood glucose level <4.0 mmol/l were accepted as valid, even if subjects reported no symptoms in association with these readings. Symptoms were recorded using a standard list (table 5.1).

Table 5.1

List of symptoms on patients' report forms.

Symptom	Description	Symptom	Description
1	Confusion	14	Blurred vision
2	Sweating	15	Hunger
3	Drowsiness	16	Thirst
4	Weakness	17	Nausea
5	Dizziness	18	Anxiety
6	Feeling warm	19	Tiredness
7	Difficulty speaking	20	Tingling
8	Pounding heart	21	Trembling
9	Impaired concentration	22	Headache
10	Shivering	23	Malaise
11	Unsteady	24	Irritability
12	Non-specific awareness	25	Other
13	Double vision	26	None

The state of hypoglycaemia awareness was assessed at entry to the study using a validated scale (170). Each month, subjects returned forms recording all hypoglycaemic episodes. If no form was received, the patient was contacted by telephone. As the intensity of hypoglycaemic symptoms is diminished following antecedent hypoglycaemia (12,14,101), any episode of hypoglycaemia occurring within 24 hours of a preceding episode was excluded from further analysis.

5.2.1 Modelling and analysis

In the statistical model developed, individuals report specific symptoms with a probability that depends on a random threshold being crossed. The behaviour of thresholds is modelled through a probability distribution whose degree of concentration around a central value provides a measure of an individual's symptom-reporting consistency.

Under a Bayesian approach, following observation of binary indicators of symptom experience (i.e. whether or not an individual experiences a given symptom), information on unobserved latent factors and the variability of the thresholds becomes available through their posterior distribution which is obtained using Markov Chain Monte Carlo (MCMC) methodology (328). Bayesian methods and MCMC techniques are used in the analysis of latent variable models in psychology (329,330). Latent variable and threshold models are commonly used in the behavioural sciences (331) and stochastic methods have been employed in diabetes to model the decision-making processes that lead to treatment of hypoglycaemia (332).

Computations were performed using the statistical package R (324). MCMC techniques were implemented using winBUGS software (333). The lack of previous similar analyses prevented formal power calculations. A pragmatic decision was made that participants should have experienced at least 2 episodes of hypoglycaemia per month on average. As follow-up ranged from 9-12 months, participants were only included if they had reported more than 18 episodes of hypoglycaemia. The data were checked for sample bias resulting from patients with more frequent episodes

potentially experiencing lower number of symptoms, but no such association was found ($\rho = -0.09$)

5.2.2 Model for intra-individual consistency

The random threshold determining the probability of an individual reporting a set of specific symptoms relates to latent variables that govern the intensity of a given symptom on a given occasion and the individual's propensity to experience that symptom. Therefore within our statistical model, assessment of intra-individual consistency is based on a principle of hierarchical symptom reporting where order is imposed by both propensity and intensity. Thus, a symptom is more likely to be reported if it is intense (e.g. profuse versus mild perspiration) and if the individual has a strong tendency to experience that symptom.

This modelling approach can be represented graphically by regarding each subject's responses as a $J \times K$ matrix of indicator variables (J = number of symptoms; K = number of episodes) where each reported symptom is represented by a marked cell. Figure 5.1a represents a hypothetical completely consistent patient who reports the same five symptoms on every episode of hypoglycaemia.

Rearranging the rows according to the frequency with which symptoms are experienced and the columns according to the number of symptoms per episode (both following a descending order from the top left corner; figure 5.1b), we obtain a representation where the degree of clustering of marked cells can be regarded as a measure of consistency and the relative frequency of embedded empty cells provides evidence of lack of consistency.

Figure 5.1 a

Example of a $J \times K$ matrix of indicator variables (J = symptoms; K = episodes) for hypothetical subject with symptoms 1-26 listed vertically and hypoglycaemic episodes listed horizontally. Each reported symptom is marked with a square.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1																			
2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
3																			
4																			
5																			
6	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
7																			
8																			
9	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
10	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
11																			
12																			
13																			
14																			
15																			
16																			
17																			
18	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
19																			
20																			
21																			
22																			
23																			
24																			
25																			
26																			

Figure 5.1b.

Rearrangement of the matrix rows and columns: rows now appear according to the frequency with which symptoms are experienced and columns according to the number of symptoms per episode (both following a descending order from the top-left corner of the table).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
6	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
9	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
10	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
18	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
1																			
3																			
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In contrast to the hypothetical completely consistent patient who reports the same symptoms on every occasion, the $J \times K$ matrix for subject number 6010 (figure 5.2) illustrates the way that symptoms for a less consistent patient cluster to a lesser extent.

Figure 5.2 a

Example of a $J \times K$ matrix of indicator variables (J = number of symptoms; K = number of episodes) for subject 6010 with symptoms 1-26 listed vertically and hypoglycaemic episodes listed horizontally. Each reported symptom is marked with a square.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	■	■					■	■	■									■	■
2	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■		
3		■								■									
4									■										
5																			
6					■	■	■				■	■		■		■	■	■	■
7									■										
8																			
9	■	■		■	■	■	■	■	■	■	■				■			■	
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Figure 5.2b.

Rearrangement of the matrix rows and columns: rows now appear according to the frequency with which symptoms are experienced and columns according to the number of symptoms per episode (both following a descending order from the top-left corner of the table).

	9	2	7	4	8	6	11	10	16	19	12	18	1	5	3	14	17	15	13
2	■	■	■	■	■	■	■	■	■		■		■	■	■	■	■	■	
9	■	■	■	■	■	■	■	■				■	■	■				■	
6			■			■	■		■	■	■	■		■		■	■		
1	■	■	■		■					■		■	■						
20		■		■			■			■	■				■				
18	■		■	■	■	■			■										
3		■						■											
7	■																		
4	■																		
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This graphical representation of symptom reporting consistency can be expressed within a parametric framework using a logistic-type latent variable model. We assume that the unobservable random thresholds τ_{ijk} (associated with individual i reporting symptom j at episode k) follow a $\log\text{-normal}(0, \sigma_i^2)$ distribution, under which the probability p_{ijk} of individual i reporting symptom j at episode k is given by

$$p_{ijk} = \Pr(\tau_{ijk} \leq \alpha_{ij}\beta_{ik}) = \Phi\left(\frac{\log(\alpha_{ij}\beta_{ik})}{\sigma_i}\right),$$

$i = 1,...,I$, $j=1,....,J$, $k = 1,...,K_i$, where α_{ij} and β_{ik} represent the propensity for symptom j and the intensity of episode k respectively for individual i, and $\Phi(\,)$ denotes

the cumulative distribution function of a standard normal variable. Therefore, the model implies that occurrence of symptoms across and within episodes depends on the relevant propensity (α_{ij}) and also on the underlying episode intensity (β_{ik}) which introduces associations among symptoms through the imposed hierarchical structure of occurrence. The information available on the frequency with which symptoms are reported through all episodes and on the total number of symptoms per episode, allows estimation of both α_{ij} and β_{ik} in our model.

The *precision parameter* σ_i^{-2} of the threshold distribution provides a measure of the symptom reporting consistency of an individual. Consistent symptom profiles are associated with low variance of the threshold distribution. For ease of interpretation σ_i^{-2} is converted to a *consistency parameter* $c_i = 100/(1+\sigma_i^2)$, which has range (0-100) with increasing values corresponding to higher symptom consistency.

5.2.3 Association between consistency and patient-specific factors

Generalised linear model (GLM) methodology was used to investigate the effect of the following ten patient-specific covariates on consistency: gender, age, type of diabetes (1 or 2), duration of diabetes, presence of retinopathy, hypoglycaemia awareness score (1 to 7, with higher scores corresponding to diminishing awareness of hypoglycaemia), body mass index, stimulated C-peptide, HbA1c, and serum ACE activity. For modeling purposes retinopathy was sub-divided into no retinopathy, background retinopathy and proliferative retinopathy (ret 1-3 respectively). A GLM with gamma errors (see appendix) was used to link estimates of the precision parameter σ_i^{-2} with the covariates, through the function

$$\begin{aligned}\log\left\{E\left(\sigma_i^{-2}\right)\right\} = {} & b_0 + b_{gen}\times \mathrm{GEN}_i + b_{age}\times \mathrm{AGE}_i + b_{type}\times \mathrm{TYPE}_i + b_{dur}\times \mathrm{DUR}_i \\ & + b_{ret1}\times \mathrm{RET1}_i + b_{ret2}\times \mathrm{RET2}_i + b_{ret3}\times \mathrm{RET3}_i + b_{awar}\times \mathrm{AWAR}_i \\ & + b_{bmi}\times \mathrm{BMI}_i + b_{cpep}\times \mathrm{CPEP}_i + b_{hba}\times \mathrm{HBA}_i + b_{ace}\times \mathrm{ACE}_i\end{aligned}$$

and the effect of each covariate was assessed using 95% equal tailed Bayesian intervals of the corresponding b coefficients.

5.3 Results

A total of 3,474 episodes of hypoglycaemia from 59 patients were examined, of which 91% were confirmed by capillary glucose readings. After exclusion of hypoglycaemic episodes occurring within 24 hour of a previous event, 2699 episodes remained for analysis. Table 5.2 summarizes the subject characteristics and hypoglycaemic episodes within each group. The most commonly recorded symptoms were weakness, decreased concentration, sweating and hunger (reported in 28.7%, 28.2%, 21.8%, and 21.1% of episodes, respectively).

The precision parameter σ_i^{-2} quantifies the degree to which a patient reports a similar set of symptoms on every episode of hypoglycaemia. The distribution of the estimated values, $\widetilde{\sigma}_i^{-2}$, is skewed, with most subjects having low consistency (fig 5.3a). Estimates of the converted consistency parameter, $\widetilde{c}_i = 100/(1+\widetilde{\sigma}_i^2)$, have mean 50.3 and standard deviation 16.7 (figure 5.3b). The main sample quartiles of $\widetilde{c}_i$ are $q_0 = 18.0$, $q_{0.25} = 37.6$, $q_{0.5} = 50.2$, $q_{0.75} = 62.7$and $q_1 = 96.7$. Posterior estimates of c_i were derived for each subject in the analysis using Markov chain Monte Carlo (MCMC) methodology (328), and are displayed in Table 5.3. Credible intervals for c_i were wide for some patients, reflecting limited information in the occurred episodes.

Table 5.2

Subject characteristics and hypoglycaemic episodes within each group. Data are given as median (range) unless otherwise stated. (T2tabs: Type 2 diabetes treated with oral agents; T2Ins<2: Type 2 diabetes treated with insulin for <2 years; T2Ins>5: Type 2 diabetes treated with insulin for >5years; T1Ins<5: Type 1 diabetes with <5 years duration; T1Ins>15: Type 1 diabetes with >15 years duration)

	T2tabs	T2Ins<2	T2Ins>5	T1Ins<5	T1Ins>15	Total
Number in original study (212)	108	89	77	50	57	381
No. of subjects with ≥ 19 hypos	1	4	9	21	24	59
Hypos per group	25	113	476	1385	1475	3474
Hypos per group after hypos <24h of each other excluded	25	104	370	1095	1104	2699
Number of hypos per patient	25 (25)	28.5 (20-36)	37 (27-146)	49 (19-210)	44.5 (19-300)	42 (19-300)
Hypos per patient after hypos <24h of each other excluded	25 (25)	25 (20-34)	31 (25-105)	44 (19-134)	37 (19-138)	37 (19-135)
Percentage of hypos confirmed biochemically	100%	95.2%	89.1%	95.1%	87.8%	91.0%
Asymptomatic episodes per group (%) after hypos <24h of each other excluded	36%	0%	9.3%	0.9%	4.5%	11.3%
No. (%) male	1 (100%)	4 (100%)	8 (89%)	14 (67%)	11 (46%)	39 (65%)
Age (years)	51 (51)	65 (60-74)	65 (57-72)	39 (22-70)	58 (34-72)	57.5 (22-74)
No. (%) with impaired awareness	0 (0%)	0 (0%)	2 (22%)	7 (33%)	13 (54%)	22 (37%)
BMI (kg/m^2)	23.7 (23.7)	27.8 (26-30.2)	27 (21.9-33)	24 (19.5-29.6)	25.3 (21.6-42.7)	25.0 (19.5-42.7)
C peptide (nmol/l)	2.22 (2.22)	0.85 (0.27-1.58)	0.24 (0.05-0.21)	0.45 (0.06-0.87)	0.09 (0.05-0.85)	26 (0.05-2.51)
HbA1c (%)	7.1 (7.1)	8.3 (7.8-8.8)	7.6 (6.3-8.9)	7.2 (5.6-10.1)	7.8 (6.1-9.7)	7.55 (5.6-10.1)
ACE (IU/l)	20 (20)	13.5 (7-24)	39 (4-71)	34 (18-94)	31.5 (3-98)	32.5 (3-98)

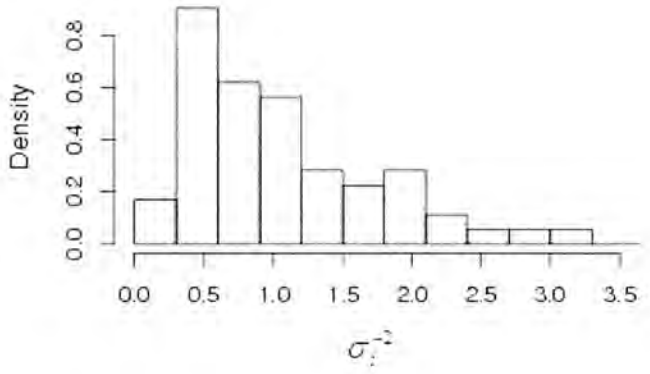
Table 5.3 Posterior estimates of consistency (mean $\widetilde{c}_i = \mathrm{E}(c_i \mid \underline{y}_i)$, std dev = $\sqrt{\mathrm{var}(c_i \mid \underline{y}_i)}$ and 95% equal-tailed interval) for all subjects.

Group	Subject	No. of hypos	No. of hypos excluding those < 24h of previous	No(%) of asymptomatic episodes	$\widetilde{c}_i$	Std dev	95% interval
T2 tabs	3046	25	25	9 (36%)	68.84	7.682	53.19, 83.24
T2 Ins <2yrs	3016	23	22	0 (0%)	73	7.495	56.95, 86.2
	6002	34	34	0 (0%)	41.3	6.986	28.9, 55.93
	6056	36	28	0 (0%)	64.92	7.89	49.38, 80.02
	6058	20	20	1 (5%)	74.29	7.048	59.25, 86.65
T2 Ins >5yrs	1055	27	27	9 (33.3%)	74.05	7.207	58.69, 86.46
	3048	37	31	0 (0%)	67.46	7.562	51.48, 80.93
	3052	146	105	14 (13.3%)	49.5	5.25	39.36, 59.91
	3057	92	51	0 (0%)	39.81	6.287	28.22, 52.48
	3065	27	26	7 (26.9%)	55.09	8.411	38.84, 71.92
	3067	32	26	2 (7.7%)	42.47	7.742	28.42, 58.19
	4072	42	39	15 (38.5%)	57.57	7.333	43.14, 72.22
	4076	33	29	0 (0%)	52.96	8.252	36.7, 69.27
	5009	40	36	0 (0%)	25.9	4.974	17.44, 36.98
T1 Ins <5yrs	1009	93	74	0 (0%)	53.16	5.902	41.57, 64.58
	1021	49	43	0 (0%)	47.72	7.065	34.3, 62.27
	1036	42	24	3 (12.5%)	51.14	8.395	35.42, 67.65
	2012	42	37	0 (0%)	29.72	6.09	19.44, 43.08
	2027	26	22	3(13.6%)	68.52	8.179	51.53, 83.29
	3001	35	31	20(6.5%)	56.94	8.089	41.18, 72.31
	3024	30	27	0 (0%)	52.05	8.046	36.88, 67.78
	3029	78	69	0 (0%)	64.71	5.951	52.68, 75.89
	3050	54	44	1 (2.3%)	49.04	6.941	36.29, 62.96
	4023	210	134	0 (0%)	22.07	2.988	16.75, 28.65
	4034	61	55	0 (0%)	59.98	6.523	46.97, 72.87
	4049	23	22	2 (9.1%)	60.79	8.303	44.17, 76.63
	4063	45	42	0 (0%)	33.95	6.241	22.85, 47.46
	5029	47	45	0 (0%)	62.07	6.905	48.07, 75.02
	5044	88	70	36 (51.4%)	40.38	5.579	29.69, 51.7
	5045	79	68	12 (17.6%)	47.46	5.902	35.97, 59.12
	5088	102	87	7 (8.0%)	24.33	3.813	17.73, 32.64
	6010	19	19	0 (0%)	63.97	8.463	46.91, 79.94
	6019	93	64	1 (1.6%)	38.13	5.327	28.27, 49.26
	6038	125	79	12 (15.2%)	28.43	4.34	20.67, 37.63
	6065	44	39	0 (0%)	50.17	7.443	36.11, 64.75
T1 Ins >15yrs	1008	124	95	14 (14.7%)	30.07	4.123	22.57, 38.78
	1015	35	27	0 (0%)	37.11	7.649	24.02, 53.68
	1025	26	25	5 (20%)	63.36	8.222	47.1, 78.24
	1028	45	42	42 (100%)	96.65	1.535	92.88, 98.65
	1039	47	43	0 (0%)	30.64	5.68	20.75, 42.81
	1086	91	67	0 (0%)	39.72	5.53	29.52, 50.89
	2009	101	86	3 (3.5%)	30.46	4.34	22.65, 39.38
	2010	102	89	32 (36.0%)	33.4	4.478	25.33, 42.55
	2013	300	138	0 (0%)	18.01	2.556	13.41, 23.37
	2015	44	32	14 (43.8%)	53.02	7.695	38.25, 68.16
	2021	33	24	4 (16.7%)	58.05	8.683	41.19, 74.89
	2022	41	36	0 (0%)	43.41	7.338	30.09, 58.72
	3015	19	19	1 (5.3%)	58.93	9.166	40.84, 76.37
	3022	23	20	1 (5%)	66.05	8.198	49.04, 81
	4003	26	23	18 (74.1%)	75.86	6.818	60.73, 87.59
	4008	67	59	0 (0%)	31.24	4.825	22.74, 41.81
	4013	55	39	0 (0%)	49.62	7.283	35.8, 64.12
	4043	36	34	0 (0%)	39.05	6.796	26.5, 52.88
	4045	46	38	0 (0%)	48.41	7.506	34.24, 63.57
	5004	28	26	3 (11.5%)	61.33	8.093	45.33, 76.98
	5023	22	20	5 (25%)	72.85	8.022	55.42, 86.8
	5026	26	24	0 (0%)	74.24	7.2	58.61, 86.96
	6018	62	39	0 (0%)	29.94	5.703	19.96, 42.12
	6023	76	59	14 (23.7%)	32.67	5.174	23.36, 43.41

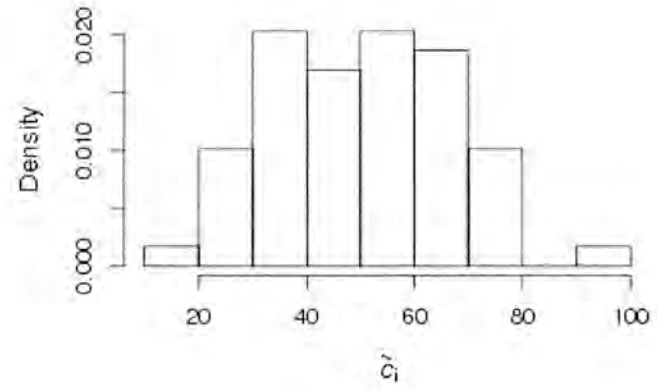
Figure 5.3

Histograms of estimated precision parameter $\widetilde{\sigma}_i^{-2}$ (5.3a) and estimated consistency parameter (5.3b) $\widetilde{c}_i = 100/(1+\widetilde{\sigma}_i^2)$.

5.3a



5.3b



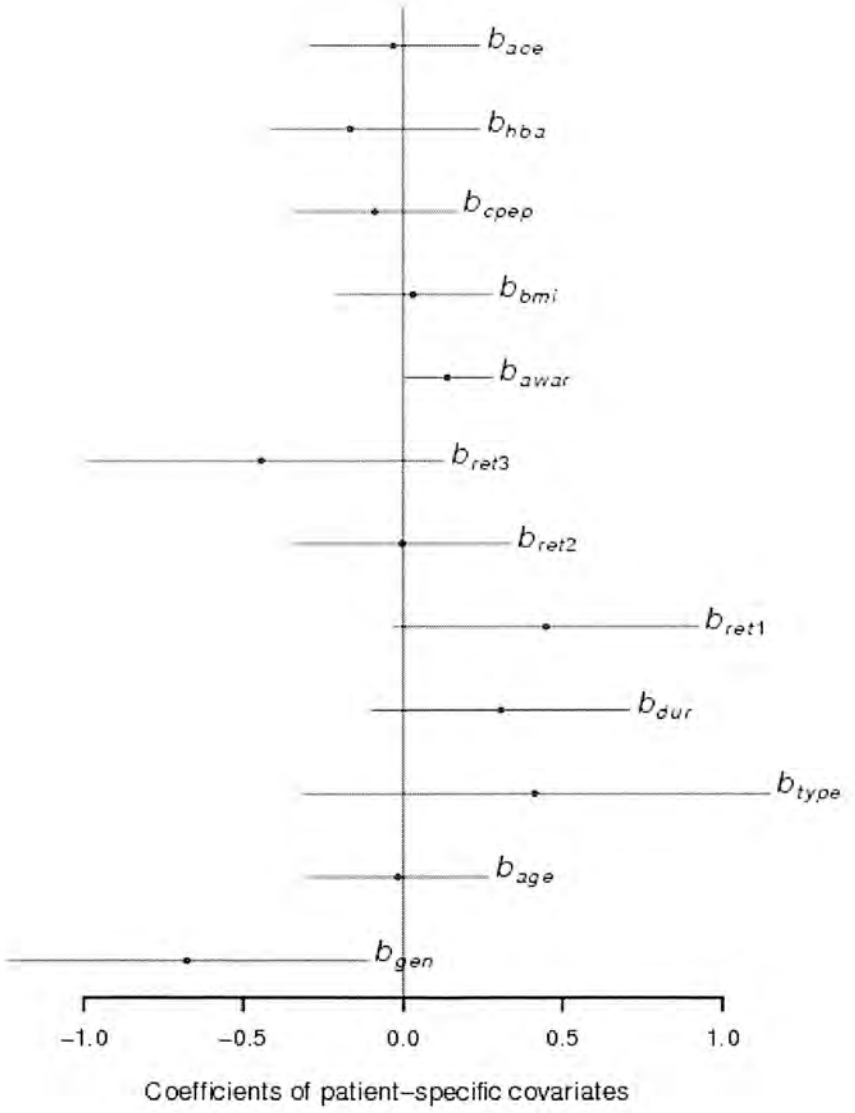
Some subjects in the study merit individual consideration. Subject 1028 (type 1 diabetes >15 years) was asymptomatic during all of his 45 recorded episodes. He had reported a hypoglycaemia awareness score (170) of 7, denoting total loss of warning symptoms, and had the highest estimated consistency (96.7 with 95% Bayesian interval 92.9 to 98.7). In Bayesian statistics, the credible or Bayesian interval plays a similar role to confidence intervals in frequentist statistics. Subject 4003 (also type 1 diabetes >15 years) had the second highest consistency score (75.86 with 95% Bayesian interval 60.7 to 87.6), was asymptomatic during 74.1% of his reported episodes and was the only other subject with an awareness score of 7. Subject 5044

(type 1 diabetes <5 years duration) had no symptoms during 51% of her reported episodes of hypoglycaemia but had a hypoglycaemia awareness score of 2, implying good awareness. Her consistency score was 40.4 (95% Bayesian interval 29.7-51.7). During the asymptomatic episodes, her median (range) blood glucose readings were 3.4 (2.4-3.9). All other subjects were symptomatic during at least 50% of their reported hypoglycaemic episodes. The single subject treated with oral agents was asymptomatic on 36% of episodes, all of which were confirmed biochemically (median blood glucose 3.4 mmol/l; range 3.1-3.5 mmol/l). All of these subjects were included in the analysis as the presence or absence of symptoms was considered to form part of the variability of their symptom profiles.

When the effect of specific covariates on the consistency measure was examined, gender and hypoglycaemia awareness were the only factors which had a systematic effect. Figure 5.4 shows 95% Bayesian intervals for all covariate coefficients. If both endpoints of the interval are positive or negative, a corresponding effect of the covariate on consistency can be inferred. The mean of the gender coefficient, b_{gen}, was − 0.677 (95% Bayesian interval − 1.239, − 0.110). This suggests that female subjects were less consistent than male subjects (gender was coded as 0=males and 1=females). The mean of the coefficient of awareness, b_{awar}, was 0.138 (95% interval 0.006, 0.284). As high values of the covariate indicate impaired awareness, the significantly positive estimate of b_{awar} implies that those with impaired awareness of hypoglycaemia recorded lower variability in their hypoglycaemic symptoms than those with higher awareness.

Figure 5.4

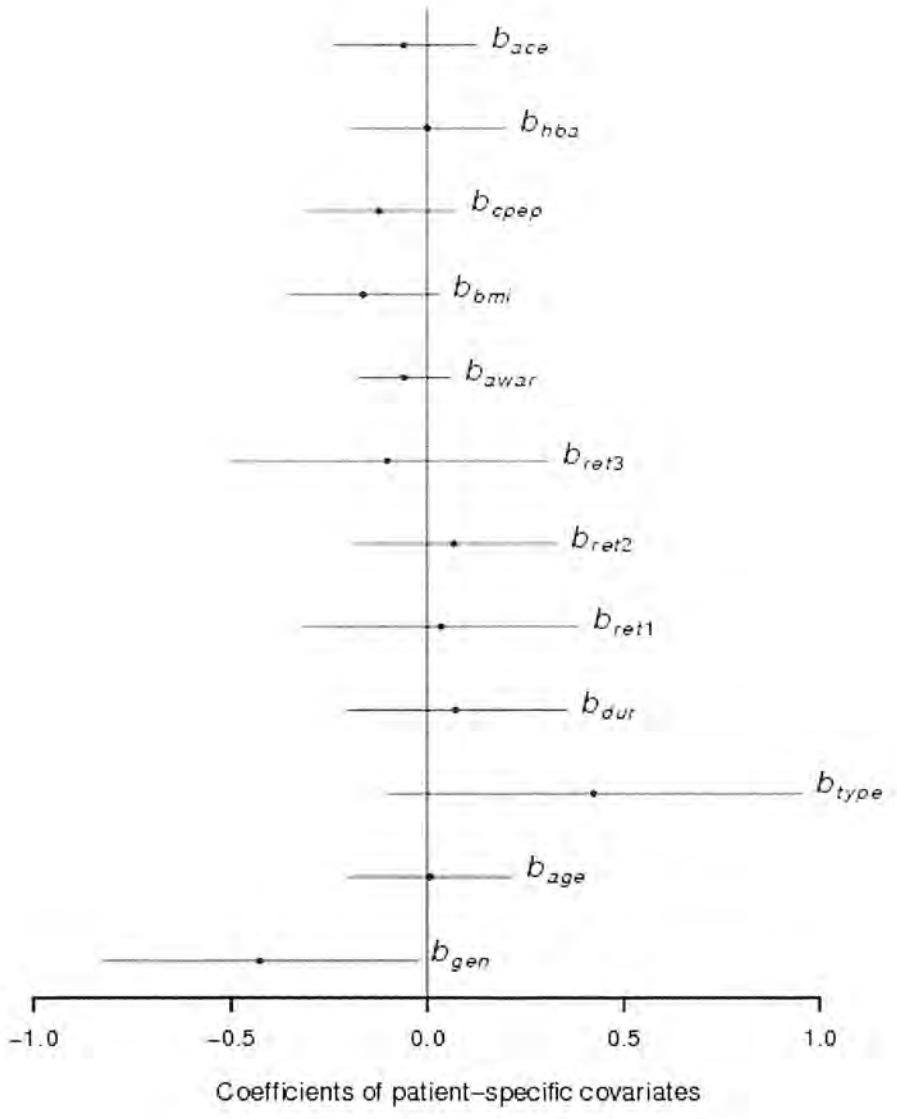
Posterior means (circles) and 95% equal tailed Bayesian intervals (bars) for standardised coefficients of patient-specific covariates (b_{ace}: serum ACE activity; b_{hba}: HbA1c; b_{cpep}: stimulated C-peptide; b_{bmi}: body mass index; b_{awar}: hypoglycaemia awareness score; b_{ret1}: no retinopathy; b_{ret2}: background retinopathy, b_{ret3}: pre-proliferative retinopathy; b_{dur}: duration of diabetes; b_{type}: type of diabetes; b_{age}: age; b_{gen}: gender).



However, if subjects 1028 and 4003 (asymptomatic on 100% and 74% of episodes respectively) are excluded, only gender has a significant effect (females less consistent than males; b_{gen}= -0.43, 95% Bayesian interval – 0.82, - 0.03) (figure 5.5).

Figure 5.5

Posterior means (circles) and 95% equal tailed Bayesian intervals (bars) for standardised coefficients of patient-specific covariates after exclusion of subjects 1028 and 4003 (b_{ace}: serum ACE; b_{hba}: HbA1c; b_{cpep}: stimulated C-peptide; b_{bmi}: body mass index; b_{awar}: awareness score; b_{ret1}: no retinopathy; b_{ret2}: background retinopathy, b_{ret3}: pre-proliferative retinopathy; b_{dur}: diabetes duration; b_{type}: diabetes type; b_{age}: age; b_{gen}: gender)



5.4 Discussion

Whereas it has long been recognized that subjective symptoms can vary in different circumstances, the present study has demonstrated and quantified episode-to-episode, intra-individual variability in symptoms of hypoglycaemia reported by adults with type 1 and type 2 diabetes. It has also sought and found some determinants of inter-individual differences in this symptom (in)consistency. The statistical method that we have developed allows patient consistency to be mapped on a continuous scale, taking into account the involved statistical uncertainty. It is accepted that each individual's hypoglycaemia symptom complex is characteristic. However, the wide range and skewed distribution of the precision parameter σ_i^{-2} demonstrates that within-subject symptom profiles vary substantially between episodes and that people show marked individual differences with respect to their consistency of symptom reporting. Conversion of the precision parameter to the normalised consistency parameter c_i on a (0, 100) scale facilitates between and within-patient comparisons of consistency estimates, although there is no pre-defined cut-off to differentiate consistent and inconsistent individuals.

The most commonly reported symptoms in this study were weakness, decreased concentration, sweating and hunger. In studies where patients have previously been asked to indicate which symptoms they most associate with hypoglycaemia, the commonest symptoms were sweating, difficulty concentrating, decreased coordination and weakness (80%, 80%, 75% and 70% of respondents respectively) (148). It has previously been noted that the earliest symptoms to develop when blood glucose falls into the hypoglycaemic range are trembling, sweating, tiredness, decreased concentration and hunger (149). The symptoms which correlate most accurately with blood glucose levels are hunger, trembling and weakness (53%, 33% and 27% of people respectively) (150). Thus the symptoms most frequently reported in the present study are those that are appreciated to be the earliest perceived symptoms of hypoglycaemia (149) and those that are most commonly (148) and accurately (150) associated with hypoglycaemia. However, the main aim of this study was not to study population similarities but rather to examine intra-individual consistency of symptom reporting.

The statistical models, methodology and analyses in the present study raise some important points for patient education and hypoglycaemia research. Firstly, the skewed distribution of the estimated precision parameter, $\widetilde{\sigma}_i^{-2}$ (fig 2a), demonstrates that most subjects in this study exhibited low symptom reporting consistency. Thus, when patients are taught that their own hypoglycaemic symptoms are idiosyncratic, they should also be informed that their symptoms are likely to vary between episodes. Reinforcing this point may reduce the possibility of failure to recognise hypoglycaemia as a result of symptom variation.

Secondly, it is probably useful for patients to have an awareness of how consistent their symptoms are, given that individuals with four or more reliable hypoglycaemic symptoms are much more likely to correctly identify low blood glucose levels than individuals with fewer reliable symptoms (148).

Finally, previous studies have relied on very few snapshots of the hypoglycaemic symptom profile, either recorded during experimental hypoglycaemia (7,161,167,334) or documented retrospectively by patients in what was thought to represent their "typical" symptom profile (6,146,160,164,165). The findings of the present study challenge the validity of the latter approach for the purpose of advising individual patients, as the degree of between-episode variability is much greater than has previously been appreciated.

Of the factors examined, only female gender and normal hypoglycaemia awareness increased symptom variability in the initial analysis. Impaired awareness is usually associated with loss of autonomic warnings and increased reliance on neuroglycopenic symptoms. Individuals with impaired awareness therefore have a more restricted range of symptoms which may explain why their symptom variability is lower. In fact, the estimated consistency measure is negatively correlated to the total number of symptoms reported throughout all episodes ($\sum_{j,k} y_{i,j,k}$) for each

patient, suggesting that higher symptom reporting activity (as would be expected with normal awareness) may be associated with greater variation of reporting thresholds.

In order to assess the extent to which the two predominantly asymptomatic individuals affected the observed results, the GLM analysis was repeated after exclusion of the two individuals with awareness scores of 7 (subjects 1028 and 4003). These two subjects were asymptomatic on 100% and 74% of occasions respectively. Although impaired awareness was associated with increased symptom consistency in the initial analysis, this effect was no longer observed once these two subjects were excluded. This illustrates that one of the limitations of this analysis is that it cannot distinguish between a completely consistent person with full symptom awareness and a consistently asymptomatic individual. However, these subjects represent the extreme end of the spectrum of impaired awareness of hypoglycaemia. For individuals with fewer asymptomatic episodes, the presence or absence of symptoms contributes to the consistency of their symptom profile and it was therefore felt important not to exclude asymptomatic episodes completely from this analysis. However, it is probably reasonable to conclude that female gender is the only factor to systematically affect consistency and the observed effect of impaired awareness in the original analysis is attributable to a consistent *lack* of symptoms rather than a consistently reported set of symptoms.

It was surprising to find that the subject treated with oral agents was asymptomatic during 36% of episodes, despite recording a normal hypoglycaemia awareness score of 1. However, as all his episodes were confirmed with appropriate capillary glucose readings, his symptom reports were regarded as valid. In the UK, patients treated with oral agents are not routinely asked to check blood glucose levels. It is therefore likely that he had not realised that his awareness was impaired prior to participation in this study.

The relationship between consistency of symptom reporting and gender has not been reported previously. However, it is recognised that differences exist between males and females in their perception of hypoglycaemia. Symptoms of hypoglycaemia develop at similar blood glucose thresholds in men and women with type 1 diabetes

(62) but the magnitude of the counterregulatory response is lower in women (107) which may influence the intensity of the symptomatic response of autonomic symptoms, some of which are enhanced by catecholamine secretion. Female counterregulatory responses are less affected by antecedent hypoglycaemia and exercise than responses in men (335). It could be hypothesised that the gender differences in this study relate to under-reporting by females as a result of lower symptom intensity. In the present study, subjects were not asked to note the intensity of symptoms, so it is not possible to establish whether symptom intensity differed between males and females. Although there are gender differences in the magnitude of the symptomatic and counterregulatory responses to hypoglycaemia, a study reviewing symptoms that were recorded during experimentally-induced hypoglycaemia in 160 adults (with and without diabetes) did not find any evidence of a gender effect on the nature of the symptoms (167)

It is possible that other factors, such as the activities engaging the individual at the time of the episode, may have an effect on symptom consistency but it would be logistically difficult to study these in greater detail. Earlier work has classified hypoglycaemic symptoms in physiological terms (6,158,160,164,165). Appropriate grouping of symptoms may be able to account for additional sources of between-group variation for an individual patient in the model, thus giving scope for including relevant effects for symptom groups in future analyses.

Not all hypoglycaemic episodes in this study were confirmed biochemically. However, the presence of typical symptoms which resolve with ingestion of carbohydrate is conventionally taken as evidence of hypoglycaemia. Insistence on biochemical corroboration would have further restricted the number of episodes available for analysis and most episodes (91%) were confirmed with capillary glucose readings.

A difficulty also arises with the definition of hypoglycaemia according to biochemical parameters when subjects are asymptomatic. Blood glucose and symptom data for this study were obtained from a multicentre epidemiological study (212), where subjects were asked to record all episodes associated with a capillary glucose <3.0

mmol/l or any episodes associated with symptoms typical of hypoglycaemia. However, as hospital clinics frequently advise patients not to let blood glucose levels drop below 4 mmol/l, patients did send in hypoglycaemia recording sheets with blood glucose readings between 3 and 4 mmol/l in the absence of symptoms. As these episodes were considered valid and were included in the epidemiological study they were also included in the statistical analysis in this study. It was felt that they could not be excluded when similar blood glucose readings from symptomatic patients were being included. However, it is possible that some of the asymptomatic readings between 3 and 4 mmol/l included in this study were essentially episodes of normoglycaemia and this represents a potential limitation of this study. For future work, a more robust approach would be to pre-define a more definite cut-off for asymptomatic hypoglycaemia, such as a blood glucose level of 3.2 mmol/l or less, as this blood glucose level is known to be associated with neuroglycopenia

In subjects with normal awareness, it would be interesting to stratify episodes according to blood glucose level to investigate whether this had an effect on symptom reporting, as it could be hypothesised that the depth of the glucose nadir might affect the range (and intensity) of the symptoms reported. However, this was not possible in the present study for several reasons. Although a fall in blood glucose in a non-diabetic adult triggers the secretion of counterregulatory hormones and the onset of cognitive and symptomatic changes at reproducible blood glucose thresholds (60,62) within a defined hierarchy (61), these thresholds become altered in diabetes and the same blood glucose level may affect individuals with diabetes in different ways. Secondly, data from field studies will never be as controlled as data generated in a lab. In the present study, confirmation of hypoglycaemia may have occurred several minutes before or after rescue carbohydrate was administered so blood glucose measurement may not have coincided exactly with the blood glucose nadir or the peak of symptom intensity. Finally, blood glucose meters are less accurate in the hypoglycaemic range and it would not have been possible to confirm these readings with venous samples outside the confines of a tightly regulated laboratory study.

The study has several strengths, including its size (2699 episodes of hypoglycaemia), its prospective design and its duration (9-12 months). Although some previous

studies have collected symptoms prospectively (4,150,336), they have not attempted to compare symptoms between episodes or to compile a representative list of symptoms from those reported during different episodes. Furthermore, prospective field data could be regarded as more generalisable than hypoglycaemia data collected under laboratory conditions.

The present study demonstrates that: intra-individual between-episode symptom variability is much greater than has been previously appreciated and that there are marked individual differences in this consistency. Caution should be exercised when interpreting patients' retrospective recall of what they regard to be their "typical" hypoglycaemic symptoms. Female gender was the only factor found to have a systematic association with increased variability of the symptom complex. Given this observed variability, clinicians should advise patients against being too dogmatic in their perception of what constitutes their cardinal hypoglycaemic symptoms, as these may vary considerably between episodes. This variability should also be considered when interpreting hypoglycaemic symptom responses under different experimental conditions or when comparing different therapeutic interventions.

CHAPTER 6: RECOVERY OF COGNITIVE FUNCTION FOLLOWING HYPOGLYCAEMIA IN ADULTS WITH TYPE 1 DIABETES AND THE EFFECT OF IMPAIRED AWARENESS OF HYPOGLYCAEMIA

The data in this chapter have been published as a multi-author paper in a peer-reviewed journal (See appendix). The first draft of this chapter was written entirely by me and I coordinated all subsequent editing. Professor Ian Deary provided advice on statistical analysis but I performed the statistical analyses and wrote up the results. Co-authors on this paper provided editorial input, corrections and comments but did not write any individual sections of this paper.

6.1 Introduction

Clinical observation has suggested that the recovery of cognitive function following hypoglycaemia often lags behind the restoration of biochemical euglycaemia. Previous studies which have attempted to quantify the time taken for cognitive recovery have been limited by factors such as recruitment of non-diabetic volunteers (279,281,282), small sample sizes and consequent lack of statistical power (282), lack of a euglycaemic control arm (281,337), the use of neurophysiological measurements rather than direct tests of cognitive function (279-281,284) or the use of cognitive tests which are not reliably affected by hypoglycaemia (277-280). Precise measurement of the time taken for recovery requires repeated testing, but many studies have restricted cognitive testing to just one or two time points (282,284,337) and therefore cannot accurately define the recovery phase. Finally, the interval between the restoration of euglycaemia and the testing of cognitive function has mostly been ill-defined, and consistency of the timing of cognitive testing between

different participants was not assured (279,280,284,337). Comparison of results from different centres is frequently hindered by methodological variation in hypoglycaemia studies (243) with relevant variables including method of measurement of blood glucose (278) and the target level for, and duration of, hypoglycaemia (284).

In addition, it has been observed that the inter-individual variation in the degree to which hypoglycaemia affects cognitive function is very wide (243). However, no consensus exists as to whether either impaired awareness of hypoglycaemia or recurrent antecedent hypoglycaemia are associated with the relative preservation of cognitive function during hypoglycaemia (62,78,273,290,292,316,317) or an exacerbation of the decrement in cognitive performance associated with hypoglycaemia (65,285,318).

The present study has examined the time taken for recovery of tests representing various domains of cognitive function in a group of individuals with type 1 diabetes, and assessed the effect of their state of awareness on the response to, and recovery from, hypoglycaemia.

6.2 Methods

The local medical research ethics committee approved the protocol. Subjects gave informed consent for participation.

6.2.1 Subjects

Subjects were eligible for inclusion if they were between 18-45 years of age with a diagnosis of type 1 diabetes of at least 2 years duration and HbA1c values between 7 and 10% within the preceding 12 months. Volunteers were excluded in the event of pregnancy, co-existent systemic disease or malignancy, a past history of head injury, epilepsy, hypoglycaemia-induced seizure, chronic alcoholism or psychiatric disorder.

Thirty-six subjects with type 1 diabetes were recruited, 20 with normal hypoglycaemia awareness (NHA) and 16 with impaired hypoglycaemia awareness (IHA), confirmed by documenting their hypoglycaemia history and using a validated hypoglycaemia awareness scale (170). Each subject was asked "Do you get warning of your hypos?" and asked to select a number on a scale from 1 ("Always") to 7 ("Never"). People who chose 1-2, and reported no history of severe hypoglycaemia or subjective change in their glycaemic threshold for symptoms, were categorised as having normal awareness. Those who scored between 3-7 were categorised as having impaired awareness

The subjects' clinical characteristics are given in table 1. Microvascular complications were defined as any clinical diagnosis of diabetic retinopathy, neuropathy or nephropathy, the latter requiring urine albumin:creatinine ratio persistently above the local reference maximum or serum creatinine > 150 μmol/l. The presence of retinopathy was determined by digital retinal photography while the presence of peripheral neuropathy was confirmed on the basis of clinical examination using a tuning fork and 10g monofilament. HbA1c was measured by high-performance liquid chromatography (Variant II haemoglobin Testing System; BioRad Diagostics Group, Hercules, CA) with a local non-diabetic reference range of 4.3-6.5%. The IHA group had a longer duration of diabetes (median [range] 33.5 [22-43]

years) compared to the NHA group (29 [19-44] years; $p<0.001$) and a higher prevalence of microvascular complications (6 patients in IHA group, 1 patient in NHA group, $\chi^2 = 5.994$, $p=0.013$). Other comparisons (sex, age, $HbA1_c$, BMI) were non-significant.

Table 6.1.

Characteristics of participants with type 1 diabetes in study. Data are mean (SD) unless stated otherwise.

Hypoglycaemia awareness status	Normal	Impaired
Number	20	16
Male : female	12 : 8	6 : 10
Median age (range) (years)	29 (19-44)	33.5 (22-43)
Median duration of type 1 diabetes (range) (years)	3.8 (1.1-20)	15.5 (2-35)
$HbA1_c$ (%) (Non-diabetic range 4.0-6.5%)	7.8 (1.3)	8.4 (1.8)
BMI ($kg\ m^{-2}$)	25.8 (2.2)	26.8 (3.6)
Number (%) with microvascular complications	1 (5)	6 (38)

6.2.2 Glucose clamp procedure

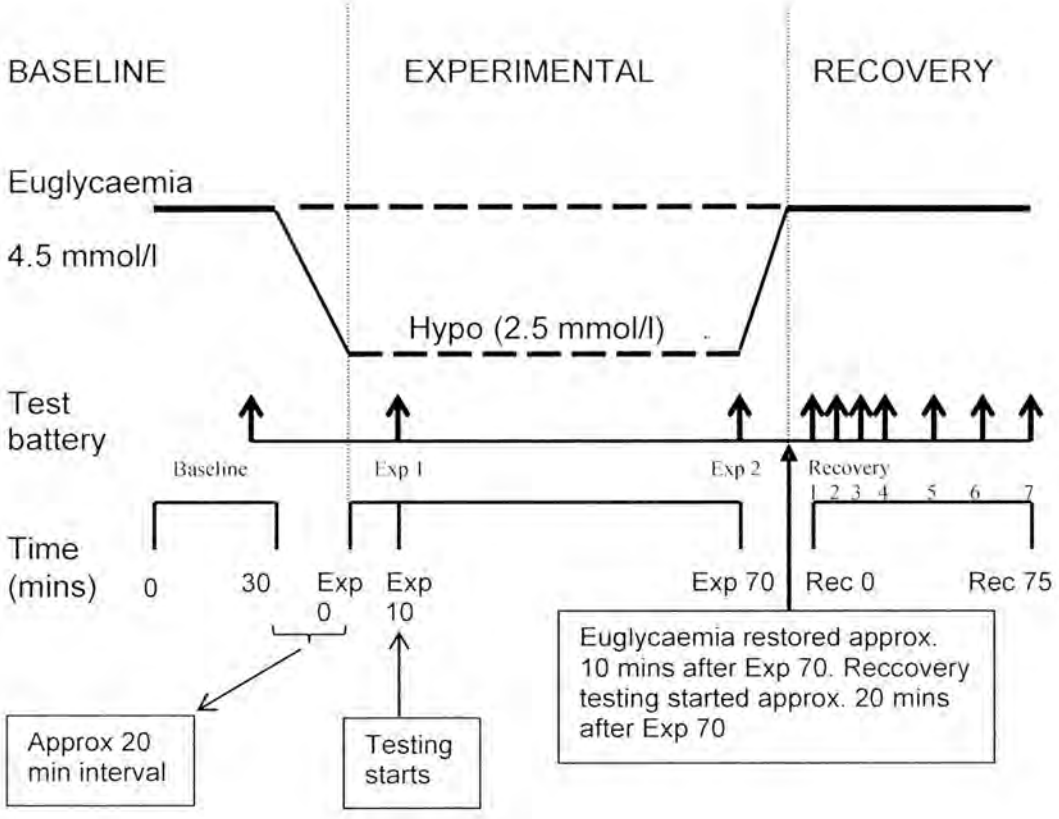
Each subject underwent one hypoglycaemic and one euglycaemic clamp, separated by at least two weeks. The order of the experimental condition (hypoglycaemia versus euglycaemia) was randomised and counterbalanced and subjects were not informed of the order of occurrence. Clamp sessions commenced at 08.00h after an overnight fast from 22.00h and subjects were asked to omit their usual morning insulin dose on study days. In order to avoid effects of antecedent hypoglycaemia, six studies were postponed (2 NHA, 4 IHA) because of symptomatic hypoglycaemia or blood glucose < 4.0mmol/l during the preceding 48h.

An ante-cubital vein and a dorsal hand vein were cannulated in the non-dominant arm for infusions and blood sampling. The dorsal hand vein was cannulated in a retrograde fashion and the hand was wrapped in a heated blanket to arterialise venous blood. Arterialised venous samples were drawn every 5 minutes, and whole-blood glucose was measured using a Yellow Springs 2300 analyser (Yellow Springs Instruments, Yellow Springs, Ohio).

The study design is illustrated in figure 6.1. Using a modified hyperinsulinaemic glucose clamp technique (74), an infusion of soluble human insulin (Actrapid, NovoNordisk) at 1.5 mU kg^{-1} min^{-1} was commenced, and 20% glucose solution was infused at a variable rate to achieve the target blood glucose levels. Blood glucose was stabilised at 4.5mmol/l (euglycaemia) and maintained for 30 minutes while subjects practised the cognitive tests (baseline phase). In the euglycaemic condition, glucose was maintained at this level during the experimental phase. In the hypoglycaemic condition blood glucose was lowered over 20 minutes to 2.5mmol/l, where it was maintained for one hour (experimental phase). It was not always possible to drop blood glucose in exactly 20 minutes so this interval is approximate. Testing during the hypoglycaemic condition began once blood glucose dropped to 2.5 mmol/l or below. Following the experimental phase, euglycaemia (>4.0mmol/l) was then rapidly restored. The recovery phase start was defined by two consecutive arterialized glucose readings $\geq$ 4mmol/l, with blood glucose tested every 5 minutes throughout the study. Cognitive testing in the recovery period commenced 10 minutes after the first of two consecutive euglycaemic readings.

Figure 6.1

Study outline. Cognitive function and symptoms were tested at baseline, at the beginning and end of the experimental condition and during the recovery period at 10, 20, 30, 40, 55, 70 and 85 minutes after euglycaemia was restored.



6.2.3 Symptom scores and cognitive function tests

The cognitive battery consisted of three tests which are sensitive to hypoglycaemia (144) and are easy to administer repeatedly. The tests were:

1. *Trail making B* (TMB): This test is taken from the Halsted Reitan battery (338) and assesses mental flexibility and executive function. A modified version was used where the subject was presented with a hand-held computer displaying a grid with randomly positioned numbers and letters (339). These must be tapped sequentially using a hand-held pen, alternating between numbers in ascending order and letters in alphabetical order. The score is the time taken to complete the task.
2. *Digit Symbol Substitution Test* (DSST): Taken from the Wechsler adult intelligence scale (340). This test of processing speed involves the

substitution of symbols for digits using a given code. Subjects are scored for the number of symbols correctly substituted within 2 minutes

3. *Four Choice Reaction Time* (CRT). In this test of processing speed, the numbers 1-4 are presented repeatedly and in random order on a small screen. Subjects are asked to press one of four numbered buttons corresponding to the number on the screen. The speed of reaction and accuracy are recorded.

Symptoms were recorded using the Edinburgh Hypoglycaemia Scale (6), which lists symptoms of hypoglycaemia and allows each to be graded in intensity on a visual analogue scale of 1-7. The cognitive battery and the Edinburgh Hypoglycaemia Scale were applied at baseline, at the beginning and end of the experimental phase, and during the recovery period at 10, 20, 30, 40, 55, 70 and 85 minutes after euglycaemia was restored (figure 1).

6.2.4 Statistical analysis

Cognitive scores were compared using general linear modelling (repeated-measures analysis of variance [ANOVA]). In the full model, including all subjects, hypoglycaemia awareness status was the between-subjects factors. The experimentally-induced state of hypoglycaemia versus euglycaemia was the within-subjects factor. Age, sex, duration of diabetes and order of exposure to hypoglycaemia had no significant effect on the results, so these fixed effects/covariables were excluded from the final model. Individuals' test scores within a single clamp study were corrected for baseline performance by subtracting their baseline score from their scores at each time point. The model compared these adjusted scores between the euglycaemic and hypoglycaemic conditions (repeated measure). The effects of hypoglycaemia in NHA and IHA groups separately are also reported. Statistical significance was accepted at $p<0.05$. Partial η^2 was used to indicate effect size. Analyses were performed using SPSS for Windows version 12.0. With 20 subjects in each group, the power of the study in detecting a 0.5 standard deviation change in any test (assuming $\alpha=0.05$, reliability of test=0.8) is 94%. Using the same assumptions, the power of detecting a 0.33 standard deviation change is 63%.

6.3 Results

6.3.1 Blood glucose results

During the euglycaemia studies, mean (SD) blood glucose concentrations were 4.5 (0.2) mmol/l in the NHA group and 4.5 (0.3) mmol/l in the IHA group (p=0.643). During the hypoglycaemia condition, blood glucose was maintained at 2.5 (0.2) mmol/l in both the NHA and IHA groups (p=0.468) (Figure 6.2).

Figure 6.2a

Mean blood glucose values during clamp study on euglycaemic and hypoglycaemic sessions: Normal awareness group

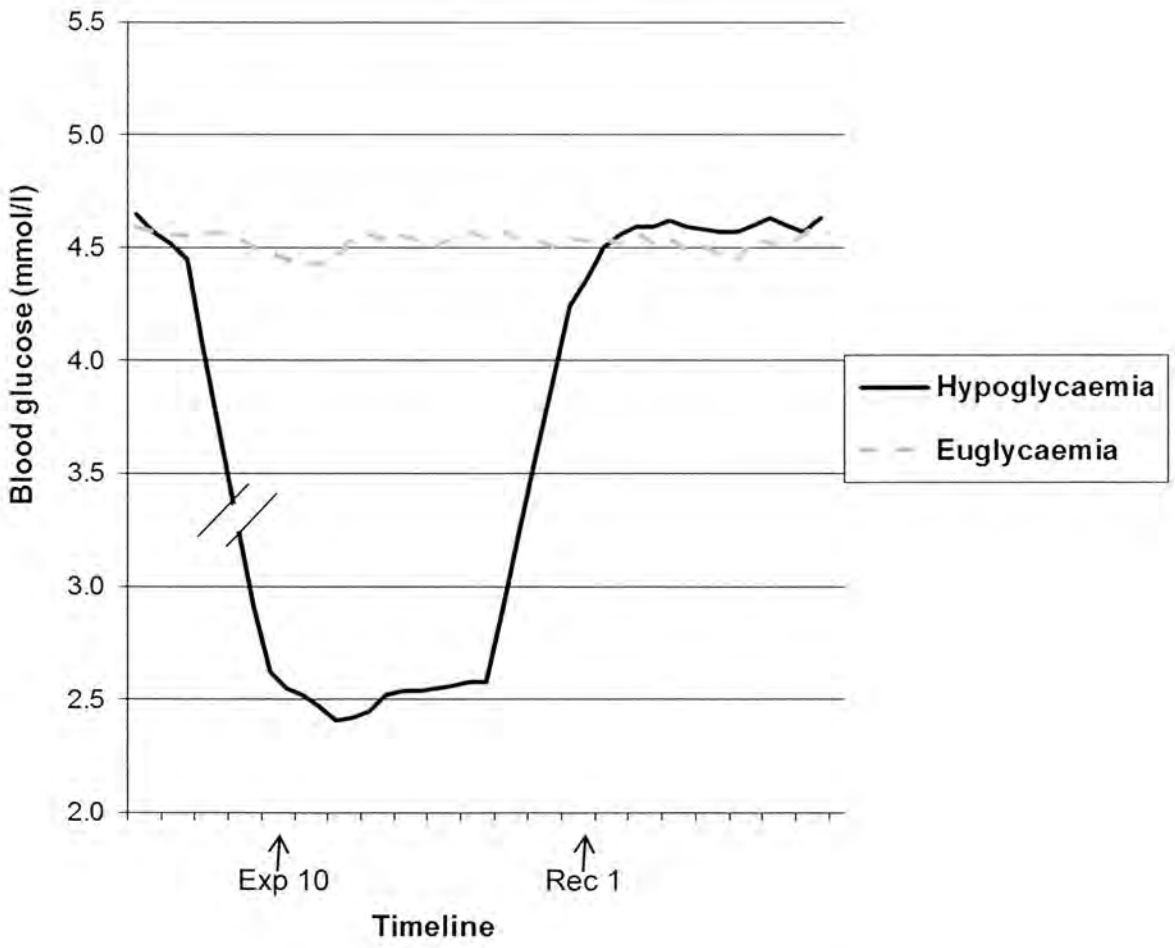
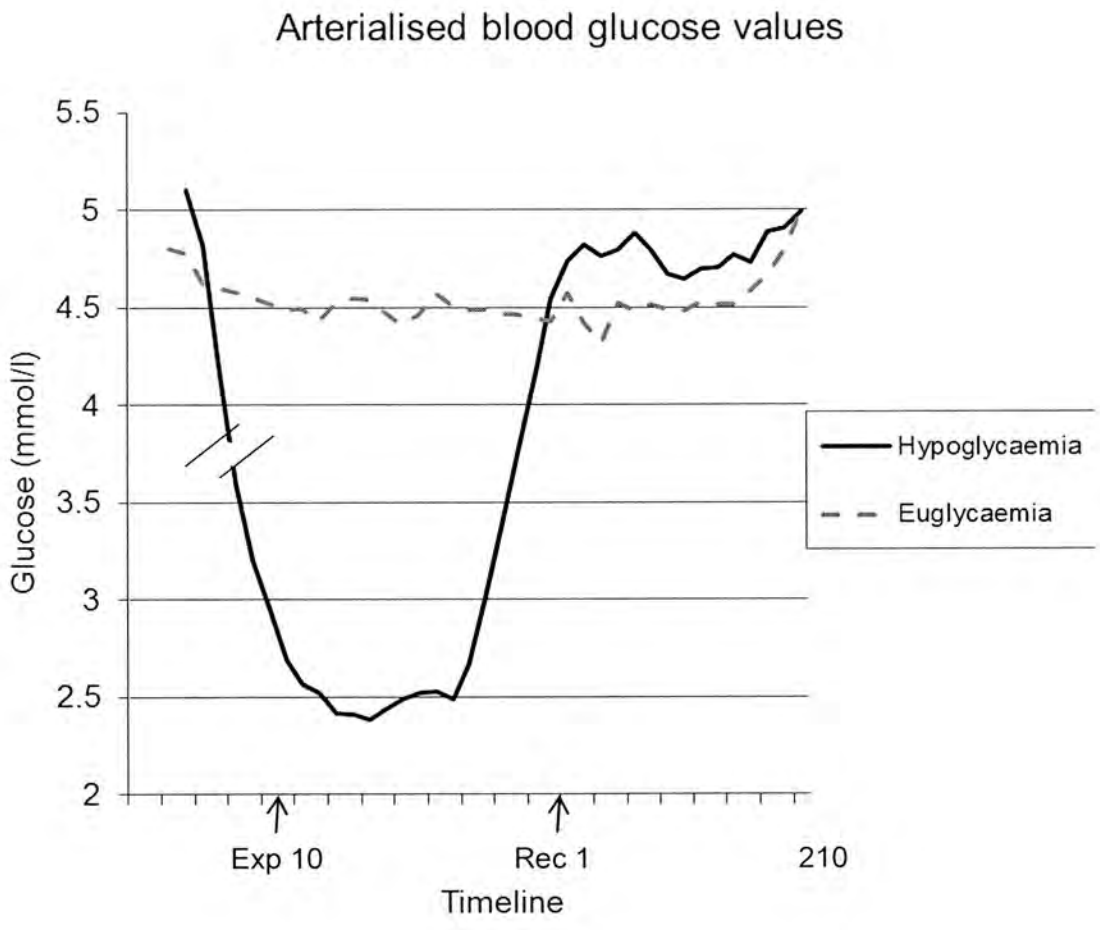


Figure 6.2b

Mean blood glucose values during clamp study on euglycaemic and hypoglycaemic sessions: Impaired awareness group



6.3.2 Symptom scores

Total symptom scores did not change during the euglycaemia condition. During the hypoglycaemia condition the mean (SD) symptom scores rose in the NHA group (baseline 23.2 (4.4) versus experimental 44.1 (22.2); $p<0.001$) and IHA subjects (baseline 22.9 (7.0) versus experimental 28.8 (8.3); $p=0.001$). The increment in symptom scores was greater in the NHA group ($p=0.002$). Symptom scores reverted to baseline immediately after euglycaemia was restored in both groups.

6.3.3 Cognitive tests

Tables 6.2, 6.3 and 6.4 show mean (SD) test scores corrected for baseline performance (i.e. each score is the difference between the baseline score and the score at that time point) for CRT, DSST and TMB test respectively. CRT and TMB scores are completion times so a lower score represents better performance. The DSST score is the number of items completed in two minutes so a higher score represents better performance. The effects of glycaemic condition were first examined *within* NHA and IHA groups, and then for all subjects combined including interaction between glycaemic condition and awareness status. A considerable practise effect was apparent on the DSST task but not on the CRT and TMB tasks. The randomised counterbalanced study design controls for practise effects.

6.3.3.1 NHA subjects

Performance on all cognitive tests was significantly impaired during hypoglycaemia in NHA subjects (tables 6.2-6.4 and figures 6.3a, 6.4a, 6.5a). Performance on DSST and TMB deteriorated significantly during hypoglycaemia but reverted to baseline as soon as euglycaemia was restored (tables 6.3-6.4). CRT remained impaired after restoration of euglycaemia, with significant differences between the hypoglycaemic and euglycaemic conditions at 20, 30, 40 and 75 minutes (table 6.2 and figure 6.3a).

Table 6.2

Mean (SD) change from baseline on Choice Reaction Time (CRT) test scores (in milliseconds) and effect of glycaemic condition and glycaemic-awareness interaction on scores. Significant differences shaded in grey. EU=Euglycaemia, HYPO=hypoglycaemia, Exp=experimental phase, Rec=recovery

	Normal awareness				Impaired awareness				All subjects combined					
	EU	HYPO	p	η^2	EU	HYPO	p	η^2	EU	HYPO	Effect of EU/HYPO		Gly/awareness interaction	
											p	η^2	p	η^2
CRT Exp1	**-8.7 (30.3)**	**51.2 (35.9)**	**<0.001**	**0.762**	5.7 (39.4)	34.1 (44.9)	0.124	0.161	**-2.3 (34.9)**	**43.6 (40.5)**	**<0.0001**	**0.435**	0.087	0.089
CRT Exp2	**-14.6 (53.6)**	**68.2 (51.8)**	**<0.001**	**0.690**	7.9 (39.0)	44.2 (54.7)	0.092	0.189	**-4.6 (48.4)**	**57.2 (53.8)**	**<0.0001**	**0.459**	**0.045**	**0.124**
CRT Rec1	-0.6 (57.8)	20.0 (45.4)	0.169	0.102	-3.4 (51.9)	13.1 (36.8)	0.283	0.082	-1.9 (54.5)	16.9 (41.4)	0.084	0.090	0.848	0.001
CRT Rec2	**-4.5 (50.8)**	**38.7 (60.8)**	**0.002**	**0.409**	-18.3 (66.7)	18.6 (23.3)	0.136	0.163	**-10.6 (57.7)**	**30.1 (48.9)**	**0.002**	**0.264**	0.672	0.006
CRT Rec3	**-15.2 (42.0)**	**23.2 (73.5)**	**0.005**	**0.360**	14.5 (44.4)	20.3 (42.5)	0.696	0.011	**-2.0 (45.0)**	**21.9 (60.9)**	**0.024**	**0.149**	0.089	0.088
CRT Rec4	**-3.8 (53.2)**	**28.0 (62.2)**	**0.010**	**0.313**	15.5 (46.1)	20.4 (40.3)	0.715	0.010	**4.8 (50.4)**	**24.6 (52.9)**	**0.040**	**0.125**	0.128	0.071
CRT Rec5	2.2 (47.0)	19.5 (44.4)	0.075	0.166	13.8 (49.0)	8.3 (44.0)	0.736	0.008	7.3 (47.5)	14.5 (43.9)	0.501	0.014	0.201	0.051
CRT Rec6	**-15.4 (55.4)**	**8.6 (50.1)**	**0.03**	**0.237**	17.4 (60.1)	18.1 (39.9)	0.974	0.000	-0.8 (59.1)	12.8 (45.5)	0.231	0.044	0.255	0.040
CRT Rec7	-9.6 (51.3	10.7 (59.7)	0.168	0.109	25.1 (57.0)	5.6 (43.2)	0.251	0.093	5.8 (55.9)	8.3 (52.1)	0.934	<0.0001	0.073	0.100

Table 6.3

Mean (SD) change from baseline on Digit Symbol Substitution Test (DSST) test scores and effect of glycaemic condition and glycaemic-awareness interaction on scores. Significant differences shaded in grey. Exp=experimental phase, Rec=recovery. DSST score is the number of symbols identified in 2 minutes.

	Normal awareness				Impaired awareness				All subjects combined					
	EU	HYPO	p	η^2	EU	HYPO	p	η^2	EU	HYPO	Effect of EU/HYPO		Gly/awareness interaction	
											p	η^2	p	η^2
DSST Exp1	**8.1 (5.3)**	**-6.1 (11.0)**	**<0.001**	**0.664**	5.6 (5.7)	1.1 (9.1)	0.082	0.201	**7.0 (5.5)**	**-2.9 (10.7)**	**<0.001**	**0.481**	**0.009**	**0.195**
DSST Exp2	**12.2 (6.0)**	**-0.9 (10.2)**	**<0.001**	**0.652**	**15.6 (8.7)**	**8.7 (11.4)**	**0.041**	**0.266**	**13.7 (7.4)**	**3.4 (11.6)**	**<0.001**	**0.474**	0.106	0.080
DSST Rec1	11.5 (8.0)	9.0 (7.9)	0.350	0.049	17.2 (10.1)	12.6 (11.3)	0.176	0.127	14.0 (9.3)	10.6 (9.6)	0.092	0.086	0.605	0.008
DSST Rec2	13.8 (9.0)	11.3 (9.1)	0.333	0.052	19.6 (13.1)	15.3 (10.7)	0.198	0.115	16.4 (11.2)	13.1 (9.9)	0.097	0.084	0.633	0.007
DSST Rec3	13.8 (9.5)	11.9 (10.5)	0.461	0.031	20.6 (13.3)	19.3 (12.8)	0.720	0.009	16.8 (11.7)	15.2 (12.0)	0.454	0.018	0.877	0.001
DSST Rec4	18.3 (7.9)	13.8 (16.2)	0.187	0.095	22.3 (14.7)	18.9 (12.6)	0.280	0.083	20.1 (11.4)	16.0 (14.7)	0.093	0.086	0.818	0.002
DSST Rec5	17.8 (8.3)	16.5 (11.4)	0.683	0.009	24.5 (15.0)	21.2 (12.4)	0.312	0.073	20.8 (12.1)	18.6 (11.9)	0.319	0.031	0.673	0.006
DSST Rec6	18.5 (7.9)	19.4 (11.0)	0.786	0.004	24.4 (14.6)	20.5 (10.3)	0.192	0.118	21.1 (11.6)	19.9 (10.6)	0.485	0.015	0.278	0.037
DSST Rec7	17.8 (11.4)	19.9 (11.6)	0.532	0.023	22.7 (15.2)	21.3 (11.5)	0.674	0.013	20.0 (13.1)	20.5 (11.4)	0.841	0.001	0.460	0.018

Table 6.4

Mean (SD) change from baseline on Trail Making B (TMB) test scores (in seconds) and effect of glycaemic condition and glycaemic-awareness interaction on scores. Significant differences shaded in grey. EU=Euglycaemia, HYPO=hypoglycaemia, Exp=experimental phase, Rec=recovery

	Normal awareness				Impaired awareness				All subjects combined					
	EU	HYPO	p	η^2	EU	HYPO	p	η^2	EU	HYPO	Effect of EU/HYPO		Gly/awareness interaction	
											p	η^2	p	η^2
TMB Exp1	**1.9 (7.0)**	**7.8 (10.5)**	**0.026**	**0.259**	2.0 (12.9)	11.4 (18.9)	0.195	0.148	**1.9 (9.9)**	**9.4 (14.4)**	**0.042**	**0.135**	0.241	0.047
TMB Exp2	**6.3 (10.4)**	**14.6 (18.1)**	**0.025**	**0.263**	-0.1 (10.9)	10.5 (12.4)	0.053	0.300	**3.6 (10.9)**	**12.8 (15.8)**	**0.003**	**0.278**	0.772	0.003
TMB Rec1	1.8 (8.9)	4.4 (10.2)	0.174	0.106	2.6 (9.7)	7.9 (10.0)	0.08	0.217	**2.2 (9.1)**	**6.0 (10.1)**	**0.024**	**0.158**	0.608	0.009
TMB Rec2	3.6 (8.1)	2.1 (9.6)	0.765	0.005	3.0 (13.2)	11.5 (17.6)	0.250	0.109	3.3 (10.5)	6.2 (14.2)	0.251	0.045	0.173	0.063
TMB Rec3	1.9 (5.7)	0.4 (6.3)	0.562	0.020	2.4 (13.9)	0.9 (9.3)	0.621	0.019	2.1 (10.0)	0.6 (7.7)	0.481	0.017	0.712	0.005
TMB Rec4	-3.5 (8.4)	-5.4 (9.2)	0.840	0.020	-7.0 (13.9)	-2.9 (12.9)	0.449	0.045	-5.0 (11.1)	-4.3 (10.9)	0.522	0.014	0.406	0.023
TMB Rec5	-2.6 (9.4)	-5.1 (8.2)	0.580	0.018	-3.7 (19.7)	-4.8 (11.9)	0.999	0.000	-3.1 (14.6)	-5.0 (9.9)	0.848	0.001	0.846	0.001
TMB Rec6	0.8 (5.2)	-4.1 (8.1)	0.087	0.172	0.2 (13.1)	1.9 (11.5)	0.841	0.003	0.5 (9.4)	-1.4 (10.1)	0.594	0.010	0.353	0.030
TMB Rec7	-0.6 (8.0)	-5.3 (9.7)	0.290	0.070	1.0 (14.0)	-0.6 (8.0)	0.671	0.014	0.1 (10.9)	-3.2 (0.1)	0.328	0.033	0.809	0.002

Figure 6.3a

Mean (SE) times on CRT test during hypoglycaemia and euglycaemia conditions in individuals with normal awareness of hypoglycaemia.

Squares=EU Triangles=HYPO

*= $p<0.05$ EU vs HYPO

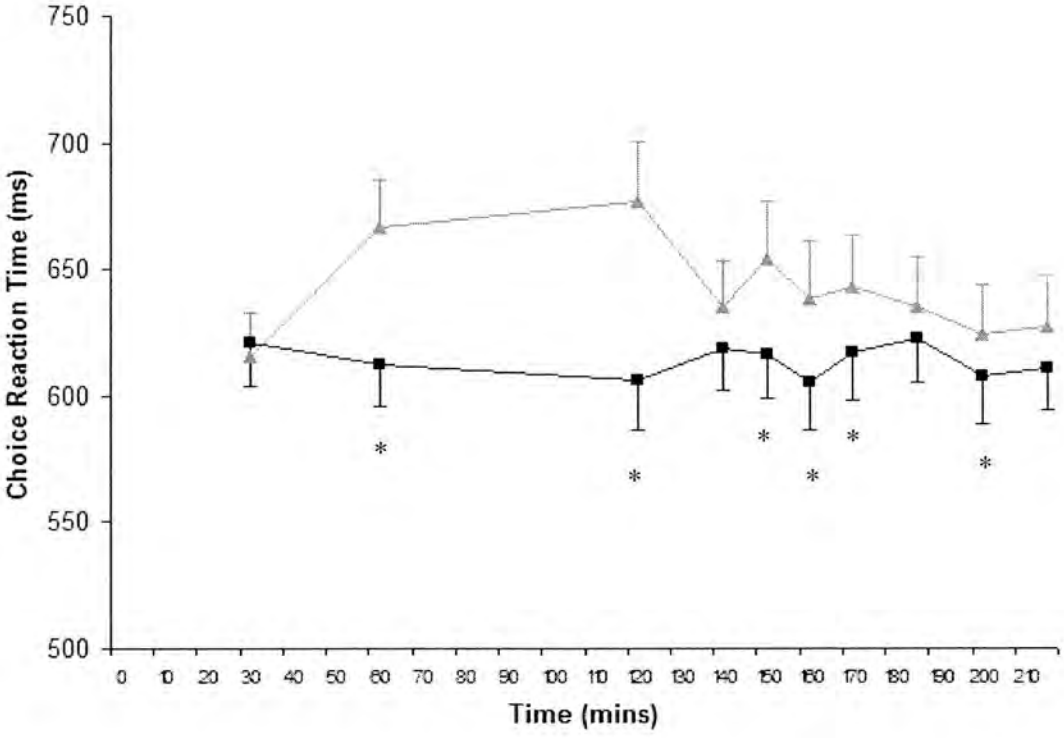


Figure 6.3b

Mean (SE) times on CRT test during hypoglycaemia and euglycaemia conditions in individuals with impaired awareness of hypoglycaemia.

Squares=EU Triangles=HYPO

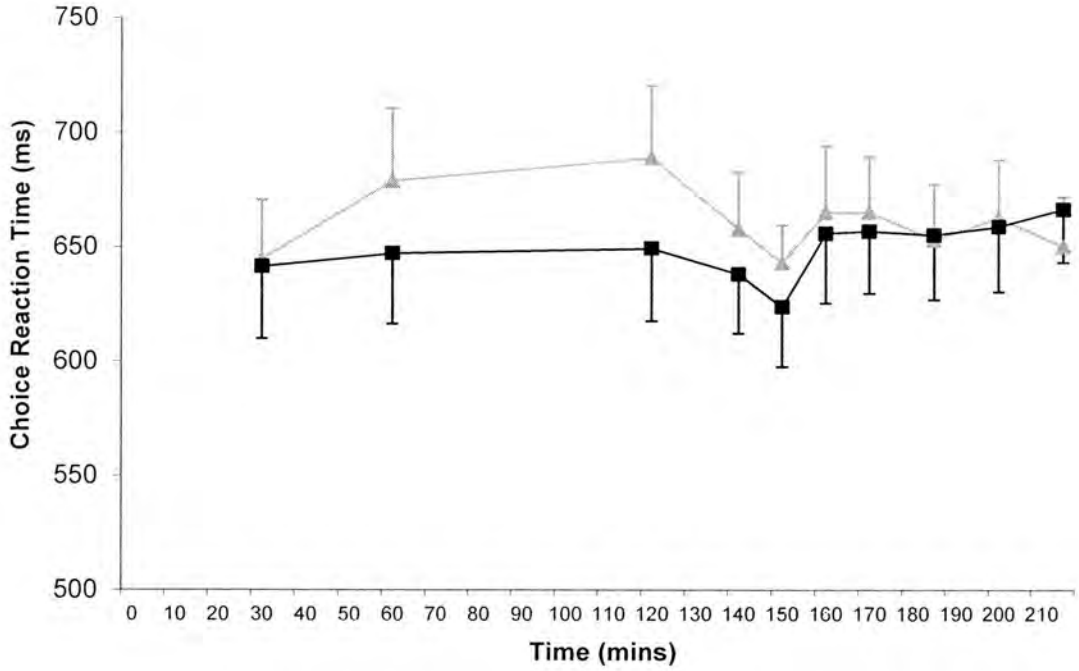


Figure 6.4a

Mean (SE) times on TMB test during hypoglycaemia and euglycaemia conditions in individuals with normal awareness of hypoglycaemia.

Squares=EU Triangles=HYPO

*= $p<0.05$ EU vs HYPO

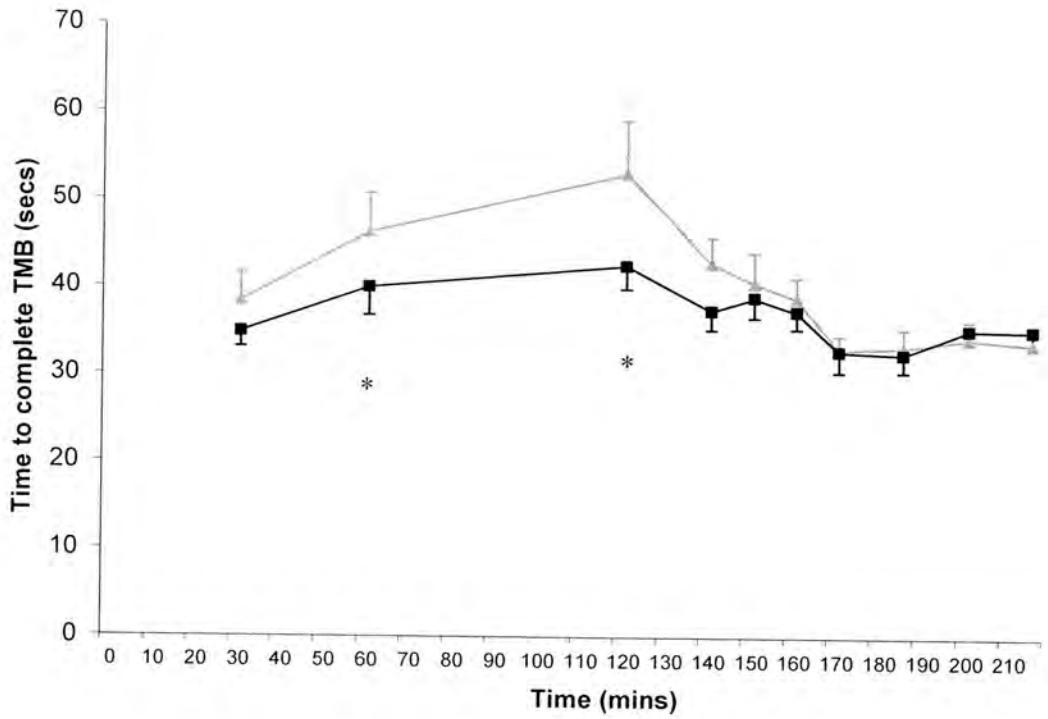


Figure 6.4b

Mean (SE) times on TMB test during hypoglycaemia and euglycaemia conditions in individuals with impaired awareness of hypoglycaemia.

Squares=EU Triangles=HYPO

◆ Data for one subject was omitted at this time point as it skewed the data markedly (required 212 seconds to complete the task during hypoglycaemia). The difference between hypoglycaemia and euglycaemia was not significant regardless of whether these data were included in the analysis.

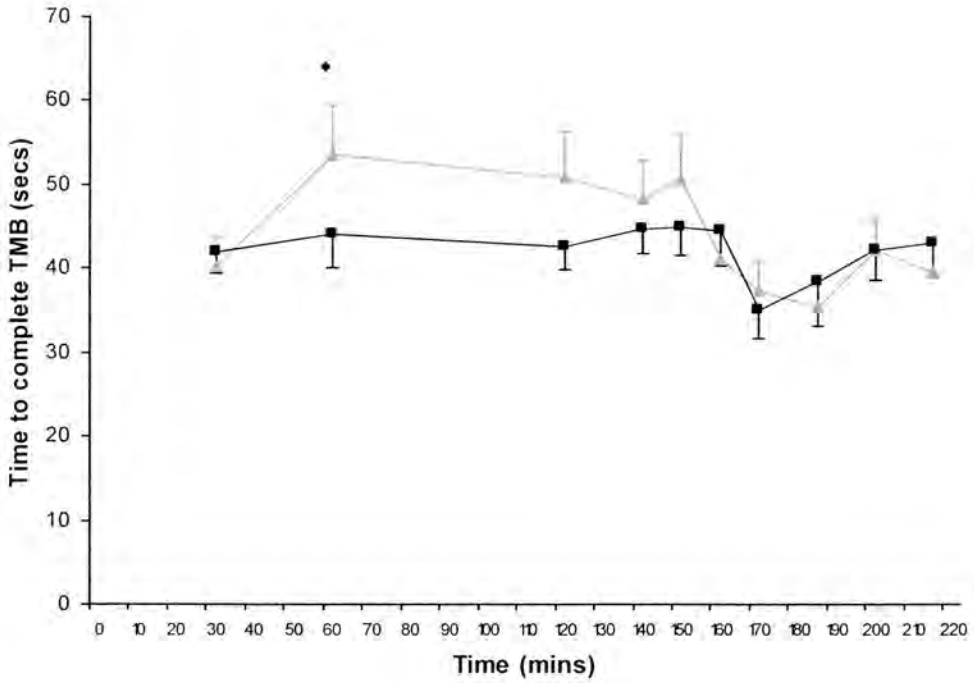


Figure 6.5a

Mean (SE) times on DSST during hypoglycaemia and euglycaemia conditions in individuals with normal awareness of hypoglycaemia.

Squares=EU Triangles=HYPO

*= $p<0.05$ EU vs HYPO

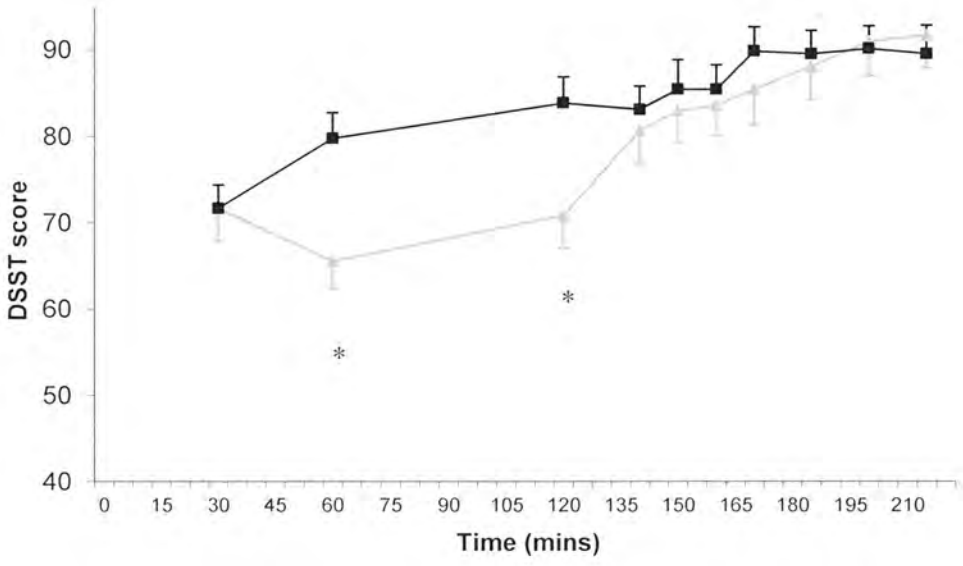
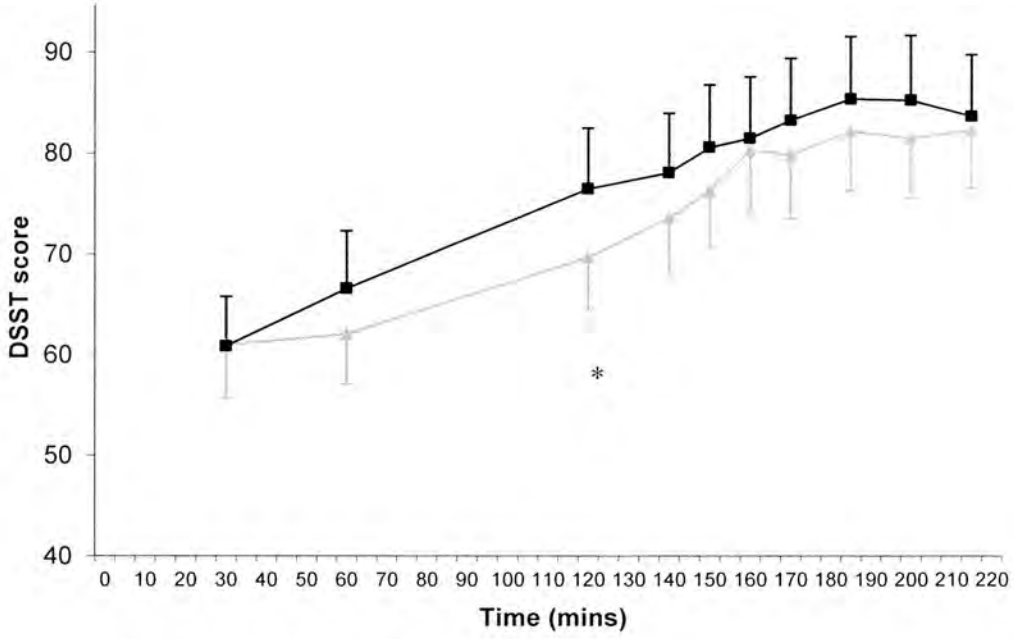


Figure 6.5b

Mean (SE) times on DSST test during hypoglycaemia and euglycaemia conditions in individuals with impaired awareness of hypoglycaemia.

Squares=EU Triangles=HYPO



6.3.3.2 IHA subjects

In IHA subjects, cognitive tests did not show significant impairment during hypoglycaemia, with the exception of the DSST task after 60 minutes of hypoglycaemia ($p=0.041$; table 6.2, figures 6.3b, 6.4b, 6.5b). There were no significant differences between the two experimental conditions during the recovery phase. Compared with NHA subjects, trends towards a smaller deterioration in performance and more rapid recovery following hypoglycaemia were observed.

6.3.3.3 All subjects

Poorer performance during hypoglycaemia versus euglycaemia was seen for all cognitive tasks. This difference persisted for CRT at 20, 30 and 40 minutes after euglycaemia was restored ($p=0.04$, $\eta^2=0.125$) (table 6.2), and for TMB at 10 minutes after euglycaemia was restored ($p=0.024$, $\eta^2=0.158$) (table 6.4). There was no persistence of impairment of DSST performance (table 6.3).

6.3.3.4 Comparison of effect of hypoglycaemia in NHA and IHA subjects

DSST: The interaction between glycaemic condition and hypoglycaemia awareness (hereafter termed the glycaemia-awareness interaction) was significant only at the start of hypoglycaemia (table 6.3) ($p=0.009$), suggesting that hypoglycaemia caused significantly greater impairment in NHA subjects than in IHA subjects.

CRT: Performance was impaired during hypoglycaemia and at 20, 30 and 40 minutes after euglycaemia was restored ($p=0.04$, $\eta^2=0.125$). The glycaemia-awareness interaction was significant only at the end of hypoglycaemia ($p=0.045$, $\eta^2=0.124$) (table 6.2). This infers that the NHA group were, relative to their baseline performance, more affected during hypoglycaemia than the IHA group, but there were no significant between-group differences during recovery.

TMB: The glycaemia-awareness interaction was not significant at any time point (table 6.4).

6.4 Discussion

Results from the present study suggest three conclusions. Firstly, in all subjects combined, cognitive performance was significantly impaired during hypoglycaemia by comparison with euglycaemia, consistent with numerous previous studies (144).

Secondly, when subjects were divided according to their state of hypoglycaemia awareness, cognitive performance was significantly impaired in NHA subjects, whereas only non-significant trends were seen in IHA subjects. This difference appears to suggest that individuals with IHA are less affected by hypoglycaemia than those with NHA. A formal NHA-IHA difference requires a significant interaction between awareness status and glycaemic condition; this was seen for CRT at the end of hypoglycaemia, and for DSST at the start of hypoglycaemia, without correction for multiple comparisons. This study therefore provides the first, but limited, evidence for a formally-tested difference in the cognitive effect of hypoglycaemia depending on state of awareness.

Thirdly, choice reaction time remained significantly prolonged up to 75 minutes after hypoglycaemia in NHA subjects (and up to 40 minutes in all subjects combined), and TMB completion time remained significantly prolonged 10 minutes after hypoglycaemia in all subjects combined. These data suggest that some aspects of cognitive function remain impaired for a clinically significant time after correction of hypoglycaemia.

The absolute differences in CRT between the groups were small. There was a trend toward improvement in CRT during the euglycaemic condition in the NHA group with a corresponding deterioration in the IHA group. This highlights the importance of the euglycaemic control arm in that each group's performance during hypoglycaemia is compared to performance during euglycaemia and not to that of a different group, thus controlling for between-group differences that may not be apparent.

The present study has a strong power for within-subjects comparisons but is less powerful at detecting between-subjects differences; high power for a medium effect size difference between groups requires over 50 subjects per group. It is impossible to exclude some overlap in hypoglycaemia awareness between the two groups, because scoring methods require some degree of subjective self-assessment. The IHA subjects also had longer duration of diabetes and more microvascular disease, although as IHA appears to be strongly associated with diabetes duration, it may be impossible to match for these characteristics. Finally, asymptomatic hypoglycaemia prior to the study cannot be excluded, particularly in IHA subjects, despite the frequent monitoring of blood glucose for the preceding 48h.

If the NHA-IHA differences are accepted, they suggest that IHA subjects develop cerebral adaptation to hypoglycaemia. This interpretation may appear to be counterintuitive as these individuals have a higher risk of severe hypoglycaemia than those with NHA (170,171). However, this adaptation may increase their susceptibility to severe hypoglycaemia by limiting the time to identify low blood glucose and allowing progression to debilitating neuroglycopenia. The degree of cognitive adaptation acquired by those with IHA is insufficient to compensate completely for the loss of physical symptoms.

Individuals with IHA are at increased risk of frequent asymptomatic hypoglycaemia. There is data to suggest that antecedent hypoglycaemia affects the subsequent physiological responses to further hypoglycaemic stimuli. The effects of antecedent hypoglycaemia on the counterregulatory responses to hypoglycaemia in people with diabetes have been well studied. In one early clamp study of 13 adults with type 1 diabetes, blood glucose was lowered to 3mmol/l and maintained at that level for 2 hours. Normoglycaemia was restored for one hour before a further hour of clamped hypoglycaemia (3mmol/l). Counterregulatory responses were reduced during the second period of hypoglycaemia (341). Similarly, counterregulatory responses to hypoglycaemia were attenuated in other studies when the interval between the antecedent episode and the studied episode of hypoglycaemia were as long as two (15) or even three days (342).

Antecedent hypoglycaemia can also affect the cognitive responses to hypoglycaemia, by shifting the thresholds for cognitive dysfunction to lower blood glucose levels in adults with type 1 diabetes (11,12,290,291). In one study, the antecedent hypoglycaemic stimulus consisted of twice weekly periods of clamped hypoglycaemia (mean glucose 2.8mmol/l for one month) (12) while in another study subjects' blood glucose was lowered to 2.6mmol/l for 3.5 hours during sleep (11). The decrement in cognitive ability during the final episode of hypoglycaemia was reduced in both these studies, indicating that a range of antecedent hypoglycaemic stimuli can alter the thresholds for cognitive dysfunction and attenuate the effects of subsequent hypoglycaemia. It has also been previously demonstrated that cognitive dysfunction begins at lower blood glucose levels in people with T1DM and IHA compared to those with NHA (78).

These effects of antecedent hypoglycaemia are not limited to individuals with diabetes. Glucose clamp studies in non-diabetic individuals have shown that 90-150 minutes of hypoglycaemia the day before cognitive testing attenuates the deterioration in short term memory, reaction time and auditory-evoked brain potentials (292-294) and avoidance of hypoglycaemia can restore the glucose thresholds for cognitive dysfunction to higher levels (62,273,274).

The suggestion therefore is that individuals with IHA experience frequent episodes of hypoglycaemia (often without symptoms), which then attenuate the effects of future episodes of hypoglycaemia. This would explain why cognitive function was not significantly affected in the IHA group in this study when performance was compared between the hypoglycaemic and euglycaemic conditions.

A smaller study from our centre by Gold and colleagues published in 1995 compared the effects of hypoglycaemia on cognitive function in 20 people with T1DM with either IHA or NHA and reported a trend towards poorer performance during hypoglycaemia in IHA subjects (285). Methodological differences exist between the

two studies, with the earlier study applying a cognitive battery of 20 minutes duration at one time point only, 10 minutes after euglycaemia was restored. This study was therefore unable to accurately quantify the time taken for recovery of cognitive function. The effect of awareness was not significant for any of the tests employed except for the Rapid Visual Information Processing (RVIP), where the results are given for RVIP hits, misses and reaction time. The result was not significant for the hits (ie correct answers) or reaction time but there was a significant effect on RVIP misses (ie there were more false positive responses in the group with impaired awareness). However, on this latter measure, the effect of the study condition (ie euglycaemia versus hypoglycaemia) was not significant. The cognitive tests used differed from the current study and CRT (the test yielding the most interesting results in the present study) was not used.

However, the study in 1995 by Gold and colleagues did employ a more robust definition of impaired awareness of hypoglycaemia than that used in the present study. The earlier study in 1994 which validated the Gold method of stratifying awareness of hypoglycaemia (170) established that people with type 1 diabetes had an almost six-fold increase in the incidence of hypoglycaemia if they had an awareness score of greater than four. In the 1995 study on the effects of hypoglycaemia unawareness on hypoglycaemia-induced cognitive dysfunction, the impaired awareness group scored 4 or more. However, in the present study, the median score for awareness was 4 (range 3-6) with seven participants selecting scores of 3. These participants clearly did not have full awareness of hypoglycaemia given that both the 1994 and 1995 Gold studies classified normal awareness as a score of 1-2. However, this group with an intermediate level of awareness may behave differently to a more clearly defined impaired awareness group. There may also be a significant degree of heterogeneity in this group as three out of the seven participants with awareness scores of 3 had not experienced any episodes of severe hypoglycaemia in the preceding year, while the remaining 4 reported 2 or 3 episodes in the preceding year. It is therefore difficult to fully ascribe the observed effects in the present study to impaired awareness when there were a number of patients with partial levels of hypoglycaemia awareness.

In addition, the present study had intended to recruit 20 participants to each arm. With 20 subjects in each group, the power of the study in detecting a 0.5 standard deviation change in any test, (assuming α=0.05, reliability of test=0.8) would have been 94%. Recruitment was halted after 16 participants were enrolled in the impaired awareness arm of the study. At this point, every patient documented to have impaired awareness on our hospital diabetes computer system had been invited to attend. In order to recruit the planned 20 subjects it would have been necessary to recruit from other centres and the result was that this study may have been slightly underpowered. However, at 36 participants, it is still a one of the largest clamp studies examining the effects of hypoglycaemia on cognitive function. For example, the 1995 study by Gold and colleagues comparing the effects of impaired awareness on hypoglycaemia-induced cognitive dysfunction only included 10 subjects with normal awareness and 10 with impaired awareness whereas the present study included a total of 36 participants.

There was inter-individual variability in the effects of hypoglycaemia on cognition, consistent with anecdotal observations. The present study was not sufficiently large to study the determinants of these differences formally. Thus, advice to individuals should not be too dogmatic given the possibility of inter-individual differences. Furthermore, although an hour of asymptomatic hypoglycaemia may occur frequently in individuals with IHA, hypoglycaemia is probably perceived and corrected earlier in those with NHA. Exposure to a shorter period of hypoglycaemia should be examined in a group of individuals with NHA to ascertain whether the duration of hypoglycaemia affects the recovery of cognitive function. It would also be interesting to examine whether other factors such as fatigue may have an additive effect on the delayed recovery of choice reaction time following hypoglycaemia.

Finally, it is important to exercise some caution when extrapolating findings of cognitive function studies to daily life. Although rapid reactions are undoubtedly required for several daily activities, most notably driving, reaction time does not operate in isolation. It is possible that other skills may be less affected following hypoglycaemia (as was the case with the TMB results in the present study) and that these may compensate for the slowing of reaction time. Recovery of cognitive

function has not been formally tested in a driving simulator. Our centre lacks the facilities to conduct such a study but a well-designed clamp study where performance on a driving simulator is assessed during and after hypoglycaemia would provide results of great ecological validity. Although these findings cannot be directly extrapolated to driving performance, they certainly support the advice currently given to patients by both the DVLA (Driver and Vehicle Licensing Agency) and health care professionals to avoid driving immediately after a period of hypoglycaemia. It is therefore of concern that in a survey of 202 insulin-treated diabetic drivers in Edinburgh, only 14% of participants would wait longer than 30 minutes after correction of hypoglycaemia before resuming driving (306).

The present study indicates that cognitive recovery is variable for different tasks but is prolonged for four-choice reaction time. It also provides evidence to support the concept of cognitive adaptation to hypoglycaemia in people with IHA, possibly as a consequence of recurrent exposure to hypoglycaemia. The delay in recovery of reaction time has implications for the safety of undertaking tasks requiring rapid responses immediately after hypoglycaemia.

CHAPTER 7: CONCLUSIONS AND FUTURE DIRECTIONS

There is already an extensive body of literature on the risk factors for severe hypoglycaemia, symptoms of hypoglycaemia and effects of hypoglycaemia on cognitive function. It is therefore pertinent to ask what the work in this thesis adds to previous studies in this area.

The first study was undertaken at a time when there was great interest in a potential role for serum ACE levels in predicting the risk of severe hypoglycaemia. Three Scandinavian studies (173,210,242) had previously suggested a strong link between increased serum ACE levels, mediated by the DD ACE genotype, and an increased risk of severe hypoglycaemia. Furthermore, it had been suggested that people with high ACE levels might be less able to function efficiently during periods of metabolic substrate deprivation. This could potentially be manifest by greater cognitive impairment during hypoglycaemia than in those with low ACE activity, which might partly explain the variable risk of developing severe hypoglycaemia amongst people with type 1 diabetes.

The concept was an attractive one. Had the link between serum ACE and severe hypoglycaemia been confirmed, it would have been interesting to conduct hypoglycaemic clamp studies in cohorts with high and low ACE levels to see whether those with high ACE levels experienced greater decrements in cognitive function than those with normal ACE levels. There are few therapeutic strategies available to reduce the risk of severe hypoglycaemia, but ACE inhibitors would have been interesting to investigate in this context.

However, the study reported in this thesis found that the association between serum ACE levels and the risk of severe hypoglycaemia was statistically significant but weak, with a low correlation coefficient. The statistical significance of the relationship was largely determined by three individuals who reported a very high frequency of severe hypoglycaemia. Furthermore, the incidence of severe hypoglycaemia did not differ significantly between subjects in the top and bottom

quartiles of serum ACE activity, suggesting that serum ACE is not sufficiently specific as a marker to allow hypoglycaemia risk stratification of people with type 1 diabetes.

Shortly before this study was published (343), two other studies were published which also suggested that there was no significant link between serum ACE levels and risk of severe hypoglycaemia in children and adolescents with type 1 diabetes (327) and in adults with type 2 diabetes (326). Although negative studies are often regarded as being less worthy of publication than positive studies, these three published studies, in different diabetic subgroups, are important in that they challenge the previously held view that serum ACE might be useful as a marker of severe hypoglycaemia. This in turn helps prevent unnecessary research into a putative link that is not strong enough to be clinically relevant.

The second study in this thesis examines the intra-individual, between-episode variability in the reporting of symptoms of hypoglycaemia. It is accepted that the symptoms of hypoglycaemia are idiosyncratic but it has also been assumed that each individual has a typical set of symptoms of hypoglycaemia. This study confirms that adults with type 1 and type 2 diabetes are much more variable in terms of symptom reporting than has previously been appreciated. This has implications for both hypoglycaemia research and for patient education.

Previous research has relied on "snapshots" of a patient's hypoglycaemia symptom profile, either recorded during experimental hypoglycaemia (7,161,167,334) or documented retrospectively by patients, who reported what they regarded as their "typical" symptom profile (6,146,160,164,165). The findings of the present study challenge the assumption that patients have a consistently reported set of hypoglycaemia symptoms because the degree of between-episode variability is much greater than has previously been appreciated.

Notwithstanding, the present study does not necessarily undermine previous research findings. For example, some of the studies where symptom profiles have previously

been gathered have contributed to the physiological grouping of hypoglycaemia symptoms in autonomic, neuroglycopenic and general malaise symptoms (6,146,160). The validity of these groupings has been confirmed by pharmacological studies which confirm the same symptom groupings. It is likely that a patient's summary of their "typical" symptoms is reasonably representative but the key point is that they will not express all these symptoms on every occasion and there may be some infrequently experienced symptoms which they do not include in their "typical" profile. It would be interesting to conduct further studies where subjects are asked to record their typical symptom profile at the start, which could then be compared to prospectively recorded symptoms over a period of time.

It is probable that the observed symptom variability is more relevant to patient education than it is to interpretation of previous research. The skewed distribution of the estimated precision parameter demonstrates that most subjects in this study exhibited low symptom reporting consistency. Thus, when patients are taught about hypoglycaemic symptoms, they should be informed that their symptoms are likely to vary between episodes in order to avoid any failure to recognise hypoglycaemia as a result of symptom variation. It is also useful for patients to have an awareness of how consistent their symptoms are, given that individuals with four or more reliable hypoglycaemic symptoms are much more likely to correctly identify low blood glucose levels than individuals with fewer reliable symptoms (148).

Of the factors examined, only female gender was consistently associated with increased symptom variability. It could be hypothesised that the gender differences in this study relate to under-reporting by females as a result of lower symptom intensity. In the present study, subjects were not asked to note the intensity of symptoms, so it is not possible to establish whether symptom intensity differed between males and females. It would be interesting to conduct further studies where subjects record both the nature and intensity of symptoms to see whether symptom intensity is related to the observed between-gender differences in the consistency of symptom reporting. Given that earlier work has classified hypoglycaemic symptoms in physiological terms (6,158,160,164,165), it would also be interesting to explore the effect of appropriate grouping of symptoms within the statistical model to see whether this

might account for additional sources of between-group variation for an individual patient.

The study has approached symptom analysis in a novel way and developed a statistical method of quantifying symptom variability. This study's size (2699 episodes of hypoglycaemia), its prospective design and its duration (9-12 months) are all notable strengths. This study has recently been published (344) and there is the potential to extend this work in future as discussed above.

The third study in this thesis examined the time taken for recovery of cognitive function following hypoglycaemia in adults with type 1 diabetes and either impaired or normal awareness of hypoglycaemia. It demonstrates a prolonged recovery for reaction time, with delayed reaction time evident in those with normal awareness up to 75 minutes after euglycaemia was restored. It also finds that subjects with impaired awareness of hypoglycaemia were less affected by low blood glucose levels, both during and after the period of hypoglycaemia, with only non-significant trends towards impaired performance evident on cognitive testing. Although the question of cerebral adaptation in those with impaired awareness remains a contentious issue, these findings are consistent with previous studies which support the concept of adaptation to hypoglycaemia as a result of repeated exposure to low blood glucose levels (65,285,318).

This study was designed to avoid the methodological limitations of earlier studies in this area. These robust results raise some further interesting questions. For example, it could be argued that an hour of hypoglycaemia would not be typical for individuals with normal awareness of hypoglycaemia, who would detect and treat the episode promptly. It is pertinent to ask whether the time taken for recovery is affected if the period of hypoglycaemia is brief. It could be hypothesised that a shorter period of hypoglycaemia might be followed by more rapid recovery of cognitive function. Following the publication of this study (250), our group is planning further clamp studies to investigate this in the future, where the study protocol would be replicated in individuals with normal awareness following a 20 minute period of hypoglycaemia.

It would also reasonable to explore the clinical significance of the observed delays in reaction time. While this study has demonstrated statistically significant delays in reaction time for a prolonged period after correction of hypoglycaemia, it is difficult to be certain what the practical relevance of these delays is. Driving is one activity which involves rapid reactions but as it also involves other domains of cognitive function, it is not possible to determine from the present study what the effect would be on aspects of driving such as braking speed or the ability to avert a collision. It would be interesting to explore the recovery of driving ability following hypoglycaemia using a well-designed clamp study and a driving simulator, but unfortunately our centre does not have the facilities to conduct such a study.

In conclusion, the current body of work adds to the existing literature on hypoglycaemia in a number of ways. Firstly, it contributes to the debate in two contentious areas by adding to the bodies of evidence which suggest that serum ACE is not a sufficiently sensitive marker of severe hypoglycaemia for clinical use and that adults with impaired awareness of hypoglycaemia can exhibit a degree of cerebral adaptation to hypoglycaemia. In addition, the data demonstrating significant delays in reaction time following restoration of euglycaemia and high intra-individual between-episode variability in hypoglycaemia symptom reporting are relevant to patient education. The work contained in this thesis has been published in high quality peer-reviewed diabetes journals and interesting questions have been raised which should open avenues to further useful hypoglycaemia studies.

Chapter 8

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APPENDIX 1:

STATISTICS FOR HYPOGLYCAEMIA SYMPTOM ANALYSIS

Model for intra-individual consistency

We have assumed that an individual's symptom profile depends on latent factors expressing the intensity of a given symptom on a given occasion and the individual's propensity to experience that symptom. The model that we develop implies that assessment of intra-individual consistency is based on a principle of hierarchical symptom reporting where order is imposed by both propensity and intensity.

To account for various sources of uncertainty associated with the process of applying the hierarchical structure described above to symptom propensity and episode intensity, a logistic-type latent variable model is used. If we let Y_{ijk} denote the indicator random variable taking the value 1 if subject i experiences symptom j at episode k and 0 otherwise, we assume $Y_{ijk} \sim \text{Bernoulli}(p_{ijk})$ for $i = 1,...,I$, $j = 1,...,J$, $k = 1,...,K_i$ (in our data $I = 59$, $J = 26$ and K_i varies from 19 to 135), where p_{ijk} gives the corresponding reporting probability and is derived as follows. We assume that individual i reports symptom j at episode k when $\alpha_{ij}\beta_{ik}$ exceeds a threshold τ_{ijk}, with latent variables $\alpha_{ij} > 0$ and $\beta_{ik} > 0$ representing the propensity for symptom j and the intensity of episode k respectively for individual i. The thresholds τ_{ijk} are considered to be random variables and we assume that for a given individual i they follow a common log-normal distribution, i.e.

$$\tau_{ijk} \sim \text{log-normal}(\mu_i, \sigma_i^2), \qquad i = 1,...,I \qquad [1]$$

where μ_i and σ_i^2 provide the mean and the variance of $\log(\tau_{ijk})$. Therefore, the probability p_{ijk} of individual i reporting symptom j at episode k is given by

$$p_{ijk} = \Pr(\tau_{ijk} \le \alpha_{ij}\beta_{ik}) = \Phi\left(\frac{\log(\alpha_{ij}\beta_{ik})}{\sigma_i}\right), \qquad [2]$$

$i = 1,...,I$, $j=1,...,J$, $k = 1,...,K_i$, where $\Phi(\,)$ denotes the cumulative distribution function of a standard normal variable. The mean of the logarithm of the thresholds, $E\{\log(\tau_{ijk})\} = \mu_i$, is

not of primary interest here and, without any loss of generality, we can set $\mu_i = 0$ for all subjects i.

Under this model Y_{ijk} are only conditionally independent, with occurrence of symptoms across and within episodes depending on the relevant propensity (α_{ij}), and also the underlying episode intensity (β_{ik}) which introduces associations among symptoms through the imposed hierarchical structure of occurrence. Also, as episodes of hypoglycaemia occurring within 24 hours of a preceding episode were excluded from this study, the model does not assume any correlation structure between intensity levels of successive episodes.

The unknown variance parameter σ_i^2 of the threshold distribution provides a measure of the symptom reporting consistency of an individual patient. To simplify notation, we use $w_i = \sigma_i^{-2}$ to denote the precision parameter of the threshold distribution throughout this appendix. Under the assumed log-normal model we have $\text{var}(\tau_{ijk}) = e^{\sigma_i^2}(e^{\sigma_i^2} - 1)$ and $\lim \text{var}(\tau_{ijk}) = 0$, as $\sigma_i \to 0$. Equivalently, $\lim \text{var}(\tau_{ijk}) = 0$ as $w_i \to \infty$. Here, to facilitate interpretation and comparisons, we use a function of σ_i^{-2} given as the rescaled consistency parameter $c_i = 100/(1+\sigma_i^2)$, with range (0, 100). For large c_i the thresholds get highly concentrated around a constant value τ_i^*, resulting in consistent reporting of symptoms associated with latent symptom propensity α_{ij} and episode intensity β_{jk} such that $\alpha_{ij}\beta_{jk} > \tau_i^*$. Therefore, consistent symptom reporting is associated with high concentration of the threshold distribution, corresponding to increasing values of the *consistency parameter* c_i.

Sensitivity to threshold distribution

The level of the consistency parameter for each subject was estimated under a Bayesian approach. The thresholds τ_{ijk} can alternatively be assumed to follow other distributions. Here we consider that, for patient i, they are drawn from a Weibull family, i.e.

$$\tau_{ijk} \sim \text{Weibull}(\nu_i, \lambda_i), \quad i = 1, \ldots, I$$

and the probability p_{ijk} of individual i reporting symptom j at episode k is is expressed though the appropriate cumulative distribution function (*cdf*) as

$$p_{ijk} = \Pr(\tau_{ijk} \le \alpha_{ij}\beta_{ik}) = 1 - \exp\{-\lambda_i(\alpha_{ij}\beta_{ik})^{\nu_i}\}$$

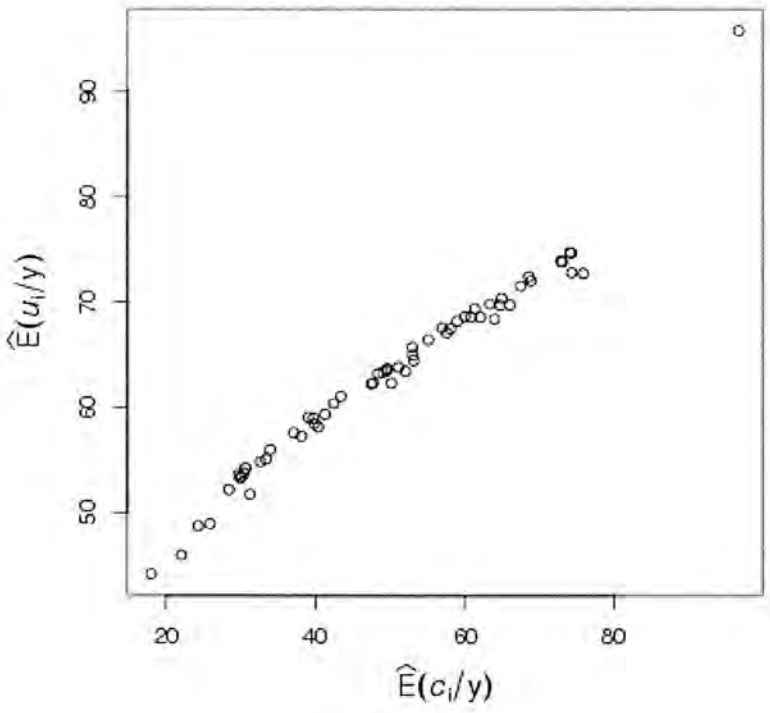
$i = 1,...,I$, $j=1,...,J$, $k = 1,...,K_i$. As with the mean parameter in the log-normal model, the scale parameter of the threshold distribution is assumed known and set to $\lambda_i = 1$. The unknown parameter of the threshold distribution provides a measure of the symptom reporting consistency of an individual patient, with $var(\tau_{ijk}) = \Gamma(1+2/v_i) - \{\Gamma(1+1/v_i)\}^2$. This gives

$$\lim var(\tau_{ijk}) = 0, \text{ as } v_i \to \infty$$

implying that high v_i values correspond to consistent symptom reporting, as was the case with c_i under the log-normal threshold distribution. Again, rescaled versions of the parameter can be used for convenience, e.g. $u_i = 100/(1+v_i^{-1})$. There is close agreement between the consistency parameter estimates under the two models, as shown in figure A1, verifying that our analysis is robust to the choice of the threshold distribution.

Figure A1:

Estimated posterior mean of $c_i = 100/(1+\sigma_i^2)$ against posterior mean of $u_i = 100/(1+v_i^{-1})$.



Posterior estimates for individual subjects

Following a Bayesian approach, we consider independent prior distributions for the latent variables $\alpha_{ij} \sim Gamma(\gamma_\alpha, \delta_\alpha)$, $i = 1,...,I$, $j = 1,...,J$, $\beta_{ik} \sim Gamma(\gamma_\beta, \delta_\beta)$, $i = 1,...,I$, $k = 1,...,K_i$, with appropriate values for $\gamma_\alpha, \delta_\alpha$, γ_β and δ_β to express relative prior ignorance (here $\gamma_\alpha = \gamma_\beta = 1$ and $\delta_\alpha = \delta_\beta = \delta_\sigma = 0.1$). As in this work we do not focus on between-individual variability of symptoms, it is not relevant to assume a hierarchical setting of common distributions for the latent propensity variables (α_{ij}). Estimation of α_{ij} and β_{ik} is informed by the frequency with which a symptom is reported throughout all episodes and the number of symptoms per particular episode. For identifiability and interpretation purposes, we have also imposed a corner-type constraint on the logarithms of these parameters ($\log \alpha_{i1} = \log \beta_{i1} = 0, i = 1,...,I$).

We also assign a relatively vague inverse gamma prior distribution to the variance parameter, $\sigma_i^2 \sim$ Inv-Gamma(γ_σ, δ_σ) , $i = 1,...,I$ where again $\gamma_\sigma = 1$, $\delta_\sigma = 0.1$.

Posterior estimates of c_i were derived for each subject in the analysis using Markov chain Monte Carlo (MCMC) methodology. Credible intervals for c_i were wide for some patients, reflecting limited information in the occurred episodes. A histogram and the empirical cumulative distribution function of $\tilde{c}_i$, the posterior means of c_i, are given in chapter 5 (figure 5.3).

Relationship between consistency measure and number of empty embedded cells

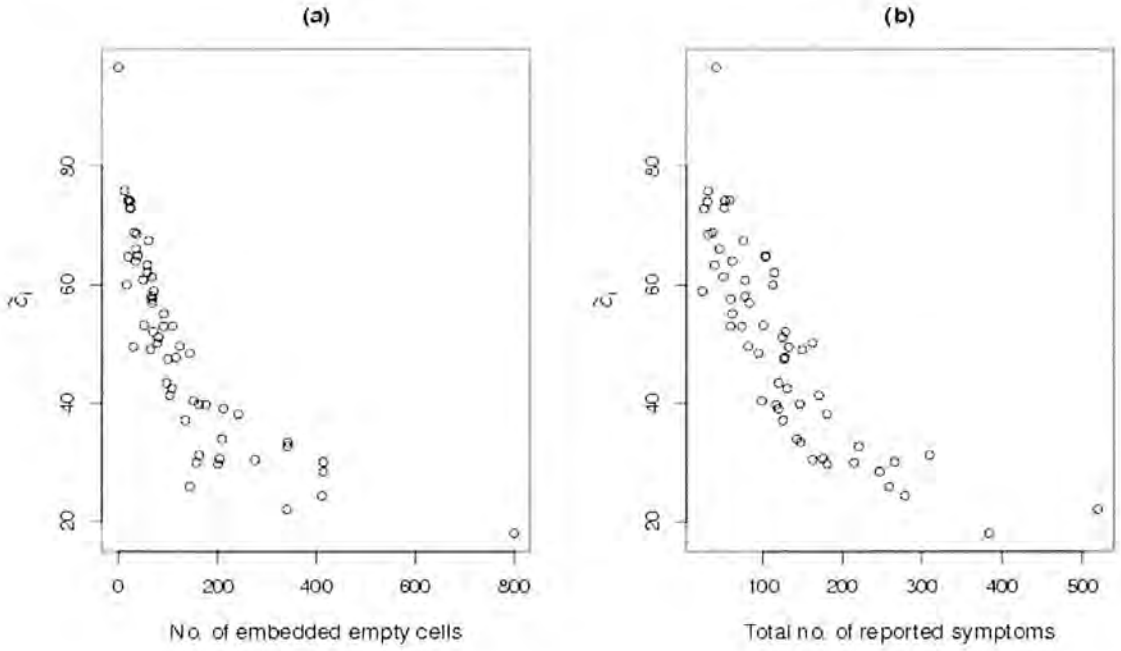
In this work, the consistency of individual patients when reporting symptoms throughout a series of hypoglycemic episodes has been associated with a principle under which symptoms are experienced according to a hierarchical order determined by their latent propensity and the latent intensity of episodes. The embedded empty cells in figures 5.1 and 5.2 (chapter 5) provide evidence of deviation from this principle in the observed complex of symptoms for each individual. Figure A2(a) illustrates that consistency, as estimated in our model, is related to the number of embedded empty cells, and therefore the consistency parameter c_i in our approach is in agreement with the principle of hierarchical symptom reporting. Figure A2(b) reveals that the estimated consistency is also negatively related to the total number of

symptoms reported throughout all episodes ($\sum_{i,k} y_{i,j,k}$) for each patient. This points towards potential presence of additional variation in the threshold level of individuals, suggesting that an extended model may also be considered in the future to allow for random effects for associated symptoms.

Figure A2:

(a) Number of embedded empty cells against $\widetilde{c}_i$

(b) Total number of symptoms reported throughout all episodes ($\sum_{i,k} y_{i,j,k}$) for each patient against $\widetilde{c}_i$.



GLM analysis for association between consistency and patient-specific factors

Generalised linear model (GLM) methodology was used to investigate the effect of the following ten patient-specific covariates on consistency: gender, age, type of diabetes, duration of diabetes, presence of retinopathy, hypoglycaemia awareness score, BMI, stimulated C-peptide, HbA1c and serum ACE activity. Gender, type of diabetes and presence of retinopathy were included in the model as categorical factors, while all other covariates assumed numerical values.

The estimated posterior mean of the precision parameter, $\widetilde{w}_i = \mathrm{E}(w_i \mid \underline{y}_i)$, is modelled as the response variable in a Bayesian gamma GLM. This is a more appropriate measure to be modelled as the GLM response variable, compared to $\widetilde{c}_i$ for which a distribution on the scaled (0, 100) range may not be naturally found or justified. Thus, we assume

$$\widetilde{w}_i \sim \mathrm{Gamma}\left(\lambda, \frac{\lambda}{m_i}\right), i = 1, \ldots, I \qquad [3]$$

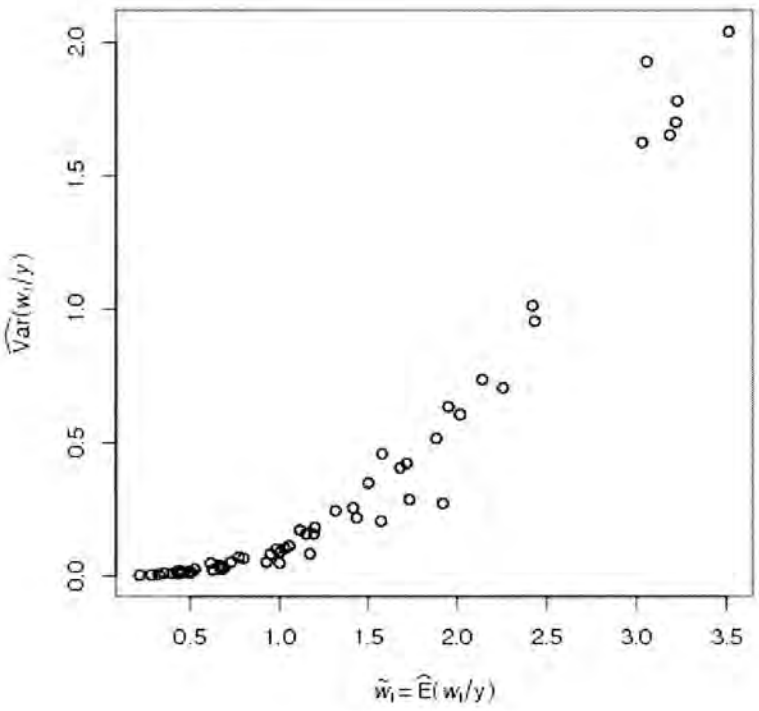
which gives $\mathrm{E}(\widetilde{w}_i) = m_i$ and $\mathrm{var}(\widetilde{w}_i) = m_i^2/\lambda$. The suitability of the gamma errors and alternative GLM assumptions are discussed later in this Appendix. The mean consistency response m_i is linked to patient-specific categorical factors and continuous covariates through a function of the form $m_i = \exp\left(\underline{x}_i^{\prime}\,\underline{b}\right)$, $i = 1, \ldots, I$, where $\underline{b} = (b_0, b_1, \ldots, b_{12})^{\prime}$ is a vector of real valued coefficients and $\underline{x}_i^{\prime} = (1, x_{1,i}, \ldots, x_{12,i})$ is the covariate vector corresponding to the order in which the covariates are given above (with the unit value giving the intercept, b_0, of the linear function). Note that gender and type of diabetes account for one coefficient each (with male patients and type 1 diabetes giving the base-line categories), while retinopathy accounts for three coefficients using a sum-to-zero constraint for comparing effects to a mean level. As before, we assume relative prior ignorance about the model parameters, using the independent priors $b_l \sim \mathrm{N}(0, \sigma_b^2)$, $l = 0, \ldots, 10$, $\lambda \sim \text{Inv-Gamma}(\gamma_\lambda, \delta_\lambda)$ with $\sigma_b^2 = 10^4, \gamma_\lambda = \delta_\lambda = 10^{-3}$. There are eight patients with unspecified records in covariates retinopathy, C peptide and HbA1c. Under a Bayesian perspective, the missing values are included in the analysis by treating the covariates as random variables to be estimated by the model. First their prior distribution is defined and then estimates can be obtained from the joint posterior distribution of the model parameters and missing values given the observed data (345). Here we assume vague prior distributions for the three covariates. Posterior estimates of the b_l coefficients are obtained through MCMC simulation. The effect of each covariate is assessed using 95% equal tailed credible intervals based on the marginal posterior densities $p(b_l \mid \underline{\widetilde{w}})$.

The gamma family was considered appropriate in the GLM analysis for two reasons: firstly, the variance of $\widetilde{w}_i$ was found to be non-constant, and in particular $\mathrm{var}(\widetilde{w}_i) \propto \mathrm{E}^2(\widetilde{w}_i)$ (see figure A3) – a typical feature of the gamma distribution; and secondly, the gamma density is

a natural choice for modelling $w_i = \sigma_i^{-2}$, that is the reciprocal of the variance of the normal distribution of $\log(\tau_{ijk})$.

Figure A3:

Mean against variance of consistency measure $w_i = \sigma_i^{-2}$. For clarity of presentation subject 1028 has been omitted from the graph, since this subject has $\mathrm{E}(w|\underline{y}) = 34.9$ and $\mathrm{var}(w|\underline{y}) = 243.7$.



An alternative GLM with log-normal errors was also considered for modelling the consistency measure $\tilde{w}_i$, with

$$\tilde{w}_i \sim \mathrm{LN}(m_i, \sigma_w^2), \qquad i = 1, \ldots, I$$

where m_i is associated to patient-specific covariates through the identity link $m_i = \underline{x}_i^T \underline{b}$, and $\underline{x}_i^T$ and $\underline{b}$ are as before. Again, relatively vague priors were assumed for all parameters. This model did not provide better fit to the data compared to the gamma formulation (eq. [3]). This is demonstrated by the lower values of both the posterior mean of the deviance and the

deviance information criterion (DIC) for the gamma model – see Table A1. A discussion on the use of these model assessment criteria is given in [3] in this Appendix (we note that since the two models have the same number of parameters, both criteria give equivalent results).

Table A1:

Posterior mean of deviance and deviance information criterion (DIC) under the two considered GLM error distributions.

Error distribution	Deviance	DIC
Gamma	152.352	163.894
Log-normal	155.444	167.277

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APPENDIX 2: PUBLISHED PAPERS

Paper 1:

- **Zammitt NN**, Geddes J, Warren RE, Marioni R, Ashby JP, Frier BM. Serum Angiotensin Converting Enzyme and frequency of severe hypoglycaemia in type 1 diabetes. Does a relationship exist? *Diabetic Medicine* **24**: 1449-1454, 2007.

Paper 2:

- **Zammitt NN**, Streftaris G, Gibson G, Deary IJ, Frier BM. Modelling the consistency of hypoglycemic symptoms: high variability in diabetes. *Diabetes Technology and Therapeutics* 13: 571-578, 2011.

Paper 3:

- **Zammitt NN,** Warren RE, Deary IJ, Frier BM. Delayed recovery of cognitive function following hypoglycemia in adults with type 1 diabetes: effect of impaired awareness of hypoglycemia. *Diabetes* 57: 732-736, 2008.

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Original Article: Clinical Care and Delivery

Serum angiotensin-converting enzyme and frequency of severe hypoglycaemia in Type 1 diabetes: does a relationship exist?

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Abstract

Aims An association has been described between elevated serum angiotensin-converting enzyme (ACE) and an increased risk of severe hypoglycaemia (SH). To ascertain whether this reported association could be replicated in a different country, it was re-examined in 300 individuals with Type 1 diabetes.

Methods People with Type 1 diabetes, none of whom was taking renin–angiotensin system blocking drugs, were recruited. Participants recorded the frequency with which they had experienced SH. Glycated haemoglobin (HbA_{1c}) and serum ACE were measured. The difference in the incidence of SH between different quartiles of ACE activity and the relationship between serum ACE and SH were examined using non-parametric statistical tests and a negative binomial model.

Results Data were obtained from 300 patients [158 male; HbA_{1c} median (range) 8.2% (5.2–12.8%), median age 36 years (16–88); duration of diabetes 14.5 years (2–49)]. The incidence of SH was 0.93 episodes per patient year. The mean incidence of SH in the top and bottom quartiles of ACE activity was 0.5 and 1.7 episodes per patient year, respectively, but this difference was not statistically significant ($P = 0.075$). Spearman's test showed a very weak, although statistically significant, association between serum ACE level and SH incidence ($r = 0.115$, $P = 0.047$). The binomial model also showed a statistically significant ($P = 0.002$), but clinically weak, relationship between serum ACE and SH.

Conclusions The present survey showed a weak relationship between serum ACE and the frequency of SH, the clinical relevance of which is unclear. This limits the proposed role for serum ACE as an index of risk for SH.

Diabet. Med. 24, 1449–1454 (2007)

Keywords hypoglycaemia, serum angiotensin converting enzyme, Type 1 diabetes

Abbreviations ACE, angiotensin-converting enzyme; HbA_{1c}, glycated haemoglobin; SH, severe hypoglycaemia

Introduction

Hypoglycaemia is a common side-effect of insulin therapy. In Type 1 diabetes, most events are mild (self-treated) with an average frequency of 2.0 episodes per week [1,2]. In northern European studies of unselected individuals with Type 1 diabetes, the estimated incidence of severe hypoglycaemia (SH; requiring assistance for recovery) ranges from 1.0 to 1.7 episodes/patient/year [2–5], with an annual prevalence between 30.0 [3,6] and 40.5% [4], similar to the Diabetes Control and Complications Trial (DCCT) [7]. The frequency of hypoglycaemia varies considerably, with most people never or rarely developing SH, while a small subgroup frequently experience SH [2].

Several risk factors for SH have been identified [7,8]. In addition to strict glycaemic control and impaired awareness of hypoglycaemia [9,10], serum angiotensin-converting enzyme (ACE) activity has emerged as a possible marker for risk assessment. Individual variation in serum ACE levels is mediated in part by gene polymorphism, via I (insertion) and D (deletion) alleles. The II genotype is associated with low serum ACE activity [11] and in Type 1 diabetes has been linked to a lower frequency and risk of SH; the DD genotype is associated with higher serum ACE activity and an increased risk of SH [12,13]. Low serum ACE and the II genotype are associated with enhanced athletic performance in events requiring stamina [14–16]. It has been postulated that a lower ACE activity confers greater ability to function efficiently during periods of metabolic substrate deprivation. Conversely, those who have a high ACE activity have more limited functional capacity when challenged by glucose deficiency.

In people with Type 1 diabetes with high ACE activity, this may be manifest by greater cognitive impairment during hypoglycaemia than in those with low ACE activity. This might explain the variable risk of developing SH within a population with Type 1 diabetes. Two Danish studies in adults, and one Swedish study in children and adolescents, all with Type 1 diabetes, have demonstrated that a high serum ACE activity is associated with an increased risk of SH [12,13,17]. However, this observation has yet to be replicated in non-Scandinavian countries. The present study therefore examined the relationship between serum ACE levels and frequency of SH in a cohort with Type 1 diabetes in Scotland.

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Patients and methods

Subjects

Three hundred adults with Type 1 diabetes attending a hospital outpatient clinic were selected at random. Inclusion criteria consisted of Type 1 diabetes of at least 2 years' duration and being over 16 years of age. Exclusion criteria consisted of pregnancy, sarcoidosis or treatment with drugs affecting the renin–angiotensin system (RAS), such as ACE inhibitors or angiotensin II receptor antagonists. The local medical ethics committee approved the study, and informed consent was obtained from all participants.

Methods

Each participant completed a questionnaire quantifying the frequency of mild hypoglycaemia (self-treated) and SH (requiring external assistance). Participants were asked to estimate the total number of episodes of SH in their lifetime (using the following categories: 0, 1–2, 3–5 or > 5 episodes of SH) and the specific number of episodes during each of the previous 2 years. Awareness of hypoglycaemia was assessed using a previously published seven-point visual analogue scale [18].

Information regarding microvascular complications was obtained from medical records. Screening for retinopathy was performed by non-mydriatic digital retinal photography in line with the standards demanded by the national retinal screening programme and was classified as absent, background, preproliferative, or retinopathy that had required laser treatment. Peripheral neuropathy was identified as being present or absent based on clinical assessment with a 10-g monofilament, while autonomic neuropathy was confirmed by autonomic function tests [19]. Nephropathy was identified by the presence of microalbuminuria (urinary albumin: creatinine ratio > 3.5 mg/mmol) or frank proteinuria on two separate early morning urine samples, or raised serum creatinine.

ACE assay

Serum ACE activity was measured using a continuous monitoring spectrophotometric assay (Sigma Diagnostics, St Louis, MO, USA) [20]. Glycated haemoglobin (HbA_{1c}) was measured by ion exchange high-performance liquid chromatography via the Bio-Rad Variant II Hemoglobin testing system (Bio-Rad Laboratories, Hercules, CA, USA). The results are DCCT-aligned and the local non-diabetic range for HbA_{1c} is 5.0–6.5%.

Statistical analyses

Primary end points were the number of events of SH reported retrospectively over the previous 2 years and the proportion of participants reporting such events. Frequency of SH was compared between the top and bottom quartiles of ACE activity using Mann–Whitney *U*-tests (assuming non-normal distribution). Serum ACE levels were compared between those with a high number of SH events in the previous year (four or more) and those with no SH in the previous year. Spearman rank correlations were calculated for the associations between serum ACE activity and both frequency of SH and awareness of hypoglycaemia. The association between SH and serum ACE was also examined with a negative binomial model using the statistical package R 2.4.1 [21]. This model takes into account the large number of zero values in the data [22]. Other analyses were performed using SPSS version 12.0 for Windows (SAS Institute, Cary, NC, USA). A *P*-value of less than 0.05 was considered to be statistically significant. A formal power calculation cannot be conducted as there are no data on the distribution of serum ACE levels within a Scottish population. However, the present study is larger than previous published studies on this subject.

Results

The clinical characteristics of the 300 participants are shown in Table 1, alongside those of the participants of the three previous relevant studies. In the present study, the mean (SD) incidence of SH in the previous year was 0.93 (2.86) episodes per patient per year. However, the frequency of SH was markedly skewed (Fig. 1), with 207 subjects experiencing no SH, while only 44 individuals had experienced two or more episodes of SH in the previous year.

The relationship between serum ACE activity and frequency of SH over the previous year is shown in Fig. 2. Data on incidence of hypoglycaemia for the previous year and lifetime frequency of hypoglycaemia are available for all 300 subjects, but the 2-year data on SH frequency was available in only 257 subjects, as several individuals felt that their recall was unreliable. One subject claimed to have experienced 175 episodes of SH during the preceding year and his data (which could not be verified) is reported as > 30 episodes of SH. The median (range) serum ACE level was 39.4 IU/l (< 12–129 IU/l). The correlation between serum ACE levels and frequency of SH was examined using Spearman's test (Table 2a). There was a small (in effect size) but statistically significant correlation between serum ACE activity and the number of episodes of SH in the previous year ($P = 0.047$, $r = 0.115$). The correlations between serum ACE activity and other estimates of frequency of hypoglycaemia all failed to reach statistical significance (Table 2a).

Table 1 Clinical characteristics of participants in the present study and in the previous studies examining an association between serum ACE and severe hypoglycaemia

Characteristics	Present study	Danish retrospective study [12]	Danish prospective study [13]	Swedish paediatric study [17]
Number of subjects	300	207*	171	86
Incidence of SH (episodes/patient/year)	0.93	1.1	1.1	1.8
Prevalence of SH	31%	39%	39%	51%
Age (years)				
Mean	38.1	43.1	44	13.0
SD	13.0	Not reported	Not reported	3.1
Median	36	Not reported	Not reported	12.8
Range	16–88	12.8	12	7.1–18.5
HbA_{1c} (%)				
Mean	8.4	8.6	8.4	6.9
SD	1.4	1.3	1.0	1.0
Median	8.2	Not reported	Not reported	6.8
Range	5.2–12.8	Not reported	Not reported	4.7–10.2
Diabetes duration (years)				
Mean	16.4	18.4	19	5.5
SD	10.4	10.9	11	3.3
Median	14.5	Not reported	Not reported	5.3
Range	2–49	Not reported	Not reported	1.2–14.7
Male/female	53%/47%	54%/46%	54%/46%	Not reported
Retinopathy				
n	95	92	Not reported	Not assessed
%	32%	46%	45%	Not assessed
Peripheral. neuropathy				
n	17	52	Not reported	Not assessed
%	6%	26%	26%	Not assessed
Autonomic neuropathy				
n	10	12	Not reported	Not assessed
%	3%	9%	7%	Not assessed
Nephropathy				
n	9	19	Not reported	Not assessed
%	3%	10%	6%	Not assessed
Awareness of hypoglycemia†				
Normal : impaired	196 : 104 (65 : 35%)	92 : 115 (44 : 56%)	70 : 101 (41 : 59%)	Not assessed
No. (%) with at least 1 SH/previous year	93 (31%)	Not reported	66 (39%)	44 (51%)

*Of the 262 patients in this study, 55 were taking ACE inhibitors or angiotensin-II receptor antagonists. Their data are excluded from this table.
†Different methods were used to estimate awareness of hypoglycaemia in the Scottish and Danish studies.
ACE, angiotensin-converting enzyme; SH, severe hypoglycaemia.

No significant association was observed between serum ACE level and the hypoglycaemia awareness score ($P = 0.701$).

The association between serum ACE levels and frequency of SH was further examined using a negative binomial model. The subject with 175 episodes of SH was treated as an outlier and omitted from the analysis, but the association remained statistically significant ($P = 0.002$). However, the frequency of SH is very skewed, as illustrated in Fig. 1. In order to assess the effect of the few individuals who experienced a high frequency of SH, the data were reanalysed using a negative binomial model but excluding two further subjects who had reported 20 and 24 episodes of SH, respectively, over the previous year. When the subject with 24 episodes was excluded, the association remained significant ($P = 0.039$), but when the subject with 20 episodes was also excluded, the result no longer achieved significance ($P = 0.141$). Adjustments were made to the model to consider stratification by age and sex. However, neither had a significant impact upon the relationship between serum ACE and frequency of SH.

The incidence of SH was determined for each quartile of ACE activity (Table 2b) and compared between top and bottom quartiles using the Mann–Whitney *U*-test. The frequency of SH did not differ significantly between these two groups ($P = 0.075$). The median serum ACE levels were compared between the subset of people who had experienced no SH ($n = 207$) over the previous year and the small group who had experienced four or more episodes of SH ($n = 18$). The serum ACE levels were significantly different between the two groups ($P = 0.009$) with median (range) ACE levels of 40.5 (12.0–129.0) IU/l and 49.3 (56.4–96.9) IU/l in the low and high SH groups, respectively ($P = 0.008$).

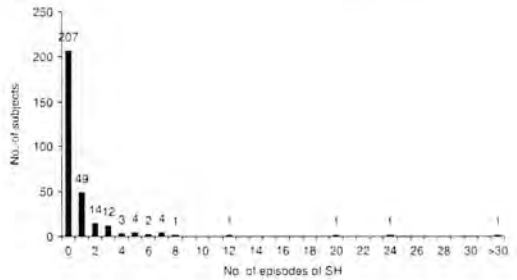

FIGURE 1 Frequency distribution of severe hypoglycaemia (SH) occurring in the previous year in 300 people with Type 1 diabetes.

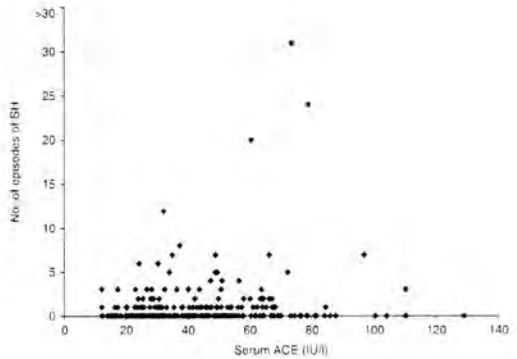

FIGURE 2 The relationship between number of episodes of severe hypoglycaemia (SH) experienced by individual participants during the previous year and their serum angiotensin-converting enzyme (ACE) levels.

Discussion

Previous studies have reported that high serum ACE activity is strongly associated with an increased risk of SH, as demonstrated in adult cohorts with Type 1 diabetes in retrospective [12] and prospective [13] studies in Denmark, and in a prospective study of children and adolescents in Sweden [17]. In the present study, a statistically significant relationship was observed between serum ACE activity and the incidence of SH, but this association was weak, with a low correlation coefficient. When the data were analysed using a negative binomial model, the statistical significance of the relationship was determined by three individuals who reported a very high frequency of SH. If the data from these three subjects are omitted from analysis, this relationship is not statistically significant. The serial removal of outliers is not a recommended statistical technique. However, it illustrates how the relationship between ACE and SH may be disproportionately affected by a small minority who have a very high incidence of hypoglycaemia.

The incidence of SH did not differ significantly between subjects in the top and bottom quartiles of ACE activity, but when the 18 subjects who reported four or more episodes of SH over 1 year were compared with those who had no SH, the serum ACE levels of these two subgroups did differ significantly. The present study examined more people than any of the Scandinavian studies and excluded those receiving treatment with RAS-blocking drugs (as did two of the Scandinavian studies [13,17]). A significant number of individuals with Type 1 diabetes are treated with such drugs and their exclusion from this study may limit the generalizability of these findings. However, we believe that the exclusion of these individuals is important to avoid confounding of serum ACE data. A study of 268 people with Type 2 diabetes in the UK has found no relationship between ACE I/D polymorphism and frequency of SH [23].

Various possibilities can be proposed to reconcile the much weaker association between serum ACE and SH observed in the present study with the results of previous studies. Retrospectively collected data may be subject to recall bias, although

Table 2 (a) Correlations between serum angiotensin converting enzyme (ACE) activity and various measures of frequency of severe hypoglycaemia (SH) and (b) number of episodes of SH in previous year for each quartile of serum ACE activity

(a)

	Serum ACE	
	Correlation coefficient (r)	P
SH in previous year	0.115	0.047
SH in penultimate year	0.022	0.725
Mean annual incidence of SH (calculated over 2 years)	0.079	0.175
Lifetime frequency of SH	0.013	0.816
Hypoglycaemia awareness score	−0.022	0.701

(b)

	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Number of subjects	77	75	73	75
ACE				
Minimum	12	28.0	39.5	51.5
Maximum	27.9	39.4	51.4	129.0
SH				
Number (%) with ≥ 1 SH	21 (27%)	21 (28%)	22 (30%)	29 (39%)
Mean (SD) episodes of SH	0.5 (1.0)	0.8 (2.1)	0.7 (1.4)	1.7 (5.0)
Median (range) episodes of SH	0 (0–6)	0 (0–12)	0 (0–7)	0 (0–30)

recall of SH over a period of 1 year has been shown to be robust and reproducible [1,24]. It is possible that the relationship of ACE to hypoglycaemia risk differs fundamentally between Danish and Scottish populations, although they share similar cultural, ethnic and genetic backgrounds, and both countries have a similar prevalence of Type 1 diabetes. Even if this is a genuine difference, it does not appear to alter the rates of SH observed in these national populations, possibly because the aetiology of SH is multifactorial and a subtle difference in one factor might be insufficient to alter the overall frequencies of hypoglycaemia. Previous studies in Denmark [1], Scotland [3,4], England [2] and the Netherlands [5] have reported very similar frequencies and distributions of SH within populations of people with Type 1 diabetes.

The discrepant results could be related to differences between the study populations. These differences (Table 1) may relate to the processes of selection and assessment rather than differences between the background populations from which subjects are recruited. For example, the Danish method of assessing hypoglycaemia awareness probably overestimates the prevalence of impaired awareness of hypoglycaemia as being 60% [24] compared with a prevalence of around 25% in other population studies [10]. If the patients in the Danish studies [12,13] had a higher frequency of impaired hypoglycaemia awareness than the present group, they would certainly have a greater vulnerability to developing SH [18]. Previous work has suggested that the predictive value of serum ACE is strongest in patients whose defence against SH is compromised, such as those with impaired hypoglycaemia awareness [12]. Although the present study differs from the others in the ages of the subjects, the two Danish studies included participants who were older than those in the present study [12,13], and the Swedish study examined adolescents and children [17], which suggests that age was not contributory. While the much younger Swedish patients had a shorter duration of diabetes and better glycaemic control [17], no consistent differences were observed between the adult participants of the present study and those in the Danish [12,13] studies, either in duration of diabetes or HbA_{1c}. However, marked differences were present in the frequencies of microvascular complications in the Danish groups compared with the present study cohort. Information about microvascular complications was not provided in the Swedish study, and these are rare in a paediatric age-group.

While it could be suggested that the difference in complication rates relates to the exclusion from the current study of individuals treated with RAS-blocking drugs, only one of the previous studies included individuals treated with these medications [12]. It could also be argued that exclusion of these individuals excludes those at higher risk of SH, but as both the Danish studies reported a frequency of hypoglycaemia of 1.1 episodes per patient per year, despite the fact that one study excluded [13] while the other included [12] people treated with these agents, this does not seem to be a likely possibility. In the latter study, the characteristics and risk of SH in those treated with RAS-blocking drugs did not differ from those of the other study participants, so the investigators concluded that ACE inhibition exerted no overall significant effect [12]. An alternative possibility is that there are differences in ACE genotype between the different study populations, as the participants in the current study did not receive genotyping. However, it has been noted previously that there is a close correlation between ACE genotype and phenotype [12,25] and that the effect of the genotype is mediated by the serum ACE levels [12], so this also seems unlikely.

In the present study, the incidence of SH did not differ significantly between subjects in the top and bottom quartiles of serum ACE activity, suggesting that serum ACE is not sufficiently specific as a marker to allow hypoglycaemia risk stratification of people with Type 1 diabetes. Although serum ACE levels differed significantly between people who had no history of SH and those who had experienced four or more episodes, this has limited clinical applicability with respect to screening for risk of SH. A previous history of SH is a recognized risk factor for further SH. A retrospective finding of high serum ACE levels in people who have already been identified as having a high risk of SH based on their previous history, has no prognostic value.

Thus, in the present study, the association between serum ACE and SH in Type 1 diabetes [12,13,17] was influenced disproportionately by a few individuals who reported a high frequency of SH, raising doubt as to the clinical significance of this finding. The present study suggests that serum ACE is not sufficiently specific to serve as a prognostic indicator of increased risk of SH. Further work is required to establish whether the association is present in ethnically different (non-Caucasian) populations and other age groups. A recent large prospective study in children and adolescents showed no such association [26].

Competing interests

None to declare.

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Modeling the Consistency of Hypoglycemic Symptoms: High Variability in Diabetes

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Abstract

Background: The aim of the present study was to examine symptoms of hypoglycemia, to develop a method to quantify individual differences in the consistency of symptom reporting, and to investigate which factors affect these differences.

Methods: Participants recorded their symptoms with every episode of hypoglycemia over a 9–12-month period. A novel logistic-type latent variable model was developed to quantify the consistency of each individual's symptom complex and was used to analyze data from 59 subjects (median age, 57.5 years [range, 22–74 years], 63% male, 77% type 1 diabetes) who had experienced 19 or more hypoglycemic episodes. The association between the calculated consistency parameter and age, sex, type and duration of diabetes, and C-peptide and serum angiotensin converting enzyme concentration was examined using a generalized linear model. Analyses were performed under a Bayesian framework, using Markov chain Monte-Carlo methodology.

Results: Individuals exhibited substantial differences in between-episode consistency of their symptom reports, with only a small number of individuals exhibiting high levels of consistency. Men were more consistent than women. No other factors affected consistency in patients with normal hypoglycemia awareness.

Conclusions: By using a novel stochastic model as a quantitative tool to compare the consistency of hypoglycemic symptom reporting, much greater intra-individual variability in symptom reporting was identified than has been recognized previously. This is relevant when instructing patients on identification of hypoglycemic symptoms and in interpreting symptomatic responses during experimentally induced hypoglycemia.

Introduction

HYPOGLYCEMIA IS A COMMON side effect of insulin treatment that carries a substantial morbidity. Rapid perception of the symptoms of hypoglycemia is essential to permit early corrective action. Field studies in which adults with insulin-treated diabetes have reported symptoms experienced during hypoglycemia have allowed the most common symptoms to be identified[1] and subdivided into autonomic, neuroglycopenic, and general malaise groups.[2]

When educating patients about the recognition of hypoglycemia, it is important to consider factors that may cause variation in their symptoms. Hypoglycemic symptoms are age-specific, in that young children have difficulty recognizing hypoglycemia[3] and often exhibit behavioral changes,[4,5] whereas neurological symptoms are prominent in elderly patients.[6] The symptom profile does not differ between type 1 diabetes and insulin-treated type 2 diabetes.[7,8] Neither the causative agent (insulin or tolbutamide)[9] nor the patient's gender[1] influences the nature of the symptoms experienced during hypoglycemia.

Some hypoglycemia-related symptoms may be more reliably associated with blood glucose levels than others, and a given symptom is not equally predictive of hypoglycemia in everybody,[10] suggesting a degree of between-subject variability. It is accepted that each individual's symptom complex is idiosyncratic.[1] However, an additional important issue is the degree to which individuals report similar patterns of hypoglycemia-related symptoms across episodes. The reliability with which these symptoms occur influences the ability to detect the onset of hypoglycemia.[11] The symptoms reported by children exhibit marked variability between episodes of hypoglycemia,[3] but it is not known whether adults exhibit similar intra-individual variability.

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The aim of the present study was to examine the symptoms of hypoglycemia recorded prospectively over a 9–12-month period by adults with type 1 and type 2 diabetes, to develop a model for quantifying the consistency of the symptom complex recorded on each occasion by every individual, and to examine what factors might produce inter-individual differences in the consistency of symptom reporting.

Subjects and Methods

Data were collected during a 12-month multicenter epidemiological study that examined the effects of type of diabetes and treatment modality on the frequency of hypoglycemia in 381 participants, 17–75 years old.[11] Subjects were recruited into five groups: (1) type 2 diabetes treated with a sulfonylurea; (2) type 2 diabetes treated with insulin for < 2 years; (3) type 2 diabetes treated with insulin for >5 years; (4) type 1 diabetes of <5 years in duration; and (5) type 1 diabetes of >15 years in duration.

The clinical diagnosis of type 1 and type 2 diabetes was corroborated by enzyme-linked immunosorbent assay measurements of glucagon-stimulated C-peptide. The presence of retinopathy was assessed using digital retinal photography. Serum angiotensin converting enzyme (considered to be a putative marker for increased risk of severe hypoglycemia at the time that the study was designed[12–14]) and hemoglobin A1c were measured in a central laboratory. Exclusion criteria were as follows: hemoglobin A1c >9%, severe diabetes complications, history of seizures, malignant disease, severe systemic disease, or pregnancy. The protocol received multicenter ethics approval. Subjects gave informed consent.

Subjects performed regular capillary glucose monitoring using a Medisense G glucose meter (Abbott Laboratories, Abbott Park, IL). All episodes of hypoglycemia were recorded on standard forms, noting the date, time, duration, symptoms, treatment received, and concurrent blood glucose. Subjects were asked to record all episodes associated with a capillary glucose ≤3.0 mmol/L (≤54 mg/dL) or any episodes associated with symptoms typical of hypoglycemia. Although subjects were encouraged to measure blood glucose, episodes were accepted as valid if typical hypoglycemic symptoms resolved with carbohydrate, even if no blood glucose measurement was available. Episodes associated with glucose levels >4.0 mmol/L were not considered valid. Symptoms were recorded using a standard list (Table 1). The state of hypoglycemia awareness was assessed with a validated scale.[15] Each month, subjects returned forms recording all hypoglycemic episodes with telephone follow-up if no form was received. As the intensity of hypoglycemic symptoms is diminished following antecedent hypoglycemia,[16–21] any episode of hypoglycemia occurring within 24 h of a preceding episode was excluded from further analysis.

TABLE 1. LIST OF SYMPTOMS OR PATTERNS REPORT FORMS

Symptom	*Description*
1	Confusion
2	Sweating
3	Drowsiness
4	Weakness
5	Dizziness
6	Feeling warm
7	Difficulty speaking
8	Pounding heart
9	Impaired concentration
10	Shivering
11	Unsteady
12	Nonspecific awareness
13	Double vision
14	Blurred vision
15	Hunger
16	Thirst
17	Nausea
18	Anxiety
19	Tiredness
20	Tingling
21	Trembling
22	Headache
23	Malaise
24	Irritability
25	Other
26	None

Modeling and analysis

In the statistical model developed, individuals report specific symptoms with a probability that depends on a random threshold being crossed. The behavior of thresholds is modeled through a probability distribution whose degree of concentration around a central value provides a measure of an individual's symptom-reporting consistency. Under a Bayesian approach, following observation of binary indicators of symptom experience (i.e., whether or not an individual experiences a given symptom), information on unobserved latent factors and the variability of the thresholds becomes available through their posterior distribution, which is obtained using Markov chain Monte-Carlo methodology.[22] Bayesian methods and Markov chain Monte-Carlo techniques are used in the analysis of latent variable models in psychology.[23,24] Latent variable and threshold models are commonly used in the behavioral sciences,[25] and stochastic methods have been used in diabetes to model the decision-making processes that lead to treatment of hypoglycemia.[26]

Computations were performed using the statistical package R.[27] Markov chain Monte-Carlo techniques were implemented using winBUGS software.[28] The lack of previous similar analyses prevented formal power calculations. A pragmatic decision was made that participants should have experienced at least two episodes of hypoglycemia per month on average. The data were checked for sample bias resulting from patients with more frequent episodes potentially experiencing fewer number of symptoms, but no such association was found ($p = 0.09$).

Model for intra-individual consistency

The random threshold determining the probability of an individual reporting specific symptoms relates to latent variables that govern the intensity of a given symptom on a given occasion and the individual's propensity to experience that symptom. Within our statistical model, assessment of intra-individual consistency is based on a principle of hierarchical symptom reporting where order is imposed by both propensity and intensity. Thus, a symptom is more likely to be reported if it is intense (e.g., profuse versus mild perspiration) and if the individual has a strong tendency to experience that

symptom. This modeling approach can be represented graphically by regarding each subject's responses as a $J \times K$ matrix of indicator variables (J = number of symptoms; K = number of episodes) (Fig. 1a), where each reported symptom is represented by a marked cell. Rearranging the rows according to the frequency with which symptoms are experienced and the columns according to the number of symptoms per episode (Fig. 1b), we obtain a representation where the degree of clustering of marked cells can be regarded as a measure of consistency.

This is expressed within a parametric framework using a logistic-type latent variable model. We assume that the unobservable random thresholds τ_{ijk} (associated with individual i reporting symptom j at episode k) follow a log-normal$(0, \sigma_i^2)$ distribution, under which the probability p_{ijk} of individual i reporting symptom j at episode k is given by

$$p_{ijk} \quad \Pr(\tau_{ijk} \leq \alpha_{ij}\beta_{ik}) \quad \Phi\left(\frac{\log(\alpha_{ij}\beta_{ik})}{\sigma_i}\right)$$

with $i = 1, \ldots, I$, $j = 1, \ldots, J$, and $k = 1, \ldots, K_i$, where α_{ij} and β_{ik} represent the propensity for symptom j and the intensity of episode k, respectively, for individual i, and $\Phi(\,)$ denotes the cumulative distribution function of a standard normal variable. Therefore, the model implies that occurrence of symptoms across and within episodes depends on the relevant propensity (α_{ij}) and also on the underlying episode intensity (β_{ik}), which introduces associations among symptoms through the imposed hierarchical structure of occurrence. The information available on the frequency with which symptoms are reported through all episodes and on the total number of symptoms per episode allows estimation of both α_{ij} and β_{ik} in our model.

The precision parameter σ_i^{-2} of the threshold distribution provides a measure of the symptom-reporting consistency of an individual. Consistent symptom profiles are associated with low variance of the threshold distribution. For ease of interpretation σ_i^{-2} is converted to a consistency parameter $c_i = 100/(1 + \sigma_i^2)$, which ranges from 0 to 100 with increasing values corresponding to higher symptom consistency.

Association between consistency and patient-specific factors

Generalized linear model methodology was used to investigate the effect of the following patient-specific covariates on consistency: gender, age, diabetes type (1 or 2), duration of diabetes, retinopathy, hypoglycemia awareness score (1–7, with higher scores corresponding to diminishing awareness of hypoglycemia), body mass index, stimulated C-peptide, hemoglobin A1c, and serum angiotensin converting enzyme activity. For modeling purposes retinopathy was subdivided into no retinopathy, background retinopathy, and proliferative retinopathy. A generalized linear model with gamma errors (see Supplementary Appendix; Supplementary Data are available online at www.liebertonline.com <http://www.liebertonline.com/dia>) was used to link estimates of the precision parameter σ_i^{-2} with the covariates, through the function

$$\begin{aligned}\log\{E(\sigma_i^{-2})\} = {} & b_0 + b_{\text{gen}} \times \text{GEN}_i + b_{\text{age}} \times \text{AGE}_i + b_{\text{type}} \times \text{TYPE}_i \\ & + b_{\text{dur}} \times \text{DUR}_i + b_{\text{ret1}} \times \text{RET1}_i + b_{\text{ret2}} \times \text{RET2}_i \\ & + b_{\text{ret3}} \times \text{RET3}_i + b_{\text{awar}} \times \text{AWAR}_i \\ & + b_{\text{bmi}} \times \text{BMI}_i + b_{\text{cpep}} \times \text{CPEP}_i \\ & + b_{\text{hba}} \times \text{HBA}_i + b_{\text{ace}} \times \text{ACE}_i\end{aligned}$$

(where the GEN represents gender, DUR represents duration, RET1 represents no retinopathy, RET2 represents background retinopathy, RET3 represents proliferative retinopathy, AWAR represents awareness of hypoglycemia, BMI represents body mass index, CPEP represents C-peptide, HBA represent hemoglobin A1c, and ACE represents angiotensin converting enzyme), and the effect of each covariate was assessed using 95% equal-tailed Bayesian intervals of the corresponding b coefficients.

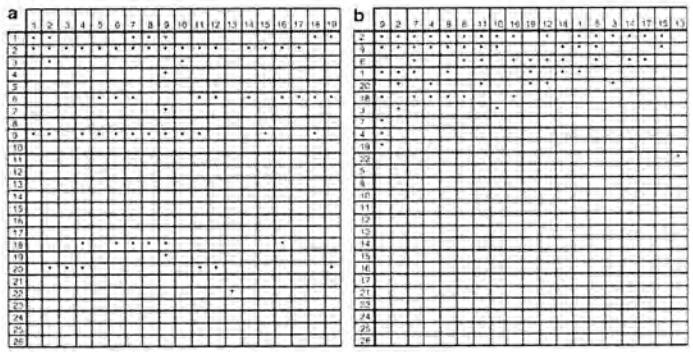

FIG. 1. (a) Example of a $J \times K$ matrix of indicator variables (J = number of symptoms; K = number of episodes) for subject 6010 with symptoms 1–26 listed vertically and hypoglycemic episodes listed horizontally. Each reported symptom is marked with a square. (b) Rearrangement of the matrix rows and columns so that rows now appear according to the frequency with which symptoms are experienced and columns according to the number of symptoms per episode (both following a descending order from the top-left corner).

Results

A total of 3,174 episodes of hypoglycemia from 59 patients were examined, of which 91% were confirmed by capillary glucose readings. After exclusion of hypoglycemic episodes occurring within 24 h of a previous event, 2,699 episodes remained for analysis. Table 2 summarizes the subject characteristics and hypoglycemic episodes within each group. The most commonly recorded symptoms were weakness, decreased concentration, sweating, and hunger (28.7%, 28.2%, 21.8%, and 21.1% of episodes, respectively).

The distribution of the estimated precision parameter $\hat{\sigma}_i^{-2}$ is skewed, with most subjects having low consistency (Fig. 2a). Estimates of the converted consistency parameter $\hat{c}_i = 100/(1 + \hat{\sigma}_i^2)$ have a mean of 50.3 and an SD of 16.7 (Fig. 2b). The main sample quartiles of $\hat{c}_i$ are $\hat{q}_{0.25} = 38.0$, $\hat{q}_{0.50} = 37.6$, $\hat{q}_{0.75} = 50.2$, $\hat{q}_{0.95} = 62.7$, and $\hat{q}_1 = 96.7$.

Some subjects in this study merit individual consideration. Subject 1028 (type 1 diabetes for >15 years) was asymptomatic during all of his 45 recorded episodes. He had reported a hypoglycemia awareness score[14] of 7, denoting total loss of warning symptoms, and had the highest estimated consistency (96.7; 95% Bayesian interval 92.9–98.7). In Bayesian statistics, the credible or Bayesian interval plays a similar role to confidence intervals in frequentist statistics. Subject 4003 (type 1 diabetes for >15 years) had the second highest consistency score (75.86; 95% Bayesian interval 60.7–87.6), was asymptomatic during 74.1% of his reported episodes, and was the only other subject with an awareness score of 7. Subject 5044 (type 1 diabetes for <5 years) had no symptoms during 51% of her episodes but had a hypoglycemia awareness score of 2, implying good awareness. Her consistency score was 40.4 (95% Bayesian interval 29.7–51.7). All other subjects were symptomatic during at least 50% of their reported hypoglycemic episodes. The single subject treated with oral agents was asymptomatic on 36% of episodes, all of which were confirmed biochemically (blood glucose ≥ 3.5 mmol/L). All of these subjects were included in the analysis as the presence or absence of symptoms was considered to form part of the variability of their symptom profiles.

When the effect of specific covariates on the consistency measure was examined, gender and hypoglycemia awareness were the only factors that had a systematic effect. Figure 3a shows 95% Bayesian intervals for all covariate coefficients. The mean of the gender coefficient, b_{gen}, was −0.677 (95% Bayesian interval −1.239, −0.110). This suggests that female subjects were less consistent than male subjects (gender was coded as 0 = males and 1 = female(s). The mean of the coefficient of awareness, b_{awar}, was 0.136 (95% interval 0.010, 0.284), which implies that those with impaired awareness recorded

TABLE 2. SUBJECT CHARACTERISTICS AND HYPOGLYCEMIC EPISODES WITHIN EACH GROUP

	T2dlo	*T2hi* = 2	*T2hi* = 5	*T1lo* = 7	*T1lo* = 15	*Total*
Number in original study ($n = 14$)	108	89	77	50	57	381
Number of subjects with ≥19 hypoglycemias	1	4	9	21	21	59
Hypoglycemias per group	25	113	476	1,385	1,475	3,474
Hypoglycemias per group after hypoglycemias <24 h of each other excluded	25	101	370	1,095	1,101	2,699
Number of hypoglycemias per patient	25 (25)	28.5 (20–36)	37 (27–146)	39 (19–210)	44.5 (19–300)	42 (19–300)
Hypoglycemias per patient after hypoglycemias <24 h of each other excluded	25 (25)	25 (20–34)	31 (25–105)	44 (19–134)	37 (19–138)	37 (19–138)
Percentage of hypoglycemias confirmed biochemically	100%	95.2%	89.1%	95.1%	87.8%	91.0%
Asymptomatic episodes per group (%) after hypoglycemias <24 h of each other excluded	36%	0%	0.3%	0.9%	4.5%	1.1%
Number (%) male	1 (100%)	4 (100%)	8 (89%)	11 (67%)	11 (70%)	39 (65%)
Age (years)	51 (51)	65 (60–74)	65 (57–72)	39 (22–70)	58 (34–72)	57.5 (22–74)
Number (%) with impaired awareness	0 (0%)	0 (0%)	2 (22%)	7 (33%)	13 (54%)	22 (37%)
Body mass index (kg/m^2)	23.7 (23.7)	27.8 (26–30.2)	27 (21.0–33)	24 (19.5–29.6)	25.3 (21.6–42.7)	25.0 (19.5–42.7)
C-peptide (nmol/L)	2.22 (2.22)	0.85 (0.27–1.58)	0.24 (0.05–0.21)	0.45 (0.06–0.87)	0.09 (0.05–0.83)	.26 (0.05–2.51)
Hemoglobin A1c (%)	7.1 (7.1)	8.3 (7.8–8.8)	7.6 (6.3–8.9)	7.2 (5.6–10.1)	7.8 (6.1–9.7)	7.55 (5.6–10.1)
ACE (IU/L)	20 (20)	13.5 (7–24)	39 (4–71)	34 (18–94)	31.5 (3–98)	32.5 (3–98)

Data are given as median (range) unless otherwise stated.

ACE, angiotensin converting enzyme; T1lo = 5, type 1 diabetes with ≥ 5 years' duration; T1lo = 15, type 1 diabetes with ≥15 years' duration; T2dlo, type 2 diabetes treated with oral agents; T2hi = 2, type 2 diabetes treated with insulin for ≥2 years; T2hi = 5, type 2 diabetes treated with insulin for ≥5 years.

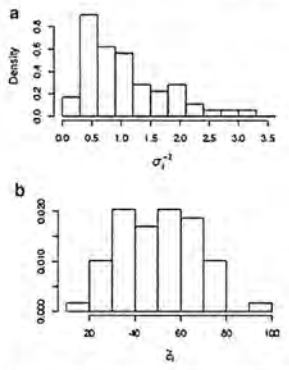

FIG. 2. Histograms of (a) estimated precision parameter $\tilde{\sigma}_i^{-2}$ and (b) estimated consistency parameter $\tilde{c}_i = 100(1 + \tilde{\sigma}_i^2)$.

lower variability in their symptoms than those with higher awareness.

However, if subjects 1028 and 4003 (asymptomatic on 100% and 74% of episodes, respectively) are excluded from the analysis, only gender has a significant effect, with females being less consistent than males ($b_{gen} = -0.43$ with 95% Bayesian interval −0.82, −0.03) (Fig. 3b).

Discussion

The present study has demonstrated and quantified episode-to-episode, intra-individual variability in symptoms of hypoglycemia reported by adults with diabetes. It has also found some determinants of inter-individual differences in this symptom (in)consistency. It is accepted that each individual's hypoglycemia symptom complex is characteristic. However, the wide range and skewed distribution of the precision parameter σ_i^{-2} demonstrate that within-subject symptom profiles vary substantially between episodes and that people show marked individual differences with respect to their consistency of symptom reporting. Conversion of the precision parameter to the normalized consistency parameter $\tilde{c}_i$ facilitates between- and within-patient comparisons of consistency estimates, although there is no predefined cutoff to differentiate consistent and inconsistent individuals.

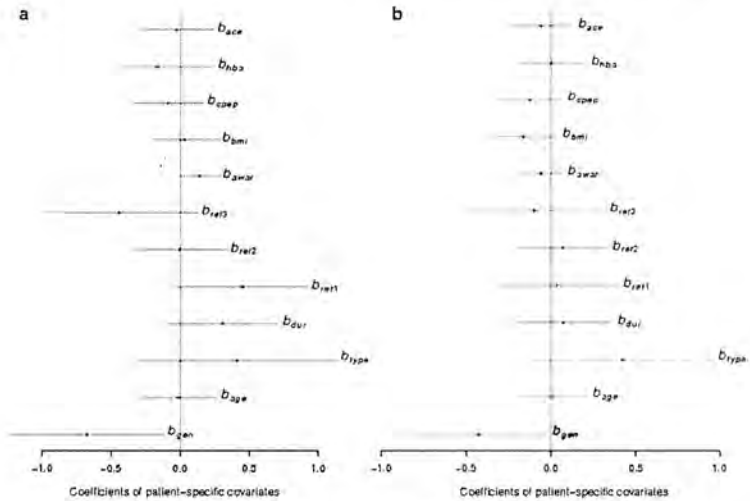

FIG. 3. (a) Posterior means (circles) and 95% equal-tailed Bayesian intervals (bars) for standardized coefficients of patient-specific covariates. b_{ace}, serum angiotensin converting enzyme activity; b_{age}, age; b_{awar}, hypoglycemia awareness score; b_{bmi}, body mass index; b_{cpep}, stimulated C-peptide; b_{dur}, duration of diabetes; b_{gen}, gender; b_{hba}, hemoglobin A1c; b_{ret1}, no retinopathy; b_{ret2}, background retinopathy; b_{ret3}, preproliferative retinopathy; b_{type}, type of diabetes. (b) Analysis repeated after exclusion of subjects 1028 and 4003.

The most commonly reported symptoms were weakness, decreased concentration, sweating, and hunger. These have previously been demonstrated to be the earliest perceived symptoms of hypoglycemia[20] and those that are most commonly[19] and accurately[12] associated with hypoglycemia. However, the main aim of this study was not to study population similarities but rather to examine intra-individual consistency of symptom reporting.

The statistical analyses in the present study raise some important points for patient education and hypoglycemia research. First, when patients are taught that their hypoglycemic symptoms are idiosyncratic, they should also be informed that their symptoms will probably vary between episodes. Reinforcing this point may avoid a failure to recognize hypoglycemia as a result of symptom variation. Second, it is probably useful for patients to have an awareness of how consistent their symptoms are because people who have at least one reliable symptom of hypoglycemia only detect blood glucose levels below 3.9 mmol/L on 50% of occasions, whereas individuals with four or more reliable symptoms recognize similar blood glucose levels on 75% of occasions.[11] Finally, previous studies have relied on very few snapshots of the hypoglycemic symptom profile, either recorded during experimental hypoglycemia[10,11,30,31] or documented retrospectively by patients in what was thought to represent their "typical" symptom profile.[2,6,8,32] The findings of the present study challenge the validity of the latter approach for the purpose of advising individual patients, as the degree of between-episode variability is much greater than has previously been appreciated.

Of the factors examined, only female gender increased symptom variability in a systematic way. Although impaired awareness was associated with increased symptom consistency in the initial analysis, this effect was no longer observed once the two individuals with awareness scores of 7 were excluded. One of the limitations of this analysis is that it cannot distinguish between a completely consistent person with full symptom awareness and a consistently asymptomatic individual. However, for individuals with a combination of symptomatic and asymptomatic episodes, the presence or absence of symptoms contributes to the consistency of their symptom profile, and it was thought important not to exclude asymptomatic episodes completely.

It was surprising that the subject treated with oral agents was asymptomatic during 36% of episodes, despite recording a normal hypoglycemia awareness score.[3] All his episodes were confirmed with glucose readings. In the United Kingdom, patients treated with oral agents are not routinely asked to check capillary glucose levels, so he had probably not realized that his awareness was impaired prior to participation in this study.

The relationship between consistency of symptom reporting and gender has not been reported previously. Symptoms of hypoglycemia develop at similar blood glucose thresholds in men and women with type 1 diabetes,[33] but the magnitude of the counterregulatory response is lower in women,[34] which may influence the intensity of the symptomatic response. Female counterregulatory responses are less affected by antecedent hypoglycemia and exercise than responses in men.[35] It could be hypothesized that the gender differences in this study relate to under-reporting by females as a result of lower symptom intensity, but it is not possible to confirm this as subjects were not asked to rate symptom intensity.

It is possible that other factors, such as the activities engaging the individual at the time of the episode, may have an effect on symptom consistency, but it would be logistically difficult to study these in greater detail. Earlier work has classified hypoglycemic symptoms in physiological terms.[2,4,6,8] Appropriate grouping of symptoms may be able to account for additional sources of between-groups variation for an individual patient in the model, thus giving scope for including relevant effects for symptom groups in future analyses.

Not all hypoglycemic episodes in this study were confirmed biochemically. However, the presence of typical symptoms that resolve with ingestion of carbohydrate is conventionally taken as evidence of hypoglycemia. Insistence on biochemical corroboration would have further restricted the number of episodes available for analysis and most episodes (91%) were confirmed.

In subjects with normal awareness, it would be interesting to stratify episodes according to blood glucose level to investigate whether this had an effect on symptom reporting. However, this was not possible in the present study for several reasons. Although a fall in blood glucose in a adult without diabetes triggers the secretion of counterregulatory hormones and the onset of cognitive and symptomatic changes at reproducible blood glucose thresholds[33,36] within a defined hierarchy,[35] these thresholds become altered in diabetes, and the same blood glucose level may affect individuals with diabetes in different ways. Second, data from field studies can never be as controlled as data generated in a laboratory. Confirmation of hypoglycemia may have occurred several minutes before or after rescue carbohydrate was administered. Thus, the blood glucose measurement may not have coincided exactly with the blood glucose nadir or the peak of symptom intensity. Finally, blood glucose meters are less accurate in the hypoglycemic range, and it would not have been possible to confirm these readings with venous samples outside the confines of a tightly regulated laboratory study. However, there is the potential to examine this question in a follow-up study.

The study has several strengths, including its size (2,600 episodes of hypoglycemia), its prospective design, and its duration. Although some previous studies have collected symptoms prospectively,[12,28,29] they have not attempted to compare symptoms between episodes. Furthermore, prospective field data could be regarded as more generalizable than hypoglycemia data collected under laboratory conditions.

The present study demonstrates that intra-individual between-episode symptom variability is much greater than has been previously appreciated and that there are marked individual differences in this consistency. Caution should be exercised when interpreting patients' retrospective recall of what they regard to be their "typical" hypoglycemic symptoms. Female gender was the only factor found to have a systematic association with increased variability of the symptom complex. Given this observed variability, clinicians should advise patients against being too dogmatic in their perception of what constitutes their cardinal hypoglycemic symptoms, as these may vary considerably between episodes. This variability should also be considered when interpreting hypoglycemic symptom responses under different experimental conditions or when comparing different therapeutic interventions.

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Supplementary Appendix

Model for intra-individual consistency

We have assumed that an individual's symptom profile depends on latent factors expressing the intensity of a given symptom on a given occasion and the individual's propensity to experience that symptom. The model that we develop implies that assessment of intra-individual consistency is based on a principle of hierarchical symptom reporting where order is imposed by both propensity and intensity.

To account for various sources of uncertainty associated with the process of applying the hierarchical structure described above to symptom propensity and episode intensity, a logistic-type latent variable model is used. If we let Y_{ijk} denote the indicator random variable taking the value 1 if subject i experiences symptom j at episode k and 0 otherwise, we assume $Y_{ijk} \sim$ Bernoulli (p_{ijk}) for $i = 1, \ldots, I$, $j = 1, \ldots, J$, and $k = 1, \ldots, K_i$ (in our data $I = 59$, $J = 26$, and K_i varies from 19 to 135), where p_{ijk} gives the corresponding reporting probability and is derived as follows. We assume that individual i reports symptom j at episode k when $\alpha_{ij}\beta_{ik}$ exceeds a threshold τ_{ijk}, with latent variables $\alpha_{ij} > 0$ and $\beta_{ik} > 0$ representing the propensity for symptom j and the intensity of episode k, respectively, for individual i. The thresholds τ_{ijk} are considered to be random variables, and we assume that for a given individual i they follow a common log-normal distribution, that is,

$$\tau_{ijk} \sim \text{log-normal}(\mu_i, \sigma_i^2) \text{ for } i = 1, \ldots, I \qquad (1)$$

where μ_i and σ_i^2 provide the mean and the variance of $\log(\tau_{ijk})$. Therefore, the probability p_{ijk} of individual i reporting symptom j at episode k is given by

$$p_{ijk} = \Pr(\tau_{ijk} \le \alpha_{ij}\beta_{ik}) = \Phi\left(\frac{\log(\alpha_{ij}\beta_{ik})}{\sigma_i}\right) \qquad (2)$$

for $i = 1, \ldots, I$, $j = 1, \ldots, J$, and $k = 1, \ldots, K_i$, where $\Phi(\,)$ denotes the cumulative distribution function of a standard normal variable. The mean of the logarithm of the thresholds, $E[\log(\tau_{ijk})] = \mu_i$, is not of primary interest here, and, without any loss of generality, we can set $\mu_i = 0$ for all subjects i.

Under this model the values Y_{ijk} are only conditionally independent, with occurrence of symptoms across and within episodes depending on the relevant propensity (α_{ij}), and also the underlying episode intensity (β_{ik}), which introduces associations among symptoms through the imposed hierarchical structure of occurrence. Also, as episodes of hypoglycaemia occurring within 24 h of a preceding episode were excluded from this study, the model does not assume any correlation structure between intensity levels of successive episodes.

The unknown variance parameter σ_i^2 of the threshold distribution provides a measure of the symptom-reporting consistency of an individual patient. To simplify notation, we use $\omega_i = \sigma_i^{-2}$ to denote the precision parameter of the threshold distribution throughout this appendix. Under the assumed log-normal model we have $\text{var}(\tau_{ijk}) = e^{\sigma_i^2}(e^{\sigma_i^2} - 1)$ and $\lim \text{var}(\tau_{ijk}) = 0$ as $\sigma_i \to 0$. Equivalently, $\lim \text{var}(\tau_{ijk}) = 0$ as $\omega_i \to \infty$. Here, to facilitate interpretation and comparisons, we use a function of ω_i given as the rescaled consistency parameter, $c_i = 100/(1 + \sigma_i^2)$ with range (0, 100). For large c_i the thresholds get highly concentrated around a constant value τ_i^*, resulting in consistent reporting of symptoms associated with latent symptom propensity α_{ij} and episode intensity β_{ik} such that $\alpha_{ij}\beta_{ik} > \tau_i^*$. Therefore, consistent symptom reporting is associated with high concentration of the threshold distribution, corresponding to increasing values of the consistency parameter c_i.

Sensitivity to threshold distribution

The level of the consistency parameter for each subject was estimated under a Bayesian approach.

The thresholds τ_{ijk} can alternatively be assumed to follow other distributions. Here we consider that, for patient i, they are drawn from a Weibull family, that is,

$$\tau_{ijk} \sim \text{Weibull}(v_i, \lambda_i) \text{ for } i = 1, \ldots, I$$

and the probability p_{ijk} of individual i reporting symptom j at episode k is is expressed though the appropriate cumulative distribution function (*cdf*) as

$$p_{ijk} = \Pr(\tau_{ijk} \le \alpha_{ij}\beta_{ik}) = 1 - \exp\{-\lambda_i(\alpha_{ij}\beta_{ik})^{v_i}\}$$

for $i = 1, \ldots, I$, $j = 1, \ldots, J$, and $k = 1, \ldots, K_i$. As with the mean parameter in the log-normal model, the scale parameter of the threshold distribution is assumed known and set to $\lambda_i = 1$. The unknown parameter of the threshold distribution provides a measure of the symptom-reporting consistency of an individual patient, with $\text{var}(\tau_{ijk}) = \{\Gamma(1 + 2/v_i) - (\Gamma(1 + 1/v_i))^2\}$. This gives

$$\lim \text{var}(\tau_{ijk}) = 0, \text{ as } v_i \to \infty$$

implying that high v_i values correspond to consistent symptom reporting, as was the case with c_i under the log-normal threshold distribution. Again, rescaled versions of the parameter can be used for convenience, for example, $u_i = 100/(1 + v_i^{-1})$. There is close agreement between the consistency parameter estimates under the two models, as shown in Figure S1, verifying that our analysis is robust to the choice of the threshold distribution.

Posterior estimates for individual subjects

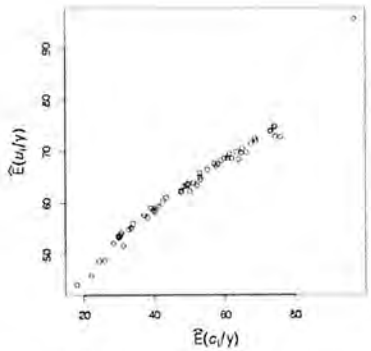
Following a Bayesian approach (e.g., reference (1) in this Appendix), we consider independent prior distributions for the latent variables $\alpha_{ij} \sim \text{Gamma}(\gamma_\alpha, \delta_\alpha)$ for $i = 1, \ldots, I$ and $j = 1, \ldots, J$, $\beta_{ik} \sim \text{Gamma}(\gamma_{\beta i}, \delta_{\beta i})$, $i = 1, \ldots, I$, with appropriate values for γ_α, δ_α, $\gamma_{\beta i}$ and $\delta_{\beta i}$ to express relative prior ignorance (here $\gamma_\alpha = \gamma_{\beta i} = 1$ and $\delta_\alpha = \delta_{\beta i} = 0.1$). As in this work we do not focus on between-individual variability of symptoms, it is not relevant to assume a hierarchical setting of common distributions for the latent propensity variables (α_{ij}). Estimation of α_{ij} and β_{ik} is informed by the frequency with which a symptom is reported throughout all episodes and the number of symptoms per particular episode. For identifiability and interpretation purposes, we have also imposed a corner-type constraint on the logarithms of these parameters ($\log \alpha_{i1} = \log \beta_{i1} = 0$, $i = 1, \ldots, I$).

We also assign a relatively vague inverse gamma prior distribution to the variance parameter, $\sigma_i^2 \sim \text{Inv-Gamma}(\gamma_\sigma, \delta_\sigma)$ for $i = 1, \ldots, I$ where again $\gamma_\sigma = 1$, $\delta_\sigma = 0.1$.

Posterior estimates of c_i were derived for each subject in the analysis using Markov chain Monte-Carlo methodology (e.g., see Tierney[22] in the main article) and are displayed in Table S1. Credible intervals for c_i were wide for some patients, reflecting limited information in the occurred episodes. A histogram and the empirical cumulative distribution function of $\hat{c}_i$, the posterior means of c_i, are given in the main article.

Relationship between consistency measure and number of empty embedded cells

In this work, the consistency of individual patients when reporting symptoms throughout a series of hypoglycemic episodes has been associated with a principle under which symptoms are experienced according to a hierarchical order determined by their latent propensity and the latent intensity of episodes. The embedded empty cells in Figure 1 in the main article provide evidence of deviation from this principle in the observed complex of symptoms for each individual. Figure S2a illustrates that consistency, as estimated in our model, is related to the number of embedded empty cells, and therefore the consistency parameter c_i in our approach is in agreement with the principle of hierarchical symptom reporting. Figure S2b reveals that the estimated consistency is also negatively related to the total number of symptoms reported throughout all episodes ($\sum_{j,k} y_{ijk}$) for each patient. This points towards potential presence of additional variation in the threshold level of individuals, suggesting that an extended model may also be considered in the future to allow for random effects for associated symptoms.



SUPPLEMENTARY FIG. S1. Estimated posterior mean of $c_i = 100/(1 + \sigma_i^2)$ against posterior mean of $u_i = 100/(1 + v_i^{-1})$

Generalized linear model analysis for association between consistency and patient-specific factors

Generalized linear model (GLM) methodology was used to investigate the effect of the following 10 patient-specific covariates on consistency: gender, age, type of diabetes, duration of diabetes, presence of retinopathy, hypoglycemia awareness score, body mass index, stimulated C-peptide, hemoglobin A1c, and serum angiotensin converting enzyme activity. Gender, type of diabetes, and presence of retinopathy were included in the model as categorical factors (see main text), while all other covariates assumed numerical values.

The estimated posterior mean of the precision parameter, $\hat{\omega}_i = E(\omega_i|y)$, is modeled as the response variable in a Bayesian gamma GLM. This is a more appropriate measure to be modeled as the GLM response variable compared with $\hat{c}_i$, for which a distribution on the scaled (0, 100) range may not be naturally found or justified. Thus, we assume

$$\hat{\omega}_i \sim \text{Gamma}\left(\tilde{\nu}, \frac{\tilde{\nu}}{m_i}\right) \text{ for } i = 1, \ldots, I \qquad (3)$$

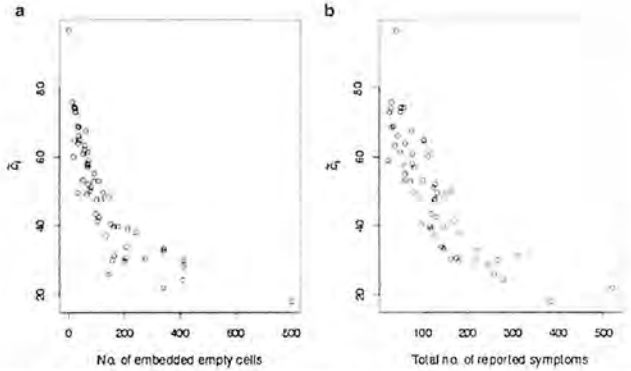
which gives $E(\hat{\omega}_i) = m_i$ and $\text{var}(\hat{\omega}_i) = m_i^2/\tilde{\nu}$. The suitability of the gamma errors and alternative GLM assumptions are discussed later in this Appendix. The mean consistency response m_i is linked to patient-specific categorical factors and continuous covariates through a function of the form $m_i = \exp(\underline{x}_i^T \underline{b})$ for $i = 1, \ldots, I$, where $\underline{b} = (b_0, b_1, \ldots, b_{10})^T$ is a vector of real valued coefficients and $\underline{x}_i^T = (1, x_{i1}, \ldots, x_{i10})$, $\underline{x}_i$ is the covariate vector corresponding to the order in which the covariates are given above (with the first value giving the intercept, b_0, of the linear function). Note that gender and type of diabetes account for one coefficient each (with male patients and type 1 diabetes giving the baseline categories), while retinopathy accounts for three coefficients using a sum-to-zero constraint for comparing effects to a mean level. As before, we assume relative prior ignorance about the model parameters, using the independent priors $b_j \sim N(0, \sigma_b^2)$, $j = 0, \ldots, 10$ and $\tilde{\nu} \sim \text{Inv-Gamma}(\gamma_{\tilde{\nu}}, \delta_{\tilde{\nu}})$ with $\sigma_b^{-2} = 10^{-2}$, $\gamma_{\tilde{\nu}} = \delta_{\tilde{\nu}} = 10^{-3}$. There are eight patients with unspecified records in covariates retinopathy, C-peptide, and hemoglobin A1c. Under a Bayesian perspective, the missing values are included in the analysis by treating the covariates as random variables to be estimated by the model. First their prior distribution is defined, and then estimates can be obtained from the joint posterior distribution of the model parameters and missing values given the observed data (see Ibrahim et al.[2]). Here we assume vague prior distributions for the three covariates. Posterior estimates of the b_j coefficients are obtained through Markov chain Monte-Carlo simulation. The effect of each covariate is assessed using 95% equal-tailed credible intervals based on the marginal posterior densities $p(b_j|\hat{\omega}_i)$.

The gamma family was considered appropriate in the GLM analysis for two reasons: first, the variance of $\hat{\omega}_i$ was found to be non-constant, and in particular $\text{var}(\hat{\omega}_i) \propto E^2(\hat{\omega}_i)$ (Fig. S3)—a typical feature of the gamma distribution; and second, the gamma density is a natural choice for modeling $\omega_i = \sigma_i^{-2}$, that is the reciprocal of the variance of the normal distribution of $\log(\tau_{ijk})$.

SUPPLEMENTARY TABLE S1. POSTERIOR ESTIMATES OF CONSISTENCY [MEAN $\hat{c}_i = E(c_i \mid y)$, STANDARD DEVIATION = $\sqrt{\text{var}(c_i \mid y)}$, AND 95% EQUAL-TAILED INTERVAL] FOR ALL SUBJECTS

Group, subject	No. of hypos	No. of hypos excluding those < 24 h of previous	No. (%) of asymptomatic episodes	$\hat{c}_i$	SD	95% interval
T2 tabs						
3046	25	25	9 (36%)	68.84	7.682	53.19, 83.21
T2 Ins < 2						
3016	23	22	0 (0%)	73	7.495	56.95, 86.2
6002	34	34	0 (0%)	41.3	6.986	28.9, 55.93
6056	36	28	0 (0%)	64.92	7.89	49.38, 80.02
6058	20	20	1 (5%)	74.29	7.048	59.25, 86.65
T2 Ins < 5						
1055	27	27	9 (33.3%)	74.05	7.207	58.69, 86.46
3048	37	31	0 (0%)	67.46	7.562	51.48, 80.93
3052	146	105	14 (13.3%)	49.5	5.25	39.36, 59.91
3057	92	51	0 (0%)	39.81	6.267	28.22, 52.48
3065	27	26	7 (26.9%)	55.09	8.411	38.84, 71.92
3067	32	26	2 (7.7%)	42.47	7.742	28.42, 58.19
4072	42	39	15 (38.5%)	57.57	7.333	43.14, 72.22
4076	33	29	0 (0%)	52.96	8.252	36.7, 69.27
5009	40	36	0 (0%)	25.9	4.974	17.44, 36.98
T1 Ins < 5						
1009	93	74	0 (0%)	53.16	5.902	41.57, 64.58
1021	49	43	0 (0%)	47.72	7.065	34.3, 62.27
1036	42	24	3 (12.5%)	51.14	8.395	35.42, 67.65
2012	42	37	0 (0%)	29.72	6.09	19.44, 43.08
2027	26	22	3 (13.6%)	68.52	8.179	51.53, 83.29
3001	35	31	20 (6.5%)	56.94	8.089	41.16, 72.31
3024	30	27	0 (0%)	52.05	8.046	36.88, 67.78
3029	76	69	0 (0%)	64.71	5.951	52.68, 75.89
3050	54	44	1 (2.3%)	49.04	6.941	36.29, 62.96
4023	210	134	0 (0%)	22.07	2.988	16.75, 28.65
4034	61	55	0 (0%)	59.98	6.523	46.97, 72.82
4049	23	22	2 (9.1%)	60.79	8.303	44.17, 76.63
4063	45	42	0 (0%)	33.95	6.241	22.85, 47.46
5029	47	45	0 (0%)	62.07	6.905	48.07, 75.02
5044	88	70	36 (51.4%)	40.38	5.579	29.69, 51.7
5045	79	68	12 (17.6%)	47.46	5.902	35.97, 59.12
5088	102	87	7 (8.0%)	24.33	3.813	17.73, 32.64
6010	19	19	0 (0%)	63.97	8.463	46.91, 79.94
6019	93	64	1 (1.6%)	38.13	5.327	28.27, 49.26
6038	125	79	12 (15.2%)	28.43	4.34	20.67, 37.63
6065	44	39	0 (0%)	50.17	7.443	36.11, 64.75
T1 Ins > 15						
1008	124	95	14 (14.7%)	30.07	4.123	22.57, 38.78
1015	35	27	0 (0%)	37.11	7.649	24.02, 53.68
1025	26	25	5 (20%)	63.36	8.222	47.1, 78.24
1028	45	42	42 (100%)	96.65	1.535	92.88, 98.65
1039	47	43	0 (0%)	30.64	5.68	20.75, 42.81
1086	91	67	0 (0%)	39.72	5.53	29.52, 50.89
2009	101	86	3 (3.5%)	30.46	4.34	22.65, 39.38
2010	102	89	32 (36.0%)	33.4	4.478	25.33, 42.55
2013	300	138	0 (0%)	18.01	2.556	13.41, 23.37
2015	44	32	14 (43.8%)	53.02	7.695	38.25, 68.16
2021	33	24	4 (16.7%)	58.05	8.683	41.19, 74.89
2022	41	36	0 (0%)	43.41	7.338	30.09, 58.72
3015	19	19	1 (5.3%)	58.93	9.166	40.84, 76.37
3022	23	20	1 (5%)	66.05	8.198	49.04, 81
4003	26	23	18 (74.1%)	75.86	6.818	60.73, 87.59
4008	67	59	0 (0%)	31.24	4.825	22.74, 41.81
4013	55	39	0 (0%)	49.62	7.283	35.8, 64.12
4043	36	34	0 (0%)	39.05	6.796	26.5, 52.88
4045	46	38	0 (0%)	48.41	7.506	34.24, 63.57
5004	28	26	3 (11.5%)	61.33	8.093	45.33, 76.98
5023	22	20	5 (25%)	72.85	8.022	55.42, 86.8
5026	26	24	0 (0%)	74.24	7.2	58.61, 86.96
6018	62	39	0 (0%)	29.94	5.703	19.96, 42.12
6023	76	59	14 (23.7%)	32.67	5.174	23.36, 43.41

Hypos, hypoglycemia episodes; T1Ins < 5, type 1 diabetes with < 5 years' duration; T1Ins > 15, type 1 diabetes with > 15 years' duration; T2Ins < 2, type 2 diabetes treated with insulin for < 2 years; T2Ins > 5, type 2 diabetes treated with insulin for > 5 years; T2obs, type 2 diabetes treated with oral agents.



SUPPLEMENTARY FIG. S2. (a) Number of embedded empty cells against c_i. (b) Total number of symptoms reported throughout all episodes ($\sum_{j,k} y_{ijk}$) for each patient against $\hat{c}_i$.

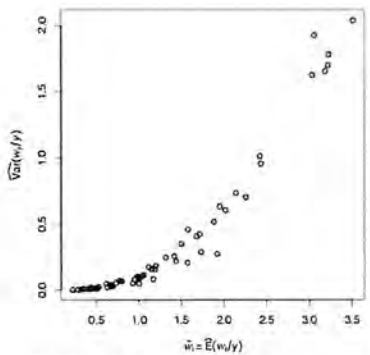
An alternative GLM with log-normal errors was also considered for modeling the consistency measure $\tilde{w}_i$, with

$$\tilde{w}_i \sim LN(m_i, \sigma^2_{(i)}) \text{ for } i = 1, \ldots, I$$

SUPPLEMENTARY TABLE S2. POSTERIOR MEAN OF DEVIANCE AND DEVIANCE INFORMATION CRITERION UNDER THE TWO GENERALIZED LINEAR MODEL ERROR DISTRIBUTIONS CONSIDERED

Error distribution	Deviance	DIC
Gamma	152.352	163.804
Log-normal	155.444	167.277

DIC, deviance information criterion.



SUPPLEMENTARY FIG. S3. Mean against variance of consistency measure $\tilde{w}_i = \sigma_i^{-2}$. For clarity of presentation subject 1028 has been omitted from the graph because this subject has $\hat{E}(w_i/y) = 34.9$ and $\widehat{var}(w_i/y) = 243.7$.

where m_i is associated with patient-specific covariates through the identity link $m_i = x_i^T \beta$, and σ_i^2 and b are as before. Again, relatively vague priors were assumed for all parameters. This model did not provide better fit to the data compared with the gamma formulation (Eq. 3). This is demonstrated by the lower values of both the posterior mean of the deviance and the deviance information criterion for the gamma model (Table S2). A discussion on the use of these model assessment criteria is given in Spiegelhalter et al.[3] (we note that because the two models have the same number of parameters, both criteria give equivalent results).

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Delayed Recovery of Cognitive Function Following Hypoglycemia in Adults With Type 1 Diabetes

Effect of Impaired Awareness of Hypoglycemia

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OBJECTIVE—Recovery times of cognitive functions were examined after exposure to hypoglycemia in people with diabetes with and without impaired hypoglycemia awareness.

RESEARCH DESIGN AND METHODS—A total of 36 subjects with type 1 diabetes were studied (20 with normal hypoglycemia awareness [NHA] and 16 with impaired hypoglycemia awareness [IHA]). A hyperinsulinemic glucose clamp was used to lower blood glucose to 2.5 mmol/l (45 mg/dl) (hypoglycemia) for 1 h or to maintain blood glucose at 4.5 mmol/l (81 mg/dl) (euglycemia) on separate occasions. Cognitive tests were applied during each experimental condition and were repeated at 10- to 15-min intervals for 90 min after euglycemia had been restored.

RESULTS—In the NHA group, performance was impaired on all cognitive tasks during hypoglycemia and remained impaired for up to 75 min on the choice reaction time (CRT) task ($P = 0.03$, $\eta^2 = 0.237$). In the IHA group, performance did not deteriorate significantly during hypoglycemia. When all subjects were analyzed within the same general linear model, performance was impaired during hypoglycemia on all tasks. Significant impairment during recovery persisted for up to 40 min on the CRT task ($P = 0.04$, $\eta^2 = 0.125$) with a significant glycemia-awareness interaction for CRT after one hour of hypoglycemia ($P = 0.045$, $\eta^2 = 0.124$). Performance on the trail-making B task was impaired for up to 10 min after euglycemia was restored ($P = 0.024$, $\eta^2 = 0.158$).

CONCLUSIONS—Following hypoglycemia, the recovery time for different cognitive tasks varied considerably. In the IHA group, performance was not significantly impaired during hypoglycemia. The state of awareness of hypoglycemia may influence cognitive function during and after hypoglycemia. ***Diabetes* 57: 732–736, 2008**

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B.M.F. has been a member of an advisory panel for and has received honoraria/consulting fees from Eli Lilly, sanofi-aventis, GlaxoSmithKline, and Takeda.

CRT, choice reaction time; DSST, digit symbol substitution test; IHA, impaired hypoglycemia awareness; NHA, normal hypoglycemia awareness; RVIP, rapid visual information processing; TMB, trail making B.

The recovery of cognitive function following hypoglycemia has not received rigorous evaluation. Previous studies examined nondiabetic volunteers (1–3) in small numbers (3), did not include a euglycemia control arm (1,4), measured neurophysiological parameters rather than cognitive function (1,2,5,6), or restricted cognitive testing to one or two time points (3–5). The interval between restoration of euglycemia and cognitive testing was usually ill defined (2,4–6). Controversy exists as to whether impaired awareness of hypoglycemia is associated with relative preservation (7–13) or exacerbation of the cognitive impairment induced by hypoglycemia (14–16). The present study examined the time taken for recovery of cognitive function in adults with type 1 diabetes and assessed the effect of their state of awareness on the response to, and recovery from, hypoglycemia.

RESEARCH DESIGN AND METHODS

The local medical research ethics committee approved the protocol, and subjects gave informed consent for participation.

Inclusion criteria were a diagnosis of type 1 diabetes and age 18–45 years. Exclusion criteria included pregnancy or any significant concurrent medical condition, history of head injury, epilepsy, or history of hypoglycemia-induced seizure.

A total of 36 subjects with type 1 diabetes were recruited: 20 with normal hypoglycemia awareness (NHA) and 16 with impaired hypoglycemia awareness (IHA) confirmed by documenting their hypoglycemia history and using a validated hypoglycemia awareness scale (17). Microvascular complications were defined as any clinical diagnosis of diabetic retinopathy, neuropathy, or nephropathy. The IHA group had a longer duration of diabetes (median 33.5 years [range 22–43]) compared with the NHA group (20 years [10–44]; $P = 0.001$) and a higher prevalence of microvascular complications (six patients in IHA group and one patient in NHA group; $\chi^2 = 5.994$, $P = 0.043$). Other comparisons (sex, age, A1C, and BMI) were nonsignificant.

Glucose clamp procedure. Each subject underwent one hypoglycemic and one euglycemic clamp separated by at least 2 weeks. Subjects were blind to clamp order, which was randomized and counterbalanced. Six studies were postponed (two NHA and four IHA) because of symptomatic hypoglycemia or blood glucose <4.0 mmol/l during the preceding 48 h. Using a modified hyperinsulinemic glucose clamp technique (18), blood glucose was stabilized at 4.5 mmol/l (81 mg/dl) (euglycemia) and maintained for 30 min while subjects practiced the cognitive tests. In the euglycemic condition, glucose was maintained at this level. In the hypoglycemic condition, blood glucose was lowered over 20 min to 2.5 mmol/l (45 mg/dl), where it was maintained for 1 h (experimental phase). Euglycemia (~4.0 mmol/l, ~72 mg/dl) was then rapidly restored. The recovery phase start was defined by two consecutive arterialized glucose readings ≥4 mmol/l (≥72 mg/dl), with cognitive testing commencing 10 min after the first of these readings.

Symptom scores and cognitive function tests. The cognitive tests were trail making B (TMB), digit symbol substitution test (DSST), and four choice reaction time (CRT), which are sensitive to hypoglycemia (20) and easy to administer repeatedly. The cognitive battery and the Edinburgh Hypoglycemia Scale (19) were applied at baseline, at the beginning and end of the experimental phase, and during the recovery period at 10, 20, 30, 40, 55, 70, and 85 min after euglycemia was restored.

Statistical analysis. Cognitive scores were compared using general linear modeling (repeated-measures ANOVA). In the full model, including all subjects, hypoglycemia awareness status was the between-subject factor. The experimentally induced state of hypoglycemia versus euglycemia was the within-subject factor. Age, sex, duration of diabetes, and index of exposure to hypoglycemia had no significant effect on the results, and these fixed effects/covariables were therefore excluded from the final model. Individual test scores within a single clamp study were corrected for baseline performance by subtracting their baseline score from their scores at each time point. The model compared these adjusted scores between the euglycemic and hypoglycemic conditions (repeated measure). The effects of hypoglycemia in NHA and IHA groups separately are also reported. Statistical significance was accepted at $P < 0.05$. Partial η^2 was used to indicate effect size. Analyses were performed using SPSS for Windows version 12.0.

RESULTS

Cognitive tasks

Table 1 shows mean ± SD test scores corrected for baseline performance. CRT and TMB scores are completion times; a lower score represents better performance. The DSST score is the number of items completed in 2 min; a higher score represents better performance. The effects of glycemic condition were first examined within NHA and IHA groups and then for all subjects combined including interaction between glycemic condition and awareness status. A considerable practice effect was apparent on the DSST task but not on the CRT and TMB tasks. The randomized counterbalanced study design controls for practice effects.

NHA subjects

Performance on all cognitive tests was significantly impaired during hypoglycemia in NHA subjects (Table 1 and Figs. 1*A*, 2*A*, and 3*A*). Performance on DSST and TMB deteriorated significantly during hypoglycemia but reverted to baseline as soon as euglycemia was restored (Table 1). CRT remained impaired after restoration of euglycemia, with significant differences between the hypoglycemic and euglycemic conditions at 20, 30, 40, and 75 min (Table 1 and Fig. 1*A*).

IHA subjects

In IHA subjects, cognitive tests did not show significant impairment during hypoglycemia, with the exception of the DSST task after 60 min of hypoglycemia ($P = 0.041$; Table 1 and Figs. 1*B*, 2*B*, and 3*B*). There were no significant differences during the recovery phase. Compared with NHA subjects, trends toward a smaller deterioration in performance and more rapid recovery following hypoglycemia were observed.

All subjects

Poorer performance during hypoglycemia versus euglycemia was seen for all cognitive tasks. This difference persisted for CRT at 20, 30, and 40 min after euglycemia was restored ($P = 0.04$, $\eta^2 = 0.125$) and for TMB at 10 min after euglycemia was restored ($P = 0.024$, $\eta^2 = 0.158$). There was no persistence of impairment of DSST performance.

Comparison of effect of hypoglycemia in NHA and IHA subjects

DSST. The interaction between glycemic condition and hypoglycemia awareness (hereafter termed the glycemia-awareness interaction) was significant only at the start of hypoglycemia (Table 2) ($P = 0.009$), suggesting that hypoglycemia caused significantly greater impairment in NHA subjects than in IHA subjects.

CRT. Performance was impaired during hypoglycemia and at 20, 30, and 40 min after euglycemia was restored ($P = 0.04$, $\eta^2 = 0.125$). The glycemia-awareness interaction was significant only at the end of hypoglycemia ($P = 0.045$, $\eta^2 = 0.124$). This infers that the NHA group, relative to their baseline performance, was more affected during hypoglycemia than the IHA group, but there were no significant between-group differences during recovery.

TMB. The glycemia-awareness interaction was not significant at any time point.

DISCUSSION

Results from the present study suggest three conclusions. First, in all subjects combined, cognitive performance was significantly impaired during hypoglycemia in comparison with euglycemia, consistent with numerous previous studies (20). Second, cognitive performance was significantly impaired in NHA subjects alone, whereas only nonsignificant trends were seen in IHA subjects. This difference appears to suggest that individuals with IHA are less affected by hypoglycemia than those with NHA. A formal NHA-IHA difference requires a significant interaction between awareness status and glycemic condition; this was seen for CRT at the end of hypoglycemia and for DSST at the start of hypoglycemia, without correction for multiple comparisons. This study therefore provides the first, but limited, evidence for a formally tested difference in the cognitive effect of hypoglycemia depending on state of awareness. Third, CRT remained significantly prolonged up to 75 min after hypoglycemia in NHA subjects (and up to 40 min in all subjects combined), and TMB completion time remained significantly prolonged 10 min after hypoglycemia in all subjects combined. These data suggest that some aspects of cognitive function remain impaired for a clinically significant time after correction of hypoglycemia.

The absolute differences in CRT between the groups were small. There was a trend toward improvement in CRT during the euglycemic condition in the NHA group with a corresponding deterioration in the IHA group. This highlights the importance of the euglycemic control arm in that each group's performance during hypoglycemia is compared with performance during euglycemia and not to that of a different group, thus controlling for between-group differences that may not be apparent.

The present study has a strong power for within-subject comparisons but is less powerful at detecting between-subject differences; high power for a medium-effect size difference between groups requires over 50 subjects per group. It is impossible to exclude some overlap in hypoglycemia awareness between the two groups because scoring methods require some degree of subjective self-assessment. The IHA subjects also had longer duration of diabetes and more microvascular disease, although as IHA appears to be strongly associated with diabetes duration, it may be impossible to match for these characteristics. Finally, asymptomatic hypoglycemia before the study cannot be excluded, particularly in IHA subjects, despite the frequent monitoring of blood glucose for the preceding 48 h.

If the NHA-IHA differences are accepted, they suggest that IHA subjects develop cerebral adaptation to hypoglycemia. This interpretation may appear to be counterintuitive, as these individuals have a higher risk of severe hypoglycemia than those with NHA (17,21). However, this adaptation may increase their susceptibility to severe hypoglycemia by limiting the time to identify low blood glucose and allowing progression to debilitating neurogly-

TABLE 1
Mean ± SD change from baseline test scores and effect of glycemia condition and glycemia awareness interaction

	Normal awareness				Impaired awareness				All subjects combined					
									Effect of euglycemia/hypoglycemia		Glycemia·awareness interaction			
	Euglycemia	Hypoglycemia	P	η^2	Euglycemia	Hypoglycemia	P	η^2	Euglycemia	Hypoglycemia	P	η^2	P	η^2
CRT Exp1	−8.7 ± 30.3	51.2 ± 35.9	<0.001	0.762	57 ± 39.4	341 ± 44.9	0.124	0.161	−2.3 ± 34.9	43.6 ± 40.5	<0.0001	0.435	<0.0001	0.089
CRT Exp2	−14.6 ± 53.6	68.2 ± 51.8	<0.001	0.690	79 ± 39.0	442 ± 54.7	0.092	0.189	−4.6 ± 48.4	57.2 ± 53.8	<0.0001	0.459	0.045	0.124
CRT Rec1	−0.6 ± 57.8	39.0 ± 45.4	0.169	0.102	−34 ± 51.9	131 ± 36.8	0.284	0.082	−19 ± 54.5	169 ± 41.4	0.081	0.090	0.848	0.001
CRT Rec2	−4.5 ± 50.8	38.7 ± 60.8	0.002	0.409	−183 ± 66.7	186 ± 233	0.136	0.163	−10.6 ± 57.7	30.1 ± 48.9	0.002	0.264	0.672	0.006
CRT Rec3	−15.2 ± 42.0	23.2 ± 73.3	0.005	0.360	−145 ± 44.1	303 ± 42.5	0.696	0.011	−2.0 ± 45.0	21.9 ± 60.9	0.024	0.149	0.089	0.088
CRT Rec4	−3.8 ± 53.2	28.0 ± 62.2	0.010	0.313	15.5 ± 46.1	204 ± 40.3	0.715	0.010	4.8 ± 50.4	24.6 ± 52.9	0.040	0.125	0.071	0.028
CRT Rec5	−35 ± 84	−54 ± 82	0.580	0.018	−70 ± 139	−29 ± 129	0.449	0.005	−50 ± 111	43 ± 109	0.022	0.014	0.406	0.025
CRT Rec6	−15.4 ± 55.4	8.6 ± 50.1	0.03	0.237	174 ± 60.1	359 ± 39.9	0.974	0.000	−0.8 ± 69.1	128 ± 45.5	0.231	0.041	0.255	0.010
CRT Rec7	9.6 ± 51.3	10.7 ± 39.7	0.168	0.109	251 ± 57.0	56 ± 48.2	0.251	0.093	5.8 ± 55.9	83 ± 52.1	<0.0001	0.073	0.100	
DSST Exp1	8.1 ± 5.3	−6.1 ± 11.0	<0.001	0.664	5.6 ± 5.7	1.1 ± 9.1	0.082	0.201	7.0 ± 5.5	−2.9 ± 10.7	<0.001	0.481	0.009	0.195
DSST Exp2	12.2 ± 6.0	−0.9 ± 10.2	<0.001	0.652	15.6 ± 8.7	8.7 ± 11.4	0.041	0.266	13.7 ± 7.4	3.4 ± 11.6	<0.001	0.474	0.106	0.080
DSST Rec1	11.5 ± 8.0	9.0 ± 7.9	0.359	0.049	17.2 ± 10.1	12.6 ± 11.3	0.176	0.127	14.0 ± 9.3	10.6 ± 9.6	0.082	0.086	0.605	0.008
DSST Rec2	13.8 ± 9.0	11.3 ± 9.1	0.333	0.032	19.6 ± 13.1	15.3 ± 10.7	0.198	0.115	16.4 ± 11.2	13.1 ± 9.9	0.084	0.097	0.633	0.007
DSST Rec3	13.8 ± 9.5	11.9 ± 10.5	0.461	0.031	20.6 ± 13.3	19.3 ± 12.8	0.730	0.009	16.8 ± 11.7	15.2 ± 12.0	0.454	0.018	0.877	0.001
DSST Rec4	13.8 ± 7.9	13.8 ± 10.2	0.987	0.000	22.8 ± 14.7	18.9 ± 12.6	0.280	0.083	20.1 ± 11.4	16.9 ± 11.7	0.093	0.086	0.818	0.002
DSST Rec5	17.8 ± 8.3	16.5 ± 11.4	0.683	0.009	24.5 ± 15.0	21.2 ± 12.4	0.312	0.073	20.8 ± 12.1	18.6 ± 11.9	0.310	0.031	0.623	0.006
DSST Rec6	18.5 ± 7.9	19.4 ± 11.0	0.780	0.004	24.4 ± 14.6	20.5 ± 10.3	0.192	0.118	21.1 ± 11.0	19.9 ± 10.6	0.485	0.015	0.278	0.007
DSST Rec7	17.8 ± 11.4	19.0 ± 11.6	0.762	0.023	22.7 ± 12.2	21.3 ± 11.5	0.674	0.013	20.0 ± 13.1	20.5 ± 11.4	0.841	0.001	0.460	0.018
DSST Rec8	19.4 ± 7.0	7.8 ± 10.5	0.026	0.259	20 ± 12.9	11.4 ± 18.9	0.195	0.118	1.9 ± 9.9	9.4 ± 14.4	0.042	0.135	0.211	0.047
TMB Exp1	6.3 ± 10.4	14.6 ± 18.1	0.025	0.263	−0.1 ± 10.9	10.5 ± 12.4	0.063	0.300	3.6 ± 10.9	12.8 ± 15.8	0.003	0.278	0.772	0.003
TMB Exp2	1.8 ± 8.9	2.6 ± 9.7	0.174	0.106	7.9 ± 10.0	0.08	0.217	2.2 ± 9.1	6.0 ± 10.1	0.024	0.158	0.608	0.000	
TMB Rec1	3.6 ± 8.1	2.1 ± 9.6	0.765	0.005	11.5 ± 17.6	6.2 ± 14.2	0.250	0.109	3.3 ± 10.3	6.2 ± 14.2	0.251	0.045	0.173	0.021
TMB Rec2	1.9 ± 5.7	0.4 ± 6.3	0.562	0.020	2.4 ± 13.9	0.9 ± 9.3	0.621	0.019	2.1 ± 10.0	0.6 ± 7.7	0.181	0.017	0.712	0.005
TMB Rec3	−3.5 ± 8.1	−2.4 ± 9.2	0.580	0.018	−7.0 ± 13.9	−2.9 ± 12.9	0.449	0.005	−5.0 ± 11.1	4.3 ± 10.9	0.522	0.014	0.406	0.025
TMB Rec4	−2.6 ± 9.4	−5.1 ± 8.2	0.280	0.048	−17.2 ± 19.7	−4.8 ± 11.9	0.999	0.000	−11 ± 14.6	−5.0 ± 9.9	0.818	0.000	0.846	0.000
TMB Rec5	−0.6 ± 5.2	−4.1 ± 8.1	0.087	0.172	0.2 ± 13.1	1.9 ± 11.5	0.841	0.003	0.5 ± 9.4	−1.4 ± 10.1	0.294	0.010	0.323	0.030
TMB Rec6	0.8 ± 5.2	−1.1 ± 8.1	0.087	0.172	0.2 ± 13.1	1.9 ± 11.5	0.841	0.003	0.5 ± 9.4	−1.4 ± 10.1	0.294	0.010	0.323	0.030
TMB Rec7	−0.6 ± 8.0	−5.3 ± 9.7	0.290	0.070	1.0 ± 14.0	−0.6 ± 8.0	0.671	0.014	0.1 ± 10.9	−3.2 ± 9.1	0.128	0.033	0.809	0.002

CRT is in milliseconds, and TMB is in seconds. DSST score is the number of symbols identified in 2 min. Boldface indicates significant differences. Exp, experimental phase; Rec, recovery.

FIG. 1. Mean (SE) times on CRT test during hypoglycemia and euglycemia conditions in individuals with normal awareness of hypoglycemia (*A*) and impaired awareness of hypoglycemia (*B*). *$P < 0.05$ for euglycemia vs. hypoglycemia. ■, euglycemia; ▲, hypoglycemia.

FIG. 2. Mean (SE) times on TMB test during hypoglycemia and euglycemia conditions in individuals with normal awareness of hypoglycemia (*A*) and impaired awareness of hypoglycemia (*B*). *$P < 0.05$ for euglycemia vs. hypoglycemia. ■, euglycemia; ▲, hypoglycemia ♦Data for one subject was omitted at this time point as it skewed the data markedly (required 212 s to complete the task during hypoglycemia; the difference between hypoglycemia and euglycemia was not significant regardless of whether these data were included in the analysis.

copenia. The degree of cognitive adaptation acquired by those with IIA is insufficient to compensate completely for the loss of physical symptoms. Cognitive dysfunction begins at lower blood glucose levels in people with type 1 diabetes and IIA compared with those with NIAA (13), and antecedent hypoglycemia in individuals with type 1 diabetes and NIA can shift the thresholds for cognitive dysfunction to lower blood glucose levels (9,10,22,23). Glucose clamp studies in nondiabetic individuals have shown that 90–150 min of hypoglycemia the day before cognitive testing attenuates the deterioration in short-term memory, reaction time, and auditory-evoked brain potentials (11,24,25), and avoidance of hypoglycemia can restore the glucose thresholds for cognitive dysfunction to higher levels (7,8,12).

A previous, smaller study from our center compared the effects of hypoglycemia on cognitive function in 20 people with type 1 diabetes with either IIA or NIA and reported a trend toward poorer performance during hypoglycemia in IIA subjects (14). Methodological differences exist between the two studies, with the earlier study applying a cognitive battery of 20 min duration at one time point only, 10 min after euglycemia was restored. The effect of awareness was not significant for any of the tests used except for rapid visual information processing (RVIP), where the results are given for RVIP hits, misses, and reaction time. The result was not significant for the hits (i.e., correct answers) or reaction time, but there was a significant effect on RVIP misses (i.e., there were more false-positive responses in the group with impaired awareness). However, on this latter measure, the effect of the study condition (i.e., euglycemia vs. hypoglycemia) was not significant. The cognitive tests used differed from those used in the current study, and CRT (the test yielding the most interesting results in the present study) was not used.

There was inter-individual variability in the effects of hypoglycemia on cognition, consistent with anecdotal observations. The present study was not sufficiently large to study the determinants of these differences formally. Thus, advice to individuals should not be too dogmatic given the possibility of inter-individual differences. Furthermore, although an hour of asymptomatic hypoglycemia may occur frequently in individuals with IIA, hypoglycemia is probably perceived and corrected earlier in those with NIA. Exposure to a shorter period of hypoglycemia should be examined in a group of individuals with NIA to ascertain whether the duration of hypoglycemia affects the recovery of cognitive function.

The present study indicates that cognitive recovery as variable for different tasks but is prolonged for four-choice reaction time. It also provides evidence to support the

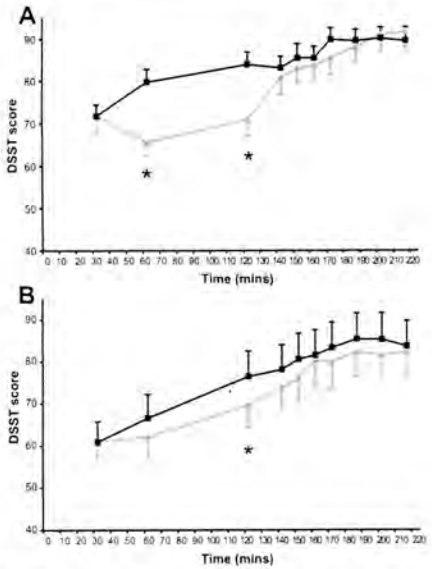

FIG. 3. Mean (SE) times on DSST during hypoglycemia and euglycemia conditions in individuals with normal awareness of hypoglycemia (*A*) and impaired awareness of hypoglycemia (*B*). *$P < 0.05$ for euglycemia vs. hypoglycemia. ■, euglycemia; ▲, hypoglycemia.

concept of cognitive adaptation to hypoglycemia in people with IHA, possibly as a consequence of recurrent exposure to hypoglycemia. The delay in recovery has implications for the safety of undertaking tasks requiring cognitive performance immediately after hypoglycemia, such as driving.

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