UNIVERSITY OF SOUTHAMPTON

The Effects of Caffeine Ingestion on the Physiological and Psychological Responses to Hypoglycaemia

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for my husband and family

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

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Doctor of Medicine

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by Joanne Marie Watson

In clinical practice, hypoglycaemia is the commonest side effect of the treatment of diabetes mellitus. If uncorrected it leads to the development of characteristic warning symptoms and impairment of higher cerebral function. For patients with diabetes such events can have major, negative impacts on both the quality of life and diabetes control. Caffeine has been shown to augment the perception of and physiological responses to hypoglycaemia in both healthy volunteers and patients with type 1 diabetes. However, these studies were laboratory based and involved caffeine abstinence for 72 hours prior to testing.

The five studies that constitute this thesis investigated both the impact of caffeine consumption on hypoglycaemic events in free-living patients, as well as the effects of tolerance to the aforementioned responses to hypoglycaemia. In addition the relationship between caffeine and hypoglycaemia was further elucidated by studying the electrophysiological effects during controlled hypoglycaemia and the recovery period.

Clinically, caffeine increased the number of symptomatic hypoglycaemic events in free-living patients. This enabled them to take action against hypoglycaemia. This change was not at the expense of increased severe hypoglycaemic events, although the number of such events was small. Pressor responses were preserved in females only but middle cerebral artery blood velocity (V_{MCA}) was most affected in males.

Complete tolerance was not demonstrated in 3 laboratory studies. Following overnight abstention only from caffeine, all responses to hypoglycaemia (2.5 mmol/l, induced by a hyperinsulinaemic glucose clamp technique) were augmented after a caffeine challenge compared to placebo. However, due to caffeine's long half-life, these effects continued into the recovery period, as the 'normal' responses to a period of hypoglycaemia were affected, namely no rise in V_{MCA} above baseline, increased time for symptom recovery and restoration of endocrine milieu; in addition possibly slower recovery of cerebral function as measured by P100 visual evoked potentials.

In two further studies, the response to 200mg caffeine challenge during euglycaemia or hypoglycaemia (induced by glucose clamp technique), with subjects either caffeine-replete or caffeine-naïve, tolerance was either not demonstrated (general cognition, symptoms, mood) or partial (V_{MCA}, blood pressure). Caffeine consumption was associated with negative aspects of mood during the euglycaemia study (which could relate to short term caffeine deprivation) and worsening verbal-logical test results during the hypoglycaemia study.

The final study illustrated that caffeine-withdrawal should not be underestimated. Even a small dose of caffeine (that in 2 cans DIET COKE) ameliorated the symptoms of caffeine withdrawal, with positive effects on mood. In addition V_{MCA} decreased and blood pressure rose following the reintroduction of caffeine.

Thus caffeine alters the responses to hypoglycaemia in both clinical and laboratory studies. Whilst it can negatively affect the recovery period, the ability for it to improve hypoglycaemia awareness proves its potential to be of benefit to patients with type 1 diabetes. Further work is required to investigate its role in the treatment of hypoglycaemia unawareness.

The prevention or rapid correction of hypoglycaemia is critical to survival of the brain and thus the individual. Philip Cryer

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ABBREVIATIONS

AUC	Area under the curve
BAEP	Brain stem evoked potentials
Caff	Caffeine
CBF	Cerebral blood flow
CNS	Central nervous system
4-CRT	4-choice reaction time
C-replete	Caffeine-replete (studies 3 + 4)
C-naive	Caffeine-naïve (studies 3 + 4)
DERA	Defence Evaluation and Research Agency
DCCT	Diabetes Control and Complications Trial
EEG	Electroencephalogram
E+C	Euglycaemia and caffeine (study 2)
EP	Evoked potential
ERP	Event related potential
G, Glc	Glucose
GH	Growth Hormone
H+C	Hypoglycaemia and caffeine (study 2)
H+P	Hypoglycaemia and caffeine (study 2)
I	Insulin
POMS	Profile of Mood States
NART	National Adult Reading Test
TB	Test battery (study 2)
UKPDS	United Kingdom Prospective Diabetes Study
V_{MCA}	Middle cerebral artery blood velocity
VMD	Visual Movement Detection
VCD	Visual Change Detection
VEP	Visual Evoked Potential
VMH	Ventromedial hypothalamus

DECLARATION

I declare that the work in this thesis is the result of my own research, with the exceptions acknowledged overleaf.

I was involved at all stages of each of the five studies, which make up this thesis; with the exception of gaining ethical approval for study 1, which was arranged by Dr. David Kerr prior to my fellowship commencing. I recruited volunteers for the studies, with help as acknowledged overleaf. I ran all of the hyperinsulinaemic clamp procedures and organised others in taking the measurements throughout. I analysed my own data, again with advice acknowledged overleaf

This work was wholly conducted whilst I was in registered postgraduate candidature, at the Royal Bournemouth Hospital, Bournemouth, University of Southampton. This work has not been previously submitted, in whole or in part to any other University for a higher degree.

Joanne Marie Watson

December 2000

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Section I HYPOGLYCAEMIA

1. General Introduction and Objectives

Since Banting and Best developed the insulin extraction process, treating their first patient with type I diabetes mellitus successfully in 1922, the management of this condition has seen many changes. Fundamentally diabetes mellitus would be an easy disease to treat if it were not for the problem of hypoglycaemia. Doctors often underestimate the frequency of hypoglycaemia as well as the anxiety and disruption it causes to patients and their relatives. From the patients' point of view, the consequences can range from mild inconvenience to a phobic avoidance of low blood glucose (Cox D. et al 1987). A consequence of this is prevention of a patient striving for good diabetic control (Surwit R. et al 1982). Even before the publication of the Diabetes Control and Complications Trial (DCCT 1993), Siperstein had concluded that 'physicians must weigh the possible beneficial effects of aggressive insulin therapy against the known harmful effects of hypoglycaemia' (Siperstein M. et al 1977).

'Hypoglycaemia is a biochemical term and the value that defines it depends on the nature of the sample' (Marks V. 1986). In clinical practice, hypoglycaemia are usually defined as the blood glucose level at which detectable physiological changes occur, which if uncorrected leads to the development of characteristic warning symptoms and impairment of higher cerebral function. Caffeine has been shown to augment the perception of and physiological responses to hypoglycaemia in both healthy volunteers and people with type I diabetes mellitus (Kerr D. et al 1993; Debrah K. et al 1996).

In the following three sections, the current literature of hypoglycaemia (the problem in general), caffeine and the relationship between the two are reviewed. The aims of this thesis include firstly to examine in greater detail the physiological effects of hypoglycaemia and caffeine, including the influence of caffeine once euglycaemia has been restored. Second, the questions of caffeine tolerance and withdrawal effects to these phenomena are explored in healthy individuals within the laboratory. Finally the influence of caffeine on the recognition and response to hypoglycaemia in free-living patients with type I diabetes mellitus is investigated. It is my overall aim to demonstrate whether caffeine has a potential role in the treatment for hypoglycaemia

unawareness, a condition with wide implications on employment, the ability to drive and quality of life for patients treated with insulin.

2. Frequency of Hypoglycaemia

When studying the frequency of hypoglycaemia, it is useful to divide the spectrum of iatrogenic hypoglycaemia into three categories:

i) Asymptomatic / Biochemical hypoglycaemia

ii) Mild- Moderate hypoglycaemia

iii) Severe Hypoglycaemia

This last category has a consensus definition which is hypoglycaemia sufficiently disabling to require the assistance of a third party (DCCT 1993). This definition is used throughout this thesis.

The true frequency of asymptomatic hypoglycaemia in type 1 diabetes is not known but almost certainly occurs in all such patients. Whilst accurate data on this matter is limited Thorsteinsson et al demonstrated that patients treated to a daytime blood glucose median of 5.0mmol/l, had 10% of serial blood glucose measurements of less than 3.0mmol/l. (Thorsteinsson B. et al 1986). Night time hypoglycaemia is a particular problem. During this time it is usual to go without food and the body is most sensitive to insulin. It has been calculated that if the blood glucose upon retiring is less than 6.0mmol/l, the likelihood of nocturnal hypoglycaemia is 80% in the patients studied and of the nocturnal hypoglycaemic episodes detected most are asymptomatic (Pramming S. et al 1985).

Symptomatic episodes of hypoglycaemia are also common and are divided into mild, moderate and severe as above. In real life the first two categories are often considered together, with the third having major effects on the individual patient's life. In a study of 411 patients with type 1 diabetes (75% using twice daily injections of insulin) there was an average of 1.8 episodes of mild - moderate hypoglycaemia per week (Pramming S. et al 1991). Similar rates were seen in the DCCT with rates of 1 or 2 episodes per week in the conventional and intensive groups respectively (DCCT 1993). Thus the average patient is likely to suffer thousands of episodes of symptomatic hypoglycaemia over a lifetime with type I diabetes.

A total of 1441 patients were followed over an average period of 6.5 years in the DCCT. 35% of those in the conventional therapy group (n = 730) and 65% in the intensive group (n = 711) suffered episodes of severe hypoglycaemia (DCCT 1995). 19% and 38% respectively suffered severe hypoglycaemia with coma or seizures. The event rates for severe hypoglycaemia have been calculated as 19 and 61 episodes per 100 patient years for the conventional and intensive groups respectively. Thus in attempting to control blood glucose close to the normal range, the rate of severe hypoglycaemia is more than trebled.

Whilst the DCCT is taken as the gold standard for modern diabetes care, it probably provides minimum estimates of the frequency of iatrogenic hypoglycaemia. This is firstly because the patients were recruited carefully and arguably represented the most motivated and physically well patients. Secondly patients who had previously experienced severe hypoglycaemia were excluded from the full trial but this is a strong predictor of further episodes of hypoglycaemia (DCCT 1997). Thirdly the DCCT patients were supported to a degree by healthcare professionals which is in excess of that available to non-trial patients. Therefore the approximate further doubling of rates for severe hypoglycaemia reported in other studies may be more representative (Cryer P. 1994; Reichard P. et al 1991). Lower rates of severe hypoglycaemia have been recorded (Bott S. et al 1997) but severe hypoglycaemia here was defined as requiring glucagon or intravenous glucose for recovery.

Pampanelli et al (Pampanelli S. et al 1996) reported one episode of severe hypoglycaemia per 100 patient-years in newly diagnosed type 1 diabetic patients started immediately on intensive insulin therapy and followed for 13 years. These data should be interpreted with caution not only because all patients were on the same drug regimen and able to contact a physician 24 hours a day but also because of the shorter duration of diabetes in these patients. Although they were followed up over a 13 year period, the duration of diabetes was still comparatively short, during this time there would also be a relatively low frequency of defective glucose counterregulation as well as the initial 'honeymoon' phase. In addition the reported rate of severe hypoglycaemia was estimated retrospectively and thus incurred the problems of limited ascertainment

due to poor recall of events. This is in contrast to the Stockholm trial where such incidents were ascertained prospectively (Reichard P.et al 1991).

3. Morbidity and Mortality associated with Hypoglycaemia

Iatrogenic hypoglycaemia causes significant morbidity, both physical and psychological, as well as mortality. Physical morbidity from an episode of hypoglycaemia ranges from unpleasant symptoms and cognitive impairment to profound neurological symptoms including seizures and coma. Whilst complete recovery can usually be anticipated from an acute hypoglycaemic event, permanent damage may also occur from chronic hypoglycaemia, e.g. cortical blindness and stroke (Gold A. et al 1996). There is a relationship between the severity and duration of hypoglycaemia and the damage inflicted. Plasma glucose levels less than 1.1mmol/l for 5 hours were required to consistently produce neurological damage in monkeys (Kahn K. et al 1971).

The incidence of permanent neurophysiological damage was not increased in the intensive therapy group in the DCCT (DCCT 1996a). Whilst there were no differences in psychiatric status between the 2 treatment groups, there was a relationship between the frequency of hypoglycaemia episodes with seizure or coma and psychiatric symptoms including depression, interpersonnal sensitivity and paranoid ideation (Diabetes 1996b). In addition a significant proportion of diabetic patients who have experienced severe recurrent hypoglycaemia develop permanent EEG abnormalities (Gold A. et al 1993). With regard to permanent cognitive impairment due to recurrent hypoglycaemia the relationship remains 'not proven' (Deary I. et al 1996).

The cardiovascular and cerebrovascular circulations are also affected by hypoglycaemia and it may be associated with myocardial ischaemia and stroke disease, although much of the evidence is anecdotal. The profound stimulus to the sympathoadrenal system may result in morbidity because of the concurrent haemodynamic changes (Hilsted J. 1993). There are case reports of hypoglycaemia-induced myocardial ischaemia or infarction (Gilbert R. et al 1946), as well as multiple cerebral effects including hemiplegia (Lala V. et al 1989).

Hypoglycaemia can provoke cardiac arrhythmias by several mechanisms:

- increased sympathetic drive
- increased plasma adrenaline
- hypokalaemia
- sympathovagal imbalance

The psychological morbidity of iatrogenic hypoglycaemia includes fear of such an episode, increased anxiety and lower levels of overall happiness (Pramming S.et al 1991; Jacobson A. 1996; DCCT 1996b; Gold A. et al 1997). Fear of hypoglycaemia can be an impediment to glycaemic control and future health.

The true hypoglycaemic mortality rate in type 1 diabetes mellitus is unknown. This is because a post mortem diagnosis cannot be made with certainty. Following death the glucose concentration rises in the central veins because of hepatic glycogenolysis and falls peripherally with post mortem glucose uptake (Gale E. 1980). The British Diabetic Association Cohort Study looked at all cause mortality in patients with type 1 diabetes mellitus incident at a young age. In a cohort of 23752 patients, there were 949 deaths in the follow up period (maximum 25 years). Of these 18% and 6% of all deaths were attributed to hypoglycaemia in males and females respectively (Laing S. et al 1999). The relationship of hypoglycaemia, if any, to the deaths of patients who appear well upon retiring but are found 'dead in bed' is unknown (Tattersall R. et al 1991). During the DCCT, there were few deaths, with one death in each group possibly attributable to hypoglycaemia. The relative risk of death was not increased in the intensively treated group compared to the conventional treatment group (DCCT 1995).

4. Risk Factors for Hypoglycaemia Development

Current insulin replacement regimens remain imperfect and thus situations will arise where there is a surplus of insulin, leading to hypoglycaemia. Whilst there may be an excess of administered insulin, an ill-timed dose or wrong type of insulin administered, this alone is not the whole problem. Complex inter-relationships between amount, type and timing of food consumption combined with endogenous glucose production and utilisation (insulin and non-insulin dependent) and also varying insulin sensitivity exist.

The DCCT demonstrated that HbA1c levels accounted statistically for only 60% of the increased risk of severe hypoglycaemia in the intensively treated group (DCCT 1997). Other studies have identified additional features which may be involved:

i) previous severe hypoglycaemic episodes (DCCT 1997; Bott S.et al 1997)

ii) long duration of type 1 diabetes (more than 6 years) (DCCT 1997; Cox D.et al 1994)

iii) absolute insulin deficiency (Bott S.et al 1997; DCCT 1997)

iv) sleep (DCCT 1997; Pramming S. et al 1985, 1990)

v) impaired hypoglycaemia awareness (Gold A. et al 1994)

vi) alcohol (Kerr D. et al 1990)

vii) activity at the time of hypoglycaemia

viii) special groups - adolescents (DCCT 1997) - pregnancy (Rosen B. et al 1995).

However, the identification of these risk factors is relatively unhelpful in prediction of future severe hypoglycaemic events. In fact only 7% of future episodes can be predicted using these factors alone (DCCT 1997). Gold et al improved this to 18%, using a structural equation model which considers history of severe hypoglycaemia, hypoglycaemia awareness and an autonomic score (Gold A.et al 1997).

Cox et al developed a *low blood glucose index* (LBGI), to more accurately predict those at risk from future severe hypoglycaemia (Cox D. et al 1994). This was established by relating the blood glucose monitoring results from 78 patients with type 1 diabetes over a 2 - 3 week period, with episodes of severe hypoglycaemia. Those subjects who recorded more variable and lower blood glucose recordings suffered more frequently from severe hypoglycaemia. From these results the authors produced the LBGI and further identified that by including LBGI and the standard deviation of blood glucose readings for the subjects in their cohort in a regression model then 43.5% of the variance in severe hypoglycaemia episodes could be accounted for. Subsequently the LBGI has been shown to provide an accurate assessment of risk in the clinical situation as well (Kovatchev B. et al 1998).

5. Recognition of Hypoglycaemia

In the non-diabetic population, hypoglycaemia is a much rarer event as blood glucose is tightly controlled by multiple homeostatic mechanisms. The pharmacodynamics and pharmacokinetics of insulin mean that glucose recovery from insulin-induced hypoglycaemia involves other factors capable of overcoming the glucose-lowering effects of insulin. This is illustrated by an intravenous injection of 0.1U/ Kg insulin causing a fall in blood glucose within minutes, reaching a nadir between 20 and 30 minutes. Blood glucose then rises over the next 2 hours, despite the concentration of insulin being at least tenfold greater than baseline insulin concentrations (Garber A. et al 1976).

The body needs to identify that blood glucose has fallen below an acceptable level and co-ordinate protective responses. Although more than one system exists, the evidence to date has identified that this is mainly within the central nervous system (Biggers D. et al 1989). A bilateral infusion of glucose into both carotid and vertebral arteries was designed to maintain cerebral euglycaemia whilst simultaneously inducing systemic hypoglycaemia. In this (canine) experiment the usual endocrine responses to hypoglycaemia were abolished (Biggers D.et al 1989). However this and similar work (Frizzel R. et al 1993) illustrated the importance of the brain in the response to hypoglycaemia but did not localise specifically the 'glucosensor'.

In a series of experiments, W. and M. Borg et al have subsequently shown that the ventromedial hypothalamus (VMH), which does not have a blood brain barrier, is a dominant but not exclusive centre within the CNS for sensing glucopenia. In rats, focal lesions of VMH abolishes the glucagon and catecholamine responses to systemic hypoglycaemia (Borg W. et al 1994), as does selective prevention of glucopenia in the VMH by glucose perfusion via stereotactically placed microdialysis probes (Borg M. et al 1997). Conversely if localised glucopenia with the infusion of 2-deoxy-glucose is established around VMH, there is a prompt increase in the counterregulatory hormones in the absence of systemic hypoglycaemia (Borg W. et al 1995). More recently this group have demonstrated that in diabetic BB rats (a close model for human type 1 diabetes), the capacity of VMH glucopenia to activate the sympathoadrenal system for

catecholamine production is only modestly decreased but that the communication between VMH and the α-cell for glucagon production is completely interrupted (Borg M. et al 1999). In this same study chronic hypoglycaemia in nondiabetic rats suppresses the ability of VMH to recognise glucopenia or activate hormonal counterregulation.

Further evidence that VMH has the ability to detect glucose concentration includes the identification of glucokinase and GLUT-2 gene-expression within (Jetton T. et al 1997). Hypothalamic neurons also contain ATP-sensitive channels which are important in signal transduction (Zini S. et al 1991). However the cellular mechanisms used by VMH to transduce the glucose signal are unknown but the evidence to date suggests that cells within VMH are intrinsically involved. It is possible that VMH may share common mechanism with the pancreatic β-cell. During et al showed that manipulation of glucose in the substantia nigra, where most ATP channels are (Zini et al 1991), by perfusion with 10mmol/l glucose via microdialysis probes in rats' brains increased GABA release twofold. Perfusion with the specific channel activator lemalkalim or 2-deoxy-glucose with oligomycin inhibited GABA release (During M. et al 1995).

Finally the existence of hepatic autoregulation means that peripheral control must also exist. A study rendering dogs hypoglycaemic (2.6 mmol/l) in the hepatic-portal circulation but systemically (cerebrally) euglycaemic have provided evidence of hepatic glucose sensors playing an important role in the sympathoadrenal response to hypoglycaemia (Hamilton-Wessler M. et al 1994). Bolli et al demonstrated a similar outcome, studying the effect of a blood glucose level of 1.7mmol/l on glucose production (Bolli G. et al 1985).

6. Physiological Responses to Hypoglycaemia

A. Endocrine Responses

The endocrine responses to hypoglycaemia are counterregulatory as they oppose the action of insulin. The most important are **glucagon**, **catecholamines**, **growth hormone** and **cortisol**, combined with the decrease in insulin secretion. Of these glucagon and catecholamines are important in rapid glucose recovery with the others' effects occurring several hours later (De Feo P. et al 1989a and 1989b). Initial stimuli to their

individual secretion will be considered first and then the integrated endocrine physiological response, including glycaemic thresholds.

Individual Hormonal Responses

- Insulin's primary mechanism for secretion control is plasma glucose; the two are inversely related. Changes are sensed by β-cells of the pancreatic islets, which also synthesise and store insulin. Other signals are also involved, for example catecholamines inhibit secretion of insulin.

- Glucagon secretion is inversely proportional to circulating glucose concentration and is also controlled by neurotransmitters (noradrenaline, acetylcholine and peptides) (Cryer P. 1996). The mechanisms of the glucagon secretory response include both intraislet and CNS-mediated stimuli to the α-cells. The former appears to be of greater importance in humans (Cryer P. 1996) and the latter in dogs (Havel P. et al 1994) and monkeys (Havel P. et al 1996). These species differences may in fact relate to technical issues involving more profound hypoglycaemia in animals as well as more complete autonomic blockade during the experiments.

- Catecholamines - it has long been recognised that hypoglycaemia stimulates production of adrenaline and noradrenaline (Goldfien A. et al 1961). Subsequently it was demonstrated that adrenaline levels rose inversely proportional to blood glucose, with a sixfold increase during mild hypoglycaemia (Garber A.et al 1976) in healthy humans. Although hypoglycaemia activates the entire autonomic system, its main effect is on adrenaline production via the adrenal medulla. This effect is mediated through the CNS, as discussed above but is interrupted in individuals in whom the efferent limb has been interrupted by spinal cord transection (Palmer J. et al 1976). Adrenal production of noradrenaline may rise from 2-8% at rest to 45% during stress in animals (Goldstein D. et al 1983). It is known that hypoglycaemia stimulates the sympathetic neurons as well as the adrenal medulla but to what extent in humans the two processes are contributory to the increase in noradrenaline during hypoglycaemia has yet to be established (Paramore D. et al 1999)

- Cortisol and Growth Hormone secretion increases during hypoglycaemia due to increased production of corticotrophin releasing hormone (adrenocorticotrophic hormone) and growth hormone releasing hormone respectively. Production of these hormones is stimulated by α-adrenoceptors and a variety of neurotransmitters including GABA.

The Integrated Endocrine Physiological Response

Cryer summarised the effects of selective pharmacological and surgical deficiencies in secretion and actions of these counterregulatory hormones on brief hypoglycaemia (Cryer P. 1981). Somatostain infusion was used to block glucagon and growth hormone secretion, this impaired glucose recovery by approximately 40%. Glucagon's importance was then demonstrated by correction of the deficit when glucagon was infused simultaneously with the somatostatin infusion but not with growth hormone infusion. Combined α- and β- adrenoceptor blockade or adrenalectomy illustrated the relative importance of catecholamines, in that there was little effect on the recovery from brief hypoglycaemia under these conditions. This was probably due to the overriding importance of glucagon. However with combined glucagon and catecholamine deficiency glucose recovery did not occur over a sixty minute period. Now it is well established that glucagon is the most potent counterregulatory hormone with catecholamines in second position. Therefore if glucagon and catecholamines are deficient, as in type 1 diabetes with hypoglycaemia unawareness, counterregulation is severely compromised (Cryer P. 1981).

Whereas recovery from brief hypoglycaemia is largely the result of stimulation of glucose production, that from prolonged hypoglycaemia involves both stimulation of glucose production and limitation of glucose utilisation. Bolli et al were the first to suggest that whilst all the aforementioned hormones participated in glucose counterregulation, during prolonged hypoglycaemia adrenaline may play a greater role (Bolli G. et al 1984). De Feo et al showed that during prolonged hypoglycaemia (12 hours at 2.8mmol/l blood glucose) glucagon was of fundamental importance increasing glucose production but had no effect on glucose utilisation (De Feo P. et al 1991a). Isolated absence of an increase in adrenergic action reduced the glucose production

response early and increased glucose utilisation later (De Feo P. et al 1991b). Both cortisol and growth hormone deficiencies had no effects initially but later caused reduced rates of glucose production and increased rates of utilisation (De Feo P.et al 1989a, 1989b). Boyle and Cryer have demonstrated that in hypopituitary patients cortisol and growth hormone were not critical to recovery from prolonged hypoglycaemia (Boyle P. et al 1991).

In early hypoglycaemia glycogenolysis accounts for about 85% of glucose production but gluconeogenesis increases progressively between 2 and 6 hours, accounting for about 85% thereafter (Lecavalier L. et al 1989). Glucagon stimulates glycogenolysis and gluconeogenesis but does not affect glucose utilisation. Adrenaline also increases hepatic glycogenolysis and it mobilises gluconeogenic precursors such as alanine, lactate and glycerol to the liver (Chu C. et al 1996). Adrenaline also limits glucose utilisation by tissues such as skeletal muscle during hypoglycaemia and glycogen deposition is reduced (Cohen N. et al 1995). Cohen suggests that the majority of glucose made available to the brain during hypoglycaemic counterregulation is due to limitation of glucose disposal. There is evidence to suggest that adrenaline stimulated lipolysis mediates the effects on hepatic glucose production and muscle glucose utilisation. For example the effect of adrenergic antagonists in reducing glucose production and limiting glucose utilisation which is associated with lower nonesterified fatty acids was partially reversed by infusion of heparin and triglycerides (Fanelli C. et al 1992).

Glycaemic Thresholds

It is the plasma glucose concentration itself during hypoglycaemia, rather than the rate of decline or the magnitude of decrease that determines the counterregulatory response (Amiel S. et al 1987). Several groups, from different institutions, have shown that the cascade of responses to hypoglycaemia are triggered at specific glucose levels (Schwarz N. et al 1987; Mitrakou A. et al 1991; Fanelli C. et al 1994a). Their results are consistent, as illustrated in table I.1.

Table I.1 Mean arterialised venous glycaemic thresholds for decrements in insulin secretion, increments in counterregulatory hormones, symptom generation and cognitive dysfunction during stepped hypoglycaemia in normal volunteers.

Response	Glycaemic Thresholds		
	Schwarz et al, 1987	*Mitrakou et al, 1991*	*Fanelli et al, 1994a*
Insulin	not measured	not measured	4.4
Glucagon	3.8	3.8	3.7
Adrenaline	3.8	3.8	3.7
Growth Hormone	3.7	3.7	3.7
Cortisol	3.2	not measured	3.6
Symptoms Neurogenic Neuroglycopenic	2.9 not measured not measured	 3.2 2.8	3.1 3.0 3.2
Cognitive Dysfunction	not measured	2.7	2.4

Thus the characteristic sequence of events is:

(i) Decreased insulin secretion- this occurs within the physiological plasma glucose range

(ii) Increased counterregulatory hormones

(iii) Symptoms

(iv) Cognitive dysfunction.

These thresholds are however dynamic, changing with physiological and pathological states. Whilst gender differences with respect to the experience of hypoglycaemia exist (Draelos M. et al 1995), the glycaemic thresholds are not similarly affected (Fanelli C.et al 1994a). Similar hormonal response to hypoglycaemia were seen in groups of young (22-26 years) and older (60-70 years) male volunteers but symptoms began at a lower blood glucose concentration in the older group, with cognitive dysfunction worsening at a higher glucose concentration, which also deteriorated to a greater degree (Matyka K. et al 1997).

Of more significance to diabetes mellitus and the condition's treatment is the effects of both hyper- and hypoglycaemia on these glycaemic thresholds. Thresholds for counterregulatory hormones and symptom generation shift to lower glycaemic thresholds (higher plasma glucose levels) in poorly controlled diabetes mellitus (hyperglycaemia) (Amiel S. et al 1988; Boyle P. et al 1988) and to higher levels with episodes of hypoglycaemia (Heller S. et al 1991; Widom B. et al 1992; Boyle P. et al 1994). This latter phenomenon is related to hypoglycaemia unawareness and is discussed later. Controversy exists as to whether cognitive dysfunction thresholds change, which similarly is considered in the section of hypoglycaemia unawareness.

B. Symptoms of hypoglycaemia

In practical terms hypoglycaemia is usually recognised by symptoms. Early reports of these following the introduction of insulin were comprehensive (Fletcher A. et al 1922; Banting F. et al 1923) and subsequently have been reproduced (Cox D. et al 1993a). Further work has confirmed that patients learn to perceive specific physical sensations, their responses being idiosyncratic.

The following symptoms are most consistently associated with actual low blood glucose concentrations (Pennebaker J. et al 1981):

- hunger (53%)
- trembling (33%)
- weakness (27%)
- light-headedness (20%)
- pounding/fast heart rate (both 17%)

However, there is considerable inter-study variation, e.g. Cox et al showed that difficulty concentrating was the most consistent symptom (Cox D.et al 1993a). Ultimately the symptoms of hypoglycaemia are traceable to glucose deprivation of neural tissue. Therefore work by several groups has established that symptoms of hypoglycaemia can be divided into 2 major categories, namely *neurogenic* and *neuroglycopenic* symptoms, with a minor category of malaise or other symptoms (table I.2) (Towler D. et al 1993; Deary I.et al 1993a).

Table I.2 Symptoms of hypoglycaemia.

Neurogenic		Neuroglycopenic		Malaise/ Other	
Deary et al, 1993a	*Towler et al, 1993*	*Deary et al, 1993a*	*Towler et al, 1993*	*Deary et al, 1993a*	*Towler et al, 1993*
sweating	sweaty	confusion	difficulty thinking/ confused	headache	headache
palpitation	heart pounding	drowsiness	tired/ drowsy	nausea	nausea
shaking	shaky/ tremulous	odd behaviour			
hunger	hungry	speech difficulty	difficulty speaking		
	tingling	inco-ordination			
	nervous/ anxious		weak		
			warm		

Neurogenic symptoms arise from stimulation of the autonomic nervous system and thus may also be referred to as autonomic symptoms. Towler et al showed that awareness of hypoglycaemia is largely dependent on the perception of these symptoms. In taking healthy individuals and rendering them hypoglycaemic (2.5 mmol/l), it was demonstrated comparing adrenergic and pan autonomic blockade with no blockade that these responses were generated mainly by muscarinic cholinergic mechanisms (Towler D.et al 1993). With respect to shaking however, this is a catecholamine dependent symptom (Fellows I. et al 1986). The extent to which the adrenergic symptoms are

mediated by noradrenaline released from the sympathetic postganglionic neurones within the target tissues, or adrenaline released from the adrenal medullae, or both is unclear.

Neuroglycopenic symptoms result from brain glucose deprivation. They are listed in table 1 and progress to more severe cognitive failure and behavioural changes, eventually leading to coma and ultimately death. Whilst patients rely on the neurogenic symptoms, this is arguably an artificial category as autonomic activation is initiated by hypoglycaemia and maintained by neural glucose deprivation i.e. neuroglycopenia.

Malaise symptoms of headache and nausea were identified as being 2 of 11 key hypoglycaemic symptoms in 295 insulin treated out-patients, by Deary et al (Deary I.et al 1993a). They are non- specific and not affected by any form of autonomic blockade (Towler D.et al 1993).

C. Cognitive Function

The effects of acute hypoglycaemia on the brain are manifested by changes in cognitive function. Whilst this was recognised soon after the introduction of insulin therapy (Fletcher A.et al 1922), the development of the hyperinsulinaemic glucose clamp (De Fronzo R. et al 1979) made investigation of the effects of hypoglycaemia more controlled and reproducible. However even this is not an exact mimic of everyday life experiences of hypoglycaemia for free-living patients with diabetes mellitus.

In a 1993 review, Deary summarised the effects of hypoglycaemia on cognitive function tests (Deary I. 1993b). Subsequent studies have added to this body of evidence and table I.3 describes the tests which have been demonstrably impaired during hypoglycaemia. A consensus statement on the effects of hypoglycaemia on mental processing is as yet not possible. This is partly because of the different experimental protocols for inducing hypoglycaemia, as well as the heterogeneity in both the subjects and the test batteries used to assess cognition.

From previous work, it can also be concluded that some cognitive function tests are not impaired. These include finger tapping, forward digit span and to a lesser extent simple reaction time. Essentially, tests that involve speed, or are more complex (i.e. demanding more attention) are more likely to show significant impairment during hypoglycaemia.

Heller and Macdonald have concluded that (Heller S. et al 1996):

- Many aspects of mental performance are impaired at blood glucose levels below 3.0 mmol/l.
- Important individual differences exist.
- Simple motor functions are preserved at near normal levels during quite marked hypoglycaemia.
- In contrast to choice reaction time (where a mental decision is needed before reacting to a stimulus)
- Speed of responding is sometimes slowed in a task in which accuracy is preserved

It can take 40- 90 minutes after restoration of euglycaemia for the brain to fully recover (Blackman J. et al 1992; Lindgren M. et al 1996).

Table I.3 Cognitive function tests impaired during acute hypoglycaemia.

Test Impaired	Study	Blood Glucose (mmol/l) [a] = arterialised
Trail making	Hoffman et al 1989	2.7
	Mitrakou et al 1991	2.4[a]
	Gold et al 1995	2.5[a]
	McCrimmon et al 1996	2.5[a]
Digit Symbol Substitution	Pramming et al 1986	2.0
	Stevens et al 1989	3.4[a]
	Kerr et al 1991	2.8[a]
	Wirsén et al 1992	1.8-2.0[a]
	Gold et al 1995	2.5[a]
	McCrimmon et al 1996	2.5[a]
Reaction Time	Holmes et al 1983	3.3
	Heller et al 1987	3.2[a] / 2.5[a]
	Holmes et al 1986	3.1
	Mitrakou et al 1991	2.4[a]
	Wirsén et al 1992	1.8-2.0[a]
	Gold et al 1995	2.5[a]
Mental Arithmetic	Holmes et al 1983	3.3
	Gold et al 1995a	2.5[a]
Verbal Fluency	Holmes et al 1984	3.3
	Mitrakou et al 1991	2.4[a]
	Wirsen et al 1992	1.8-2.0[a]
	Kerr et al 1993b	2.0[a]
Stroop Test	Mitrakou et al 1991	2.4[a]
	Boyle et al 1994	3.0[a]
Grooved Peg Board	Kerr et al 1991	2.8[a]
Pursuit Rotor	Hoffman et al 1989	2.7
Letter Cancellation	Pramming et al 1986	2.0
	Mitrakou et al 1991	2.4[a]
Delayed Verbal Memory	Mitrakou et al 1991	2.4[a]
Backward Digit Span	Mitrakou et al 1991	2.4[a]
Story Recall	Pramming et al 1986	2.0

With respect to the variation seen on individuals during hypoglycaemia, the following factors may increase a person's degree of cognitive impairment during acute hypoglycaemia:

- male sex (Draelos M.et al 1995)
- age (Matyka K.et al 1997)
- high IQ (Draelos M.et al 1995; Gold A. et al 1995a)
- impaired hypoglycaemia awareness (Gold A. et al 1995b); although this is controversial as the cognitive threshold may actually increase in hypoglycaemia unawareness (Fanelli C. et al 1998; Ovalle F. et al 1998)
- type 1 diabetes mellitus (Wirsén A. et al 1992)

The impairments of mental test performance are likely to have implications for everyday life. This is illustrated by Cox et al's study into the complex decision-making process of driving (Cox D. et al 1993b). They demonstrated significant impairment of driving skills during controlled hypoglycaemia (blood glucose 2.6 mmol/l), using a sophisticated driving simulator. Subjects' performance in steering ability, car control and road positioning were decreased. They also drove more slowly during hypoglycaemia, perhaps as a compensatory mechanism. Worringly, 35% of patients were globally affected during moderate hypoglycaemia but only 50% of these recognised the decreased ability to drive.

At a time when investigation into hypoglycaemic cognitive dysfunction was developing Holmes raised the problem that as yet remains unsatisfactorily answered (Holmes C. et al 1984). The problem is that for many of the psychological tests referenced in table I.3 the processes involved are unknown. This limits their interpretation as it is not clear to what extent they index abilities that are important in everyday cognition (Anastasi A. 1990).

An exception to this is the specific aspects of information processing, which have been examined during acute hypoglycaemia in normal volunteers. McCrimmon et al studied visual information processing and showed that whilst visual acuity and stereoscopic vision were not affected by hypoglycaemia (2.5mmol/l) other aspects of vision were

(McCrimmon R. et al 1996). These included contrast sensitivity as well as three aspects of visual information processing. In this study inspection time, visual change detection and visual movement detection were all significantly decreased during hypoglycaemia. These tests are psychophysical measures of the efficiency of the early stages of visual information processing for high contrast stimuli (Phillips W. 1974). The authors conclude that 'as many decisions are made under conditions of limited perceptual time and low vision contrast (e.g. driving), the disruptive effect of moderate insulin-induced hypoglycaemia on visual perception will have important practical implications in diabetic humans exposed to this metabolic stress' (McCrimmon R.et al 1996). Similar deterioration occurs in auditory temporal information processing (McCrimmon R. et al 1997). Simultaneously one test of simple auditory processing decreased significantly during hypoglycaemia (single tone loudness). Due to the importance of hearing, these changes during hypoglycaemia have important implications especially for diabetic people employed in audio-related jobs.

D. Neurophysiological Changes

Hypoglycaemia was one of the first metabolic disturbances to be investigated by means of electroencephalography (EEG) (Davis P. 1943). Quantitative studies have subsequently demonstrated a change in α- waves from fast to slow, primarily in the frontal regions (Harrad R. et al 1985; Tamburrano G. et al 1988) with increased θ and δ wave activity (Harrad R.et al 1985). Similar findings were demonstrated in type 1 diabetic patients during hypoglycaemia (Tribl G. et al 1996). In this study differences between patients with good or poor awareness of hypoglycaemia were only evident during euglycaemia and mild hypoglycaemia (3.0 mmol/l). The performance decrements described by the studies using cognition testing do not indicate which function is being affected. For example reaction time is the result of the time used by several cognitive processes such as selective attention and motor-related processes, even when a simple reaction time test is used. Electrical neurophysiological investigation provides another approach into the effects of hypoglycaemia on the brain and cognition.

Evoked potentials (EP) represent the neuronal response to a given stimulus. Visual evoked potentials (VEP) record the response to an external visual stimulus usually a reversing checkerboard pattern. The first and most prominent deflection over the occipital cortex is positive and seen at 100ms after the stimulus, thus it is called P100. Measurements are taken by electrodes around the scalp placed in standardised positions.

Electrophysiology studies of the early stages of visual function have reported the effects of hypoglycaemia on visual sensation. Harrad et al found that the P100 latency increased (mean 10.8 ms) during hypoglycaemia (mean 2.0 mmol/l) in both healthy volunteers and type 1 diabetic patients (Harrad R.et al 1985). In a later study no significant change was demonstrated during hypoglycaemia (2.38 mmol/l) (Tamburrano G.et al 1988). These different findings could be explained either by the more severe condition of hypoglycaemia in the former study (absolute nadir 1.5 mmol/l) or the fact that in this study euglycaemia was not adequately controlled for; blood glucose prior to testing was reported as 7.1 ± 5.35 mmol/l for both the patients and healthy subjects.

Event related potentials (ERP) which is the neuronal response to an external stimulus when the subject is selectively attentive to that stimulus, distinguishing the target from a group of related stimuli. An investigation which combines these two elements is the *oddball* task. Here the subject is presented with a sequence of two distinguishable audible or visual stimuli, one occurring frequently and the other, the oddball, infrequently. The subject listens to (or watches) these, whilst counting mentally. A response is only required on presentation of the oddball stimulus. Electrical responses are recorded to the frequent and oddball stimuli and averaged separately. In the auditory test the response to the former consists of a series of waves. In the audio test, this relates to activity in the auditory pathway, which are divided into early-, mid- and long-related responses. The early response is also called the Brain Stem Auditory Evoked Potential (BAEP) and reflects activity in the peripheral and brain-stem auditory structures. It is divided into components I-V. Waves I, III, V correspond to activity in the acoustic nerve, pons and brain stem respectively. The latencies between them

reflect neuronal conduction. Abnormal prolongation of these relates to a disturbance of central auditory conduction.

The electrical activity to the oddball stimulus is characterised by the N200 (negativity at 200ms) or P300 (positivity at 300ms) waves. The N200 is thought to be related to a cognitive process involved in automatic feature extraction of a stimulus (Naatanen R. et al 1986). P300 is thought to be related to the cognitive process of stimulus evaluation or context updating (Donchin E. et al 1988).

Several studies have used the oddball task (Blackman J. et al 1990; Jones T. et al 1990; Ziegler D. et al 1992; Munte T. et al 1995). Ziegler (Ziegler D.et al 1992) observed increases in the inter-peak latencies as well as an increase in the amplitude of waves III and IV of BAEP in a group of type 1 diabetic patients (blood glucose 1.7 mmol/l). Using a stepped hyperinsulinaemic glucose clamp technique, Jones et al demonstrated that the threshold for change in BAEP was 3.0 mmol/l in healthy volunteers (Jones T.et al 1990). This was the same as for symptom generation but higher than that for the counterregulatory hormone response. Interestingly in both studies central as opposed to peripheral activity of BAEP was affected.

Blackman et al showed that both auditory and visual P300 waves to the oddball stimulus were significantly affected at 2.6 mmol/l glucose and suggested that hypoglycaemia was disrupting different sensory systems to the same degree (Blackman J.et al 1990). In this and a subsequent study the investigators demonstrated that there was a lag time of up to 75 minutes before P300 returned to baseline after the restoration of euglycaemia (Blackman J.et al 1990; Blackman J.et al 1992).

Hypoglycaemia has sometimes been found to increase the latency of the P300 and decrease its amplitude, and sometimes only decrease its amplitude (Zeigler D. 1992). These inconsistent results are difficult to interpret because of the different methods of latency and amplitude assessment, differences in subject groups and experimental design. Other problems are more specific and include consideration of the relationship between change in the overall electrophysiological state of the brain and its effect on

performance (Rugg M. et al 1995). Furthermore the N200 and P300 waves are actually composed of a number of small deflections which are sensitive to distinct experimental factors, the functional interpretation of which is not clear (Naatanen R.et al 1986; Rugg M.et al 1995).

Smid et al sought to demonstrate that new developments in ERP research had made it possible to assess the timing of two major cognitive processes in the brain: stimulus selection and response selection. A distinguishing feature of these methods is that they are concerned with difference potentials which have clearly defined relationships to differential cognitive processing (Smid H. et al 1997). This group studied 24 healthy individuals; 12 went through the euglycaemia-hypoglycaemia (2.6 mmol/l)-euglycaemia arm of the study, with the other 12 in the euglycaemia-euglycaemia-euglycaemia arm. During hypoglycaemia ERP measurements of selective attention, response choice and reaction time were all delayed compared to baseline performance. With the restoration of blood glucose the onset of selective attention returned to baseline, whereas the lateralised readiness potential (response selection) was still delayed. The authors suggest that hypoglycaemia delays both stimulus selection and motor-response selection and that the former recovers more quickly. They conclude that hypoglycaemia affects mainly the executive processes of the frontal lobes, citing EEG and regional blood flow evidence in support. This may be presumptive not least because they did not study more basic aspects of vision during hypoglycaemia but also the study was insufficiently powered as it was not a crossover study.

E. Emotions

Mood change is part of the experience of hypoglycaemia. The UWIST Mood Score measures three basic moods (Matthews G. et al 1990):

Energetic arousal - feelings of being lively and active
Tense arousal - feelings of anxiety and nervous
Hedonic tone - feeling happy

In the laboratory setting, hypoglycaemia changes all 3 aspects of mood (McCrimmon R. et al 1999a) with people feeling less energetic, more tense and less happy. Some people also become irritable and angry (McCrimmon R. et al 1999b). Low energy feelings take the longest time to be restored (about 30 minutes).

In addition, altered mood may account for the symptom of 'odd behaviour' from the Edinburgh hypoglycaemia symptom score (Deary I.et al 1993a). Altered mood may be a factor in the behavioural disturbances which are a primary feature of hypoglycaemia in children (McCrimmon R. et al 1995).

F. Haemodynamic Changes

Haemodynamic changes during hypoglycaemia are in response to activation of the sympathetic nervous system and are largely attributable to catecholamine mediated β-receptor stimulation. It has previously been established that hypoglycaemia in healthy individuals causes a rise in heart rate, systolic blood pressure and a decrease in diastolic blood pressure (French E. et al 1955; Hilstead et al 1993). Increasing vagal tone counteracts the increase in heart rate so this rise is transient. Cardiac output is increased by a raised stroke volume, which is augmented by direct β2 stimulation (Fisher B. et al 1987). Peripheral resistance is reduced (Hilsted J. 1993). It is this combination which causes the rise in systolic blood pressure and fall in diastolic, which leads to a widening of the pulse pressure but no change in mean arterial pressure.

G. Cerebral blood flow

Whilst changes in blood flow during hypoglycaemia have been observed in multiple tissues and organs (Patrick A. et al 1989; Maggs D. et al 1994), of particular relevance here is the changes in cerebral blood flow (CBF).

CBF increases during hypoglycaemia (Tallroth G. et al 1992) but this phenomenon is not seen until blood glucose has fallen to 2.2 mmol/l, a level well below the threshold for cognitive dysfunction (Powers et al 19996). Boyle et al has demonstrated that in both healthy individuals exposed to recurrent hypoglycaemia (2.5mmol/l) and type 1 diabetic patients with good control (average HbA1c 7.2%) normal brain glucose uptake

is maintained during a hypoglycaemic insulin clamp (Boyle P.et al 1994; Boyle P. et al 1995). This could be due to capillary recruitment increasing CBF or an increase in the number and activity of GLUT 1 transporters. The latter is associated with chronic hypoglycaemia (Hargreaves R.etal 1986; Kumagai A. et al 1995). As a corollary, increasing cerebral blood flow (and thus substrate delivery) with a bolus of acetazolamide by 30% at the onset of acute hypoglycaemia both the counter-regulatory hormonal responses to and perception of hypoglycaemia are attenuated (Thomas M. et al 1997). This indicates that the brain is acutely sensitive to substrate delivery. Furthermore a caffeine-induced decrease in CBF augments the usual hypoglycaemic responses to hypoglycaemia in both healthy individuals and type 1 diabetic patients (Kerr D.et al 1993a; Debrah K.et al 1996).

Eckert et al demonstrated that CBF increased by more than 10% during 66 minutes of hypoglycaemia (2.2mmol/l) and remained at this level for more than 90 minutes after the restoration of euglycaemia (Eckert B. et al 1998). This was also in the presence of basal concentrations of counterregulatory hormones after the hypoglycaemic stimulus. Additional, as yet unidentified factors are involved with this vasculatory response which are not coupled to cerebral metabolism directly.

Abnormal CBF and cerebro-vascular reactivity without experimental hypoglycaemia is recognised in type 1 diabetic patients. Lower CBF may actually be associated with increasing duration of disease (McCall A. 1992). Cerebro-vascular reactivity is abnormal in some patients; for example Rodriguez et al showed that some type 1 patients respond poorly to acetazolamide induced changes in regional blood flow (Rodriguez G. et al 1993). As a whole this evidence suggests that diabetic patients could be more vulnerable to hypoglycaemia as the usual response of increasing CBF in moderate/ severe hypoglycaemia is attenuated. MacLeod et al demonstrated that an alteration in the pattern of baseline regional cerebral blood flow exists in type 1 diabetic patients. There was an increase to the frontal lobes with a relative posterior reduction. The differences were particularly marked in the group who had a history of recurrent severe hypoglycaemia, thus postulating the idea that these permanent changes may represent an adaptive mechanism to protect these regions from such a problem but

causing cumulative cerebral damage (MacLeod K. et al 1994). Interestingly a study of normal humans showed a similar result in differential regional cerebral blood flow when hypoglycaemia was compared to the euglycaemic state (Tallroth G. et al 1992). This study compared regional blood flow during both euglycaemia and hypoglycaemia. During hypoglycaemia frontal areas showed a strong increase in regional blood flow at the expense of posterior regions. (Acute hypoglycaemia may lead to capillary recruitment and glucose transporter mobilisation (Hargreaves R. et al 1986). See before.)

7. Hypoglycaemia- associated autonomic failure - Clinical Relevance

The syndromes of *defective glucose counterregulation* and of *hypoglycaemia unawareness* are distinct concepts, but with significant overlapping features. The former is characterised by a compromised physiological defence with an absent glucagon response. The latter's key feature is a deficient behavioural (symptomatic) response, attributable to reduced autonomic (sympathetic, neural and adrenomedullary) responses with hypoglycaemia.

Both syndromes are associated with a high frequency of iatrogenic hypoglycaemia and share several pathophysiological features including elevated glycaemic thresholds (lower plasma glucose concentrations) required for endocrine and symptomatic responses to hypoglycaemia. Affected patients exhibit reduced adrenomedullary, parasympathetic neural and perhaps sympathetic neural (neurogenic symptoms) responses to a given level of hypoglycaemia. Therefore it has been suggested that these syndromes can be coupled together as examples of *hypoglycaemia- associated autonomic failure* in type 1 diabetes (Cryer P. 1992). To summarise the two basic components of this phenomenon each will be considered in turn.

Defective Glucose Counterregulation

The diagnosis of type 1 diabetes means that an important counterregulatory step against hypoglycaemia has been lost already in that insulin production can not be decreased. Glucagon is the major counterregulatory hormone in non-diabetic individuals. However just as a patient with type 1 diabetes becomes totally deficient of insulin over the first

few years of disease duration, so is the hypoglycaemic response of glucagon lost (Gerich J. et al 1973; Bolli G. et al 1983). This is a selective deficit of glucagon production, as response to other stimuli remain largely intact (Wiethop B. et al 1993). The exact mechanism of damage is unknown but is thought to be a loss of signalling mechanism as opposed to alpha cell destruction. The tight relationship of insulin deficiency and loss of this response imply that glucagon release is signalled by a decrease in ß cell insulin (Samols E. et al 1996). Alternatively as insulin is usually an inhibitor of glucagon release and supraphysiological levels of insulin have been shown to impair glucagon release (Kerr D. et al 1991), the hyperinsulinaemia associated with treatment of diabetes may actually stimulate the loss of the glucagon response.

The alpha cells are however also directly stimulated by circulating glucose and the autonomic nervous system. Even with the implementation of good control of plasma glucose there is not restoration of the glucagon response (Bolli G. et al 1982). It has been found that in patients with a pancreatic transplant the glucagon response is substantially improved to near normal levels (Kendall D. et al 1997). Fundamentally, whatever the mechanism, the counterregulation against hypoglycaemia is flawed in established type 1 diabetes. Physiological defence is adequate in this situation providing that catecholamine responses are intact.

However this third physiological defence is also compromised when the adrenaline response to a low blood glucose is attenuated in patients with type 1 diabetes. Like the absent glucagon response, this is stimulus specific as catecholamine responses to other stimuli (exercise) are not significantly different (Bottini P. et al 1997). Although evidence is also presented by Bottini et al to show that when diabetic autoneuropathy is present the defect is non-selective. However, in deficient catecholamine production, there has been an elevation of the threshold such that a more profound stimulus (i.e. lower glucose level) to augment the catecholamine production is required (Dagogo-Jack S. et al 1993). Other factors involved include recurrent antecedent hypoglycaemia (Davis S. et al 1997), peripheral autonomic neuropathy (Bottini P.et al 1997) and a central neurological defect such that recognition is impaired (Borg W.et al 1994).

Finally the other 2 hormones directly involved in counterregulation against hypoglycaemia are cortisol and growth hormone. These have roles in prolonged hypoglycaemia (De Feo P.et al 1989a; De Feo P.et al 1989b), as described before. Whilst abnormalities exist with regard to their production in people with type 1 diabetes, these defects are not as prevalent in patients with type 1 diabetes as defective catecholamine production. Gerich and Bolli have estimated the frequency of abnormal cortisol and growth hormone responses to rise from 0% at 5 years disease duration to 25% at >10 years (Gerich J. et al 1993). It is sagacious to remember that an intact hypothalamic-pituitary-adrenal axis is essential for adequate counterregulation.

Hypoglycaemia Unawareness

Whilst no formal definition has yet been widely accepted, the phenomenon of hypoglycaemia unawareness (Heller S. et al 1987; Hepburn D. et al 1990; Clarke W. et al 1991; Pramming S.et al 1991; Gold A.et al 1994) represents the loss of the warning symptoms of developing hypoglycaemia that previously prompted the patient to act (e.g. eat carbohydrate in a relatively pure form) to prevent progression to severe hypoglycaemia. Since awareness of hypoglycaemia is normally largely due to the perception of autonomic symptoms (Towler D.et al 1993), hypoglycaemia unawareness is most reasonably attributed to loss of these symptoms of developing hypoglycaemia. In the absence of these manifestations of a falling blood glucose, neuroglycopenia may develop to a critical level whereby the patient is unable to take avoiding action. In a prospective study, affected patients are estimated to be at a six fold risk for severe hypoglycaemic episodes (Gold A.et al 1994).

Hepburn et al (Hepburn D.et al 1990) subdivided hypoglycaemia awareness into 3 categories, which represent a continuum of the problem:

- **Normal** awareness means that an individual is aware of hypoglycaemia onset
- **Partial** awareness occurs when symptoms, whilst still present are reduced in intensity or number or absent on occasion
- **Absent** awareness is when symptoms present just before or do not occur until the onset of severe hypoglycaemia

Total unawareness is rare (Gold A.et al 1994; Clarke W. et al 1995), with the neuroglycopenic symptoms usually remaining, albeit significantly reduced. The problem may also vary over time, presumably because of the importance of environmental and emotional factors in this phenomenon. Impaired hypoglycaemia awareness is common in patients treated with insulin. Surveys of the prevalence have been remarkably consistent (table I.4).

Country	No. of patients	Impaired awareness of hypoglycaemia (%)	Reference
Scotland	302	23	Hepburn 1990
Germany	523	25	Muhlhauser 1991
Denmark	411	27	Pramming 1991
USA	628	20	Orchard 1991

Table I.4 Population studies of type 1 diabetes mellitus showing the prevalence of hypoglycaemia unawareness.

This contrasts with estimates of decreased catecholamine production (defective counterregulation) during hypoglycaemia being present in 66% of the type 1 diabetic population after 10 years of the disease (Gerich J.et al 1993). Duration of disease is relevant too, as hypoglycaemia unawareness increases such that about 50% of patients experience hypoglycaemia without warning after 25 years or more of treatment (Pramming S.et al 1991).

Although this problem is more prevalent in type 1 diabetes, it is not confined to this disease. A similar problem has been shown to occur in type 2 diabetes when patients have been on insulin for several years (Hepburn D. et al 1993). With the increasing use of insulin following the publication of the UKPDS data, more investigation will be required to further characterise this problem in type 2 diabetes mellitus.

The exact pathogenesis of hypoglycaemia unawareness is unknown but is likely to be multifactorial. The most important mechanisms identified is antecedent hypoglycaemia. Recurrent episodes of hypoglycaemia lead to reduced autonomic and symptomatic responses in healthy individuals and people with type 1 diabetes mellitus. Marked hypoglycaemia is not required to for this phenomenon to develop. For example, Davis et al found that two 2 hour episodes of hypoglycaemia held at 3.9mmol/l on day 1 were sufficient to blunt the adrenaline, glucagon and muscle sympathetic activity responses to hypoglycaemia on day 2, in healthy volunteers (Davis S.et al 1997). This effect was increased when day 1 involved hypoglycaemia of 3.3mmol/l and included a decrease in other responses. There was no further augmentation when day 1 hypoglycaemia was induced down to 2.9mmol/l. The minimum requirement for antecedent hypoglycaemia to effect awareness is 2 hours (Heller S.et al 1991), although recurrent 1 hour episodes on 4 consecutive days has a similar effect (Widom B.et al 1992); transient (15 minutes), recurrent (over 4 days) reduction does not influence this problem; although this has only been demonstrated in healthy volunteers (Peters A. et al 1995). As so many episodes of hypoglycaemia are nocturnal and not properly recognised, this may result in hypoglycaemia unawareness (in waking hours) for patients who give no history of recurrent hypoglycaemia (Veneman T. et al 1993).

Ultimately the glycaemic thresholds are altered and lower plasma glucose concentrations are required to elicit autonomic and symptomatic responses to hypoglycaemia during intensive effective treatment therapy (Amiel S.et al 1988). It has been subsequently demonstrated that diabetic patients self-reporting hypoglycaemia unawareness were able to mount a sympathoadrenal response to hypoglycaemia but that this occurred at a lower level of plasma glucose than in patients with hypoglycaemia awareness or even patients with autonomic neuropathy (Hepburn D. et al 1991). Conversely chronic hyperglycaemia in diabetes mellitus is associated with lower response thresholds (Schwarz N.et al 1987; Amiel S.et al 1988; Mitrakou A.et al 1991).

Hypoglycaemia unawareness is reversible by relatively short-term avoidance of iatrogenic hypoglycaemia (Fanelli C. et al 1993; Cranston I. et al 1994; Dagogo-Jack S. et al 1994; Fanelli C. et al 1994b). Dagogo-Jack et al showed that over a three day period of hypoglycaemia avoidance the symptoms improved in patients with hypoglycaemia unawareness and these became indistinguishable from normal after about three weeks. Increased albeit not entirely normal plasma adrenaline responses to hypoglycaemia were found in most studies but glucagon responses were hardly affected. Interestingly the results were not in agreement with respect to cognitive dysfunction. Fanelli et al (Fanelli C.et al 1993; Fanelli C.et al 1994b) showed that glycaemic thresholds for a variety of cognitive tests were improved towards 'normal' but Cranston et al (Cranston I.et al 1994) showed that the impairment threshold for four-choice reaction time was unaltered. Plasma glucose concentrations required to elicit cognitive dysfunction did not appear to be lower following a preceding episode of hypoglycaemia in another study (Dagogo-Jack S.et al 1993).

This leads onto the question as to whether the changes in hypoglycaemia awareness (glycaemic thresholds) for symptoms and adrenaline responses are adaptive or maladaptive during intensive treatment of type 1 diabetes (Amiel S.et al 1988). If the cognitive thresholds shift this could be protective and explained by the a generalised increase in blood-to-brain glucose transport. The evidence for elevation of the glycaemic threshold of cognitive dysfunction after recent hypoglycaemia is mixed, as the shifts in glycaemic thresholds appear to be more difficult to describe. This may reflect technical issues such as the details of the antecedent hypoglycaemia and the variety/ sensitivity of tests used (Amiel S. 1998). Although studies have been published in support of the glycaemic threshold for cognitive dysfunction not being altered (Cranston I.et al 1994; Dagogo-Jack S.et al 1994), an increasing number of studies have suggested that it does so subtlely, in both healthy individuals and patients with type 1 diabetes mellitus (Veneman T.et al 1993; Fanelli C.et al 1998; Ovalle F.et al 1998).

Adrenaline responses to hypoglycaemia invariably become blunted in long term type 1 diabetes (Bolli G.et al 1983), even if recent antecedent hypoglycaemia is meticulously

prevented (Fanelli C.et al 1994b). In the shorter term (8 years disease duration) a more recent cross-sectional study in type 1 diabetes on intensive insulin therapy with nearly optimal blood glucose regulation since diabetes onset showed normal adrenaline responses to hypoglycaemia (Pampanelli S.et al 1996). Hypoglycaemia associated autonomic failure (Cryer P. 1994), autonomic neuropathy (Bolli G.et al 1983), and diabetes per se (Dagogo-Jack S.et al 1993) have been proposed as possible causes to the longer term effects, although their individual contribution remains largely unknown. Bottini et al demonstrated that in the presence of autonomic neuropathy, independent of antecedent hypoglycaemia, adrenaline responses to hypoglycaemia were further reduced in type 1 diabetic patients (Bottini P.et al 1997). This was demonstrated even when autonomic neuropathy was confined to predominant parasympathetic involvement (i.e. cardiovascular reflexes normal). At this stage the deficit has also ceased to be selective as there is also a decreased adrenaline response to exercise. Noradrenaline responses were decreased in these patients without postural hypotension, suggesting decreased sympathetic activity was already established but not identified.

Thus in summary following the diagnosis of type 1 diabetes mellitus a patient has lost the glucagon response to hypoglycaemia and thus has defective glucose counterregulation. Whilst this is of key importance, the defect is further exaggerated as a result of decreased adrenaline production in hypoglycaemia. Recurrent iatrogenic hypoglycaemia results in hypoglycaemia unawareness, serving to elevate glycaemic thresholds for symptoms and also catecholamine responses. Whilst the full pathogenesis of these processes are not understood, autonomic neuropathy augments the inability to detect hypoglycaemia and it remains to be seen whether long term intensive insulin therapy may prevent the onset of autonomic neuropathy and loss of the adrenaline response to hypoglycaemia.

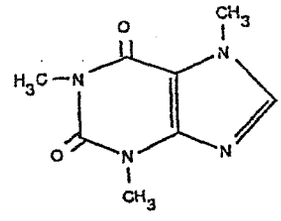
Section II CAFFEINE

1. General Introduction

Caffeine is a chemical substance which has been isolated from approximately sixty plants. In its pure form it is a bitter-tasting, white, odourless, crystalline powder. Its botanical function is thought to be both an antifungal and insecticidal agent.

Caffeine was first isolated in 1820, by Runge and van Giese. It was included into the medical vocabulary in 1823 but it was not until 1875 that its structural formula was described by Medicus. Caffeine has a purine base, its chemical name is 1,3,7-trimethylxanthine:

Figure II.1: Chemical structure of caffeine: $C_8H_{10}N_4O_2$- molecular weight 194.19



Caffeine is one of the most widely consumed substances in the world and in moderate doses generally considered to have the effect of a 'mild stimulant, helpful in temporarily relieving minor fatigue and boredom with little risk of any harmful effects' (Graham D. 1978). Caffeine is also a multi-million dollar, multi-national business, an enormous investment has been made to examine health issues around its regular use. Much debate has ensued. Thus there follows an over view of caffeine, including its physiological and psychological effects to place in context the hypothesis behind this thesis.

2. Caffeine Consumption

Throughout the world caffeine is consumed by 80% of the adult population. There are various forms: some of are internationally recognised such as tea and coffee but others are only locally consumed e.g. maté in South America and miang in Thailand. Worldwide the average caffeine consumption is estimated at 50mg/day (Gilbert R. 1984).

The properties of caffeine have been known of since preliterate times when its use was confined to specific areas- those where coffee plants grew wild (e.g. Ethiopia), until the time of European colonisation, in the 17th century. At this time caffeine containing products were cultivated and subsequently became widely distributed.

The combined physiological and psychological impact of caffeine depends on the pattern and degree of exposure to the drug. Thus there has been extensive investigation into the caffeine content of widely consumed beverages and their consumption.

Caffeine Content of Beverages and Food

There is marked variation in the estimates of caffeine content in different beverages (Barone J. et al 1996). This arises from differences in:

- Methods of preparation- e.g. methods of brewing tea and coffee
- Product source- e.g. black or green tea
- Reference volumes
- Analytical methods

Throughout this thesis, the following standards are used with respect to caffeine content (table II.1):

Table II.1 Caffeine content of beverages and foodstuffs (Barone J.et al 1996).

Drink	Caffeine content
Coffee:	
Ground Roast	85mg/ 150ml
Instant	60mg/ 150ml
Decaffeinated	3mg/ 150ml
Tea:	
Leaf or bag	30mg/ 150ml
Instant	20mg/ 150ml
Cola	18mg/ 180ml
Caffeine-free cola	0
Cocoa (hot chocolate)	4mg/ 150ml
Chocolate milk	4mg/ 150ml
Chocolate candy	5- 20mg/ 100g

Caffeine Consumption Studies

It is estimated that 80% of the adult population in the majority of countries worldwide consume caffeine on a regular (daily) basis. Variation exists in the nature of the caffeine vehicle. In UK 81% of adults drink tea, whilst in France 81% drink coffee. In the USA it is estimated that only 52.1% drink coffee but more soft beverages are consumed than in other countries. Between 1960 and 1982 Gilbert recorded a 231% increase in the consumption of soft drinks in the USA (Gilbert R. 1984). This rise is unequaled in the developed world.

Data for estimating a population's caffeine intake have been available from prospective and retrospective studies. In general the former has gained more credence and it has been shown repeatedly that there is good correlation between reported caffeine intake and subsequent appropriate measurement of caffeine (James J. et al 1988) or caffeine metabolite levels (Klebanoff M. et al 1999).

In 1988 the International Sweetners Association sponsored a dietary survey of foods consumed in the UK. 644 people, representative of the national population participated. Each kept a detailed food diary for 7 consecutive days. From these diaries it was estimated that 95% consumed a caffeine-containing beverage once a day. The mean intake was 4 mg/ Kg per day (Barone J.et al 1996). Another UK study estimated the intake to be 5.1mg/ Kg, in adult caffeine consumers (Scott N. et al 1989). This compares to 3mg/ Kg (Gilbert R. 1984) and 7mg/ Kg (Barone J.et al 1996) in the USA and Denmark populations respectively.

Finally patterns of caffeine consumption are continually changing. The above describes the most current estimates, and thus are most relevant to this work. As an example of the changing pattern in 1984 Gilbert estimated the average UK consumption of caffeine as 444mg per day (Gilbert R. 1984). Now this has been revised to 359mg per day (Scott N.et al 1989).

3. Pharmacokinetics of Caffeine

Absorption

Caffeine absorption from the gastrointestinal tract is rapid. About 90% of the caffeine in a cup of coffee is cleared from the stomach in 20 minutes, although the stomach is also able to absorb this substance (Chvasta T. et al 1971). Wide variation of time to reach peak plasma concentration has been reported in humans (i.e. 15- 120 minutes) (Arnaud M. et al 1982; Bonati M. et al 1982). However, as absorption may be slowed by the presence of food in the intestine (Arnaud M. 1987) or when large amounts of the drug are ingested (Passmore A. et al 1987), on an empty stomach the time taken is around 40 minutes (Rall T. 1990; Liguori A. et al 1997), with 99% absorbed after 45 minutes (Marks V. et al 1973). The rate of caffeine absorption is similar for tea and coffee (30 minutes) (Marks V.et al 1973). Although Marks et al demonstrated slower absorption for a caffeinated soft-beverage (120 minutes), Liguori et al more recently showed cola and coffee absorption to be similar (39 and 42 minutes respectively) (Liguori A.et al 1997). It has also been demonstrated that the effects of hot tea and coffee are mediated through the caffeine and hot water with the addition of milk playing a modulatory role (Quinlan P. et al 1997).

Distribution

Once absorbed caffeine, due to its hydrophobic properties, can pass through all biological membranes, including the blood brain barrier. Salivary caffeine concentrations have been shown to correlate closely with plasma concentrations, reaching up to 85% of plasma levels (Setchell K. 1987).

After oral ingestion of 5-8 mg/Kg in humans the peak plasma caffeine concentration equals 8-10 mg/l (Arnaud M.et al 1982; Bonati M.et al 1982). From this it can be estimated that ingestion of a single cup of coffee, which provides a dose of 85 mg, would give a peak concentration of 2.0 mg/l in an avareage adult (Pfeifer R. et al 1988). In normal adults the plasma half life is between 3 and 7 hours (Pfeifer R.et al 1988; Rall T. 1990). Whilst there is little or no difference with increasing adult age, premature and full-term neonates show a markedly increased plasma half-life due to lower activity of cytochrome P450 (Arunda J. et al 1979) and the relative immaturity

of some elimination pathways (Aranda J. et al 1974). This state continues for up to 12 months and is then essentially stable throughout life.

Whilst the clearance rate is not different overall between men and women (Patwardhan R. et al 1980), it is related to the menstrual cycle, being slower in the luteal phase (6.85 hours luteal vs. 5.54 hours follicular) (Lane J. et al 1992). Patwardhan et al also demonstrated a significant lengthening effect by the oral contraceptive pill (OCP) (Patwardhan R.et al 1980). Six month use of OCP lead to a doubling of the half-life (10.7 hours) compared to non-users (5.2 hours). The authors suggest that this effect is due to cytochrome P450 inhibition. The third trimester of pregnancy leads to a significant prolongation of the plasma half-life, up to 15 hours (Knutti R. et al 1981). In adult males who smoke, caffeine half life is decreased by up to 50% compared to non-smokers (Parsons W.et al 1978). Interestingly (poorly controlled) diabetes mellitus does not alter the half-life or pharmacokinetics of caffeine (Zysset T. et al 1991)

Caffeine Metabolism

Caffeine is metabolised by the cytochrome P450 monoxidase enzyme system, specifically 3-methylcholanthrene inducible isoenzyme. The major process is demethylation at the C3 position to produce paraxanthine which accounts for up to 80% of caffeine metabolism (Arnaud M.et al 1982). The other 24 excretory products include theobromine (3,7-dimethylxanthine), theophylline (1, 3- dimethylxanthine) and both uric acid and uracil derivatives. Many of the metabolic steps may be saturable in humans as the elimination half-time for caffeine and some of its metabolites is dose-dependent (Kaplan G. et al 1997). Less than 2% of the ingested dose of caffeine is recoverable from the urine unchanged (Arnaud M. 1987).

The abundance of paraxanthine as a metabolite of caffeine has lead investigators to consider the importance of this compound in the overall effects of caffeine (Dulloo A. et al 1994). Dulloo et al have shown that paraxanthine mimics the effects of caffeine on thermogenesis (Dulloo A.et al 1994). More recent work showed that after long-term caffeine administration the levels of theophylline in the mouse brain may be higher

than those of caffeine during a substantial part of the day and almost always higher than paraxanthine (Johansson B. et al 1996). The authors speculate that caffeine in the brain is metabolised in part by specific, local enzymatic pathways which lead to high cerebral concentrations of theophylline. This is of possible major importance as most of the effects of caffeine are thought to be mediated via adenosine receptor antagonism (see later) and theophylline is 3-5 times more potent, with paraxanthine equipotent to caffeine. Further studies are needed to see if this situation is relevant to humans.

Leading on from this, as much of the background data is from animal experiments, extrapolation to humans is difficult. Interspecies comparisons are firstly complicated by marked differences in:

1. **Kinetic and metabolic profiles-** kinetic similarity between species does not necessarily indicate metabolic similarity and vice versa. For example, whilst the half-life of caffeine is similar in humans and some primates, the major metabolites are paraxanthine and theophylline respectively. Conversely, paraxanthine is also the major metabolite in mice but the half-life is about 25% that of humans (Bonati M. et al 1984-85).

2. **Experimental protocols-** in animal experiments caffeine is often administered as a high dose bolus, which differs to usual human caffeine consumption being distributed throughout the day.

This has lead to the idea of metabolic weight being used as a correction factor. It is now generally assumed that 10mg/Kg in a rat is equivalent to 3.5mg/Kg in a human i.e. 250mg in a person weighing 70Kg or 2-3 cups of drip-brewed coffee.

4. Mechanism of Caffeine Action

Caffeine exerts a variety of pharmacological actions at diverse sites, both central and peripheral. Whilst several processes have been investigated as being responsible for these effects, the mechanism must be activated by caffeine consumption within normal human amounts. This effectively rules out some hypotheses (Table II.2).

Table II.2: Mechanisms of Caffeine Action.

Potential Mechanism of Caffeine Action	Reference	Relative caffeine concentration required for effective activation (1= a cup of coffee)
Adenosine receptor antagonism	Fredholm 1980	x1
Inhibition of phosphodiesterase	Smellie et al 1979	x10
GABA receptor blockade	Marangos et al 1979	x 20
Mobilisation of intracellular Calcium	McPherson et al 1991	x 500

Thus the general consensus now is that the only known mechanism that is significantly affected by the relevant doses of caffeine (i.e. able to achieve extracellular concentration of 10-50 μM) is binding to adenosine receptors and competitive antagonism of adenosine (Fredholm B. 1980). Although the effects of caffeine on behaviour have been well documented, understanding of its cellular mechanisms of action remains incomplete. The following is a summary of the current literature.

Adenosine Receptors

Adenosine receptors are widely distributed throughout the body, which befits the mechanism of caffeine action because of the substance's wide range of effects. For example adenosine causes bronchoconstriction, negative inotropic effects on the heart, and inhibits gastric secretions, lipolysis and renin release. All of which are the opposite to the effects of caffeine. Centrally mediated effects of adenosine are also the reverse of caffeine.

Receptor Subtypes: 4 distinct adenosine receptors have been identified and cloned. They are A_1, A_{2A}, A_{2B} and A_3 (Fredholm B. et al 1994).In humans it is A_1 and A_{2A} that

have been shown to be important, as the other 2 are only stimulated with toxic concentrations of caffeine. A_1 and A_{2A} are both G- protein coupled. The A_1 receptor is coupled to pertussis toxin sensitive G-proteins and activation of these receptors can cause inhibition of adenyl cyclase and N- and Q-voltage sensitive calcium channels and activation of certain K-channels, phospholipases C and D (see (Fredholm B.et al 1994)). A_{2A} receptors associate with G_s proteins and activation of these receptors is associated with activation of adenyl cyclase and calcium channels (L-type). Thus A_1 and A_{2A} receptors can have opposing actions at the cellular level. Therefore although these receptors can be coexpressed on the same cell, their individual, specific distributions are important.

Receptor Distribution: Adenosine A_1 receptors are widely distributed, although higher concentrations are in the hippocampus, cerebral and cerebellar cortex and thalamic nuclei (Fastbom J. et al 1987). A_{2A} receptors are distributed more conservatively, being associated with dopaminergic regions (caudate-putamen, nucleus accumbens, olfactory tubercle and globus pallidus) (Paxinos G. et al 1986). Whilst caffeine is equally active at adenosine A_1 and A_{2A} receptors, it has recently been shown that the stimulatory central action of caffeine is mainly mediated through an inhibition of transmission of adenosine A_{2A} receptors (Svenningsson P. et al 1997).

Role of Dopamine: Various findings suggest an involvement of dopamine systems in the central effects of caffeine but the actual mechanisms are ambiguous (Ferre S. et al 1992). It would appear that low caffeine doses increase and high caffeine doses decrease both dopamine release and locomotor activity (Morgan M. et al 1989). A_2 receptors co-localise and functionally interact with D2 dopamine receptors. This has been demonstrated by a number of experiments, including demonstration of decreased affinity of the D2 receptor for dopamine combined with decreased signal transduction in the presence of a specific A_2 receptor agonist (Ferre S. et al 1991). Similarly A_1 receptors appear to negatively interact with D1 dopamine receptors on striatal neurons (Ferre S. et al 1994; Ferre S. et al 1996).

The importance of the involvement of the dopamine system is in the explanation of the behavioural effects of caffeine in both animals and humans (see below).

5. Physiological Effects of Caffeine- General

The effects of caffeine are legion. Table II.3 summarises the effects of caffeine on the body, with the following sections describing the physiological effects on the cardiovascular, central nervous and neuroendocrine (including adrenomedullary) systems' function. Psychological effects are considered separately.

Table II.3: Summary of the physiological effects of caffeine on the body: from Debry 1994 (Debry G. 1994).

	Effect of Caffeine
Gastrointestinal System	• increases gastric acid secretion in susceptible individuals, not related to gastric ulcers • hepatic dysfunction increases caffeine half life • digestive absorption of alcohol is increased when caffeine is consumed prior
Respiratory System	• relaxes bronchial smooth muscle • increases CNS sensitivity to carbon dioxide
Renal System	• stimulate renin release • diuresis due to increased glomerular filtration rate and decreased tubular reabsorption
Adipose Tissue	• Stimulates lipolysis
Bone Tissue	• in large amounts coffee can contribute to negative calcium balance in those with a low calcium intake

6. Physiological Effects of Caffeine on the Cardiovascular System

The effects on caffeine on the cardiovascular system have been extensively researched in both experimental and epidemiological studies. It has generated much interest because of the debate regarding the involvement of caffeine in cardiovascular disease. The experimental studies have investigated the effects of controlled caffeine exposure, while epidemiological ones have addressed the relationship of caffeine to cardiovascular disease.

Caffeine and Blood Pressure

The major cardiovascular effect of caffeine is peripheral vasoconstriction (Pinacomb G. et al 1985; Sung B. et al 1994), resulting in elevated blood pressure which is not accompanied by an increase in cardiac output (Whitsett T. et al 1984; Sung B. et al 1990; Pincomb G. et al 1991). Acute tolerance to the pressor response does occur in regular consumers but is reinstated by a brief period of abstinence, as demonstrated by the referenced studies. This may be as short as 3 hours (Sung B.et al 1994). Other evidence includes a study by Lane et al (Lane J. et al 1989) which demonstrated that a second cup of coffee induced a pressor effect albeit diminished. Finally ambulatory blood pressure measurements on days of caffeine intake compared to placebo days was higher in usual caffeine consumers (Jeong D. et al 1990; Green P. et al 1996). Both systolic (Daniels J. et al 1998) and diastolic (Green P.et al 1996) blood pressures have been reported as being more significantly affected by an acute caffeine challenge.

In addition caffeine also increases the cardiovascular responses to stress. This includes the pressor response to mental or physical stress (Lane J.et al 1989; Jeong D.et al 1990; Sung B.et al 1990), as well as other responses e.g. cardiac output (Pincomb G. et al 1988) and forearm blood flow (Daniels J.et al 1998).

Adenosine causes vasodilatation and suppresses renin activity, antagonsim of adenosine receptors would account for the main effects of caffeine on blood pressure. The A_2 receptors in the periphery mediate vasodilatation (Rongen G. et al 1997). In addition stimulation of A_1 receptors are responsible for decreasing renin secretion,

resulting in reduced production of angiotensin II (Rongen G.et al 1997). This substance theoretically could account for part of the effects of caffeine during stress:

- angiotensin II is a cutaneous vasoconstrictor (Pang C. 1983)
- angiotensin II increases myocardial blood flow and enhances reductions in splanchnic and renal blood flow (Stebbins C. et al 1995).

Again there is the possibility of low dose caffeine increasing and higher doses decreasing blood pressure. Excitatory effects of adenosine include stimulation of carotid and aortic chemoreceptors (Biaggioni I. et al 1987) and sensory nerves of the kidneys (Katholi R. et al 1996), heart (Dibner-Dunlap M. et al 1993) and skeletal muscle (Costa R. et al 1994). If caffeine modified these responses blood pressure and heart rate would fall. Thus it could be postulated that caffeine levels do not reach adequate concentrations to stimulate these reflexes.

Caffeine and Heart Rate

The effect of caffeine on heart rate is less well defined. Consumption within normal limits has been reported as being associated with a decrease (Smits P. et al 1985), an increase (Gould L. et al 1973) or no change in heart rate (Bender A. et al 1997; Daniels J.et al 1998). Whilst caution is advised with regard to tachyarrhythmias induced by caffeine, this is associated with excessive doses or underlying cardiac disease (Sutherland D. et al 1985). Interestingly it is widely believed that caffeine is associated with palpitations, tachycardia and arrhythmias, by physicians (Hughes J. et al 1988).

Inotropic Effects of Caffeine

Bender et al demonstrated a positive inotropic effect of caffeine on cardiac function (Bender A.et al 1997). In 12 young, caffeine-withdrawn adults echocardiographic results following an acute caffeine challenge showed a significant increase in ventricular contractility compared to placebo, which was sustained over 4.5 hours. This work replicated previous work in adults (Scholz H. 1984) and preterm infants (Walther F. et al 1990). However Pincomb et al had demonstrated an increase in systemic

vascular resistance without an increase in cardiac contractility (Pincomb G.et al 1985). This difference may be due to differences in technique as earlier studies made no correction for heart rate, or cardiac pre-/ afterload.

Caffeine consumption can also influence the cardiovascular autonomic responses. These effects tend to be subtle and are not detected on the most common clinical investigations of Valsalva manoeuvre and deep breathing tests (Piha S. 1994). However differences have been observed with a lower heart rate on standing up with caffeine use before testing, as well as stronger isometric hand grip (Piha S. 1994). As caffeine has pressor effects it has been recommended as a treatment for orthostatic (Hoeldtke R. et al 1986) and postprandial (Heseltine D. et al 1991) hypotension. When investigating for autonomic neuropathy prior caffeine consumption should be avoided.

7. Physiological Effects of Caffeine on the Central Nervous System

The physiological effects on the central nervous system are manifold. In this section the relevant aspects to this thesis will be described. These include the effects of the caffeine on cerebral blood flow, cerebral metabolism and electrophysiological activity. The pyschological effects and the phenomenon of tolerance will be considered separately.

Cerebral Blood Flow

Caffeine causes cerebro-vasoconstriction 30 minutes after ingestion (Mathew R. et al 1983), via antagonism of the vasodilatory properties of endogenous adenosine. This effect was first demonstrated over 60 years ago (Gibbs F. et al 1935). 250mg caffeine causes a decrease in CBF of 15-18 % as measured by the Xenon inhalation technique (Mathew R. et al 1985a; Mathew R. et al 1985b). Mathew et al in an earlier study showed no further effect by high dose caffeine (500mg) (Mathew R.et al 1983). The effects of caffeine on CBF have been demonstrated to last for 90 minutes (Mathew R. et al 1985a). These changes are independent of mood and arterial partial pressure of carbon dioxide (Mathew R.et al 1985a).

Caffeine is associated with differential regional responses to changes in CBF (Mathew R.et al 1985b). In this study, whilst there was an overall decrease in CBF, frontal lobe blood flow actually increased. This was after a 250mg caffeine challenge in 8 high caffeine consumers only who were compared to 6 light users after 2 hours of caffeine abstinence. Conversely animal experiments have demonstrated a reduction in local blood flow where it simultaneously increases brain metabolism (see below) (Grome J. et al 1986). This can either be considered to cause an imbalance in supply and demand of cerebral metabolism or a reseting of the relationship between CBF and glucose requirements.

Cerebral Metabolism

Following caffeine administration the stimulant effects on the CNS are associated with changes in local rates of cerebral energy metabolism. In rat studies a caffeine dose of 10mg/ Kg causes increases in the motor and limbic systems, as well as the thalamus (specific nuclei only), substantia nigra, ventral tegmental areas, locus ceruleus and raphe nuclei. These changes in glucose utilisation correlate with changes in locomotor activity in rats and other behavioural modifications (Nehlig A. et al 1986).The multiple areas of the brain which are affected by caffeine mean that although the primary action of caffeine is to block adenosine (a neuromodulator), other neurotransmitters than dopamine are also affected:

- **noradrenaline:** caffeine increases both the rate of synthesis and turnover of noradrenaline, as well as increasing the spontaneous electrical activity of noradrenaline neurons (Grant S. et al 1982).
- **serotonin:** studies on rodents show that caffeine can increase both concentration and utilisation of serotonin, especially within the limbic system (Hadfield M. et al 1989)
- **acetylcholine:** less work has been done with this neurotransmitter and caffeine. In one study caffeine and theophylline increased acetylcholine transmitted activity in the cerebral cortex of rats at high doses (>15 mg/kg) (Phillis J. et al 1980).
- **glutamate/GABA:** caffeine administered initially at 0.5 mg/ml in drinking water for 7 days and then 1.0 mg/ml for the next 14 increased the amount of glutamine in the

whole brain of mice whilst simultaneously decreasing concentrations of GABA and glycine (Debler E. et al 1989). These changes could potentially lead to increased CNS excitability.

Neuroelectrophysiology

EEG: Caffeine is a cerebral stimulant and thus its effects can be measured in the EEG. The most robust results from a number of studies is that moderate doses of caffeine effect a decrease in spectral power in the lower α and θ ranges (Bruce M. et al 1986; Dimpfel W. et al 1994). Bruce et al also demonstrated using 250 and 500mg caffeine that these changes in the EEG were dose related (Bruce M.et al 1986). These changes, especially reduction in alpha power, reflect heightened cortical arousal by caffeine. Other changes have also been observed but are less consistent. Kenemans et al demonstrated a decrease in the delta and low beta ranges (Kenemans J. et al 1995), whereas Newman et al also reported an increase in alpha dominant frequency (Newman F. et al 1992). It would seem logical that these inconsistencies are more sensitive to differences in experimental variables. Conversely caffeine withdrawal is also associated with EEG changes. In people with a minor EEG dysrhythmia (diffuse paroxysmal slowing) increases in frequency of this abnormality were observed during caffeine abstinence (4 days) but was restored to baseline frequency with caffeine ingestion (Reeves R. et al 1995).

Evoked and Event-related potentials: P300 amplitude increases with confidence, attention, the arousal level and reward (Donchin E.et al 1988). Lorist et al demonstrated that caffeine can induce such a change as well (Lorist M. et al 1996). In this study 15 young and 15 older healthy volunteers who had abstained from their usual caffeine consumption for at least 12 hours were tested with 250mg caffeine or placebo. Reaction time to a visual stimulus significantly improved and a P300 amplitude increased, as did that of N100 and N200 in all subjects in the caffeine condition only. This would suggest that caffeine affects the 'output' stages of reaction time as latency is not affected. This is supported by another study which involved elegant manipulation of the ERP tracings to demonstrate that sensory discrimination ('input' stage) was not affected by caffeine ingestion (Kenemans J.et al 1995). However the situation is not so

easily described because in 2 further studies by Lorist et al showed that reaction time improved with a complicated visual task and changes in the ERP results supported the idea that caffeine increases cortical arousal and perceptual sensitivity (Lorist M. et al 1994a; Lorist M. et al 1994b).

With regard to aural stimuli, caffeine causes an increase in P300 amplitude during the oddball but not single tone paradigms which lasts for less than 210 minutes (Kawamura N. et al 1996). Here reaction time was not improved by caffeine and the authors suggest that the increase in amplitude of P300 is due to increased 'allocation of attentional resources' i.e. affects the 'input' stage. Studies with respect to aural tests are less consistent than visual ones. For example, caffeine causes a decrease in amplitude of N100, N200 and P200 in auditory evoked potentials (Bruce M. et al 1992). These changes were augmented in patients with generalised anxiety disorder. Finally in this section, both auditory and visual P300 evoked potentials showed a decrease with caffeine withdrawal in a recent study: no change in latency was observed (Reeves R.et al 1999).

8. Physiological Effects of Caffeine on Neuroendocrine Function

The effects of caffeine in humans on the endocrine system appear to be confined to the endocrine response to stress, especially production of catecholamines and cortisol although other hormones may be important too e.g. growth hormone. Plasma concentrations and urinary excretion of adrenaline and noradrenaline is increased by caffeine consumption (Bellet S. et al 1969; Robertson D. et al 1978; Van Soeren M. et al 1993). Although the methods of augmentation have not been described in detail, in the case of adrenaline, adenosine attenuates its release from the adrenal medulla. It has been shown that in tetraplegic individuals in whom sympathoadrenal responses are blunted, caffeine ingestion (6mg/kg) is not associated with a rise in plasma adrenaline levels (Van Soeren M. et al 1996). This suggests that caffeine-stimulated release of adrenomedullary catecholamines is mediated by peripheral sympathetic nerves. Thus caffeine antagonism may result in increased sympathetic stimulation of the adrenal gland leading to increased thyroxine hydroxylase activity and thus catecholamine synthesis (Snider S. et al 1974; Van Soeren M.et al 1993).

A_1 adenosine receptor activation causes presynaptic inhibition of noradrenaline release from postganglionic sympathetic nerves (Wennmalm M. et al 1988). However it is likely that this mechanism is not as important as autoreceptor control via noradrenaline acting on α_2 adrenergic receptors (Fredholm B. 1995). Therefore like increased adrenaline production, noradrenaline synthesis is controlled centrally by sympathetic outflow. Lane et al demonstrated that caffeine (3.5 mg/Kg) augmented the response to psychological stress in both light or habitual users of caffeine (Lane J. et al 1990). In this study additive effects of stress and caffeine were demonstrated with noradrenaline production and potentiation of the production of adrenaline during a stressful task compared to placebo.

Cortisol production is similarly augmented by caffeine and stress (Pincomb G.et al 1988; Lane J.et al 1990).This is thought to be mediated by an increase in adrenocorticotrophin (Bellet S.et al 1969). This action could also be partly due to caffeine's action on prostaglandins (Avogaro P. et al 1973). The effects of caffeine on growth hormone and prolactin are less well defined.

9. Psychological Effects of Caffeine Consumption

Having previously discussed the effects of caffeine at a molecular and neuronal level, this section goes on to the effects of caffeine on psychological responses. A large number of functions are affected, which would be beyond the range of this review, thus three specific ones are considered.

Behaviour

The first demonstration of an adenosine-dopamine interaction on behaviour was the finding that several adenosine receptor antagonists (caffeine, theophylline and isobutyl-methylxanthine) could increase dopamine receptor activated rotation behaviour in rodents (Fredholm B. et al 1976). This effect has been subsequently repeated and further investigated (Ferre S.et al 1992; Nehlig A. et al 1992; Daly J. 1993). The effect of caffeine appears to have a threshold of 1 to 3 mg/kg in rats with a maximal effect around 30 mg/kg (Garrett B. et al 1994). This study also demonstrated that the effects of caffeine are synergistic with dopamine agonist injected into the nucleus accumbens

(Garrett B.et al 1994). More evidence that there is a close relationship between the changes in animal behaviour induced by dopamine and caffeine is the correlation between changes observed with apomorphine (dopamine agonist) and caffeine (Casas M. et al 1989). However although there is evidence that adenosine receptor antagonsim is involved (Fredholm B.et al 1976), this is not the only mechanism. Evidence includes the finding that although CGS 15943 (a non-selective, non xanthine adenosine receptor antagonist) mimics caffeine action in increasing animal rotational behaviour, it is much less potent than caffeine in doing so (Garrett B.et al 1994). A_{2a} receptors appear to be of major importance in enhancing the dopaminergic system (Ferre S. et al 1997; Svenningsson P.et al 1997). This is important as it suggests the possibility of a new line in treatment of Parkinson's disease (Richardson P. et al 1997). A_1 adenosine receptors modulate the process, as demonstrated by Okada et al. The authors showed using microdialysis techniques that direct stimulation of the striatal adenosine receptors blocked the rise in dopamine levels when specific A_1 agonists were used but not A_{2A} agonists (Okada M. et al 1997): it must be noted that this study used high concentrations of caffeine however.

Of more relevance to human effects of caffeine is drug discrimination and drug self-administration. For example rodents can be trained to discriminate positively for caffeine (Mumford G. et al 1991). The discriminative stimulus for caffeine at low doses (10 mg/kg) are blocked by selective D1 and D2 dopamine receptor antagonists but not for higher doses (56 mg/kg) (Powell K. et al 1996). The situation is similar in humans as the discriminative stimulus for caffeine appears to be different for low and high doses. This is illustrated by the results of studies on low dose discrimination (20-200mg caffeine) reporting that subjects make the discrimination based on improvements in positive aspects of mood (Mumford G. et al 1994). This compares to the parameter used by subjects in high dose studies (up to 800mg) of increased anxiety, jitteriness and stomach-upset (Evans S. et al 1991).

A drug is considered to be reinforcing if it maintains behaviour on which the delivery of the drug is dependent. Caffeine is erratically self-administered in animal models of drug self-administration. However there seems to be a reinforcing effect between

caffeine and cocaine, as caffeine increases the rates of self-injection of a low dose of cocaine (Schenk S. et al 1994; Griffiths R. et al 1995). In humans both self-administration and choice procedures have shown that caffeine acts as reinforcer (Griffiths R. et al 1988a; Griffiths R.et al 1995; Hale K. et al 1995; Hughes J. et al 1995). In choice studies, subjects typically first sample caffeine or placebo and later have the opportunity to choose to self-administer one. Caffeine was chosen in preference to placebo in subjects with or without a prior history of caffeine consumption and is especially marked in those with a history of drug abuse (Griffiths R.et al 1995). Although the avoidance of caffeine-withdrawal may be important (James J. 1994b), evidence that caffeine acts as a reinforcer in the absence of physical dependence is from a study by Silverman et al (Silverman K. et al 1994). They studied subjects who had withdrawn from caffeine consumption for at least 6 weeks. 100mg caffeine acted as a reinforcer when subjects were required to perform a computer vigilance performance task. Under these conditions caffeine was also positively associated with improvements in mood.

Performance

Due to the enormous consumption of caffeine worldwide, there has been a vast amount of work on the performance effects of caffeine. It is far from clear how the benefits and drawbacks of its consumption balance out. In this section the evidence for the psychostimulant effects of caffeine on performance are considered, as they are widely held to be the main benefits of caffeine use.

Effects of acute caffeine ingestion of performance:

Numerous placebo controlled studies have been published. Although the results are varied with some studies showing no effect of caffeine (as reviewed by James (James J.1997b)), taken as a whole they confirm a psychostimulant action of caffeine. Specific positive examples of performance with caffeine vs. placebo are summarised in Table II.4.

Table II.4: Positive Effects of Caffeine on Performance Tasks.

Performance Task	Reference
Tapping Speed	Azcona O. et al 1995 Clubley M. et al 1979
Simple Reaction Time	Azcona O.et al 1995 Bruce M.et al 1986
Digit Symbol Substitution	Kaplan G.et al 1997
Vigilance	Lieberman H. et al 1987
Memory	Arnold M.E. et al 1987
Logical Reasoning/ Learning	Miller L. et al 1996 Suenaga N. et al 1997
Perceptual Judgement	Gupta U. et al 1994
Simulated Driving	Regina E. et al 1974

Net Effects of Caffeine on Performance:

Whilst a wide spectrum of cognitive tests have been investigated in the above studies under different protocols, some of the specific points can be summarised:

- subjects were young (usually between 18 and 30 years) and otherwise healthy
- subjects were low or moderate habitual users of caffeine
- prior abstinence of caffeine was required for at least overnight
- amount of caffeine in the challenge was relatively high up to 600mg

These points limit the relevance to caffeine consumption in everyday life. The problem with caffeine deprivation prior to testing, especially for a short period means that symptoms of caffeine withdrawal can be invoked. Thus it is not possible to describe the net effects of caffeine with these studies especially as caffeine withdrawal has also been shown to decrease performance in both humans (finger tapping) (Silverman K. et al 1992a) and animals (Griffiths R. et al 1988b). Strong evidence for the negative effects of caffeine deprivation of performance comes from a study comparing the performance of heavy caffeine users to non users in a choice reaction time test (Rizzo A. et al 1988). With 4 hours of caffeine deprivation there was no difference in

performance. However, with 48 hours caffeine deprivation the users were markedly impaired compared to non users. The pharmacokinetics of caffeine as previously described would predict that caffeine withdrawal effects would be worse after 48 than 4 hours without caffeine ingestion.

However evidence in this area is inconclusive as demonstrated by Richardson et al. The authors investigated the effects of a caffeine challenge (70 and 250 mg) on caffeine deprived (1.5hours, 13 hours and 7 days) subjects who were both users and non users of caffeine (Richardson N. et al 1995). 70 mg caffeine significantly decreased simple reaction time under all conditions but not 250 mg caffeine. Similarly, in a study of heavy coffee drinkers an abrupt change to decaffeinated coffee gave subjects symptoms of caffeine withdrawal but no significant change in a short duration psychomotor test (Griffiths R. et al 1986a).

However, another standpoint in this discussion is founded on the demonstration of improved psychomotor performance in non caffeine deprived individuals. A number of studies have reported performance enhancement when caffeine vs. placebo is administered to individuals who have consumed caffeine previously (Frewer L. et al 1991; Smith A. et al 1994; Warburton D. 1995). In the first two of these studies higher doses of caffeine (200-500mg) were used to show that caffeine significantly enhances performance even in caffeine replete individuals (Frewer L. et al 1991; Smith A. et al 1994). However, Warburton used 75 and 150 mg caffeine in subjects deprived of caffeine for 1.5 hours only and demonstrated improved performance in attention, logical reasoning and delayed recall tasks (Warburton D. 1995). It is possible that pre-treatment only partially eliminates the withdrawal effect (James J. 1997b). However in a large (9003 subjects) correlational study, Jarvis showed that there was a relationship between habitual caffeine consumption and performance (reaction time - simple and choice, verbal memory and visuospatial reasoning) even when all variables were controlled for (Jarvis M. 1993).

Interactions between Caffeine and Degraded Performance:

A significant body of evidence suggests that caffeine is most beneficial when baseline performance is degraded by factors other than caffeine abstinence. One example is the interaction between caffeine and benzodiazepines (File S. et al 1982). Lorazepam alone significantly decreased a symbol copying task. When given with caffeine this effect was negated. Similarly cyclizine decreased performance in an arithmetic test. When caffeine was simultaneously administered this effect was counteracted, although caffeine had little effect alone (Clubley M.et al 1979). Other such interactions include caffeine and alcohol (Azcona O.et al 1995) and caffeine and the common cold (Smith A. et al 1997). In the former study Azcona et al demonstrated an antagonistic relationship between caffeine (400 mg) and low dose (0.8g/kg) alcohol in simple reaction time and visual ERP. The results of other studies in this field are conflicting. For example Oboune and Rogers demonstrate that 150 mg caffeine given with 2.2 ml /Kg alcohol produced a more significant decrease on reaction time than alcohol alone (Oborne D. et al 1983). It has also been demonstrated that older (55^{+} years) people are more susceptible to the performance enhancing effects of caffeine (Jarvis M. 1993), although this was not confirmed in a later placebo-controlled trial (Rogers P. et al 1998).

Mood

The effects of caffeine on mood have been studied extensively and the results of these studies have been more consistent than in other areas of caffeine research. Caffeine in lower doses (up to 250 mg) has positive effects on mood in both caffeine deprived (Bruce M.et al 1986; Griffiths R. et al 1990; Silverman K.et al 1992b) and caffeine tolerant studies (Warburton D. 1995). From these and many other studies the benefits of acute caffeine ingestion on mood include an increase in energetic, confident and alert feelings, as well as motivation to work, concentrate or socialise. Similar effects have been described in children whom consume caffeine mainly from soft-drinks (Goldstein A. et al 1997). Here the authors demonstrated that in school children whom consumed more than 50 mg caffeine/day, they reported a higher degree of wakefulness than those who consumed less than 10 mg/ day.

A similarity to the effects of caffeine on deteriorated performance has also been demonstrated with mood. Smith et al showed that feelings of alertness improved when caffeine was ingested during a common cold in a group of (usually) health volunteers (Smith A.et al 1997).

With regard to the relationship between caffeine and psychological (psychiatric) disturbances, much work has been done too. In 1974 Greden described 'caffeineism' in patients with symptoms of generalised anxiety who consumed more than 1000 mg caffeine/ day (Greden J. 1974): this diagnosis has subsequently been included in DSM III and DSM IV, although is now referred to as 'caffeine intoxication'. Interestingly a population study by Eaton and McLeod showed no clear relationship between caffeine consumption and anxiety in the public at large (Eaton W. et al 1984). Later evidence supports such a direct relationship (Bruce M.et al 1992); in high doses human subjects report increased anxiety and jitteriness (Evans S.et al 1991). Strong evidence for the pharmacological effects of caffeine on anxiety came from an animal study in *Nature* (Ledent C. et al 1997). This group showed that mice with a specific, isolated disruption to adenosine A_{2A} receptors suffered increased anxiety.

Two recent studies have considered the effect of caffeine on depression. Rihs et al demonstrated that for in-patients there was a direct relationship between caffeine intake and depression (Rihs M. et al 1996). Typically the second showed an opposite result. In a study of Japanese, female medical students, caffeine intake negatively correlated with depression and suicide (Kawachi I. et al 1996).

Other Influences on the Psychological Effects of Caffeine

Further complications into examining the psychological effects of caffeine are due to the variability in subject personality and time of day (Revelle W. et al 1980). A subsequent study showed that the effects of caffeine are related to level of arousal (Anderson K. et al 1982) with a U-shaped relationship. Thus an increase in arousal improves performance with relatively few information sources which require monitoring, particularly when time to respond is important. When however multiple sources of information have to be used arousal and attention selectivity have no

apparent benefit and may decrease performance (Kenemans J.et al 1995). This study went on to conclude that caffeine increases cortical activation whilst simultaneously increasing the accumulation of information and quality of the selection process.

Time of day also influences the effects of caffeine on performance. Smith et al demonstrated that caffeine ameliorates the post-lunch performance dip (Smith A. et al 1990). In a more complex study Robelin and Rogers demonstrated a flat dose response relationship for the psychoactive effects of caffeine (86-258 mg caffeine) given in 1 to 3 divided doses throughout the day (Robelin M. et al 1998). Performance can also relate to 'morning' or 'evening' type people (Horne J. et al 1976). In this study the authors showed a relationship between those who found it hard to get up in the morning (evening types) and those who did not (morning types). Elucidation of the caffeine withdrawal syndrome however makes it more likely that evening types are actually experiencing caffeine withdrawal rather than a different aspect of personality. Finally caffeine's psychological effects are not influenced by gender. Of the wide variety of investigations carried out into the effects of caffeine only a few have demonstrated any difference between males and females e.g. Arnold et al demonstrated improved memory recall in females (Arnold M.et al 1987).

With the extensive work that has been done in the area of psychological effects of caffeine, it can be concluded that there is an improvement in mood and performance with caffeine consumption.. This however has to balanced against the negative effects of caffeine withdrawal (reviewed below) as well as increased blood pressure, reduced quality of sleep (Landolt H.-P. et al 1995) and decreased hand steadiness. Thus the overall effects of caffeine are probably negative but when the enormous world wide consumption of caffeine is considered its overall effect is considerably greater.

10. Caffeine Withdrawal

The phenomenon of the caffeine withdrawal syndrome is well substantiated and characterised in detail (Griffiths R.et al 1990; Hughes J. et al 1991; Silverman K.et al 1992a). Symptoms include headache, lethargy, fatigue, muscle pain/ stiffness and

dysphoric mood states. There follows a full list of symptoms identified by Griffiths to occur in the caffeine withdrawn state (table II.5).

Table II.5: Symptoms of caffeine withdrawal (Silverman K.et al 1992a)

Increased	Decreased
irritable/ cross/ grumpy	Well-being
blurred vision	desire to talk/ socialise
drowsy/ sleepy	urge to do work related tasks
yawning	energy/ active
lethargy/ fatigues/ tired/ sluggish	content/satisfied
muzzy/ foggy/ not clear headed	flu-like feelings
headache	self-confidence
heavy feeling in arms and legs	
hot or cold spells	

Silverman et al investigated the prevalence of symptoms when caffeine consumption was stopped abruptly in a double-blinded, placebo controlled study (Silverman K.et al 1992a). 62 healthy subjects were recruited who consumed 235 ± 126mg daily (mean ± SD). They were maintained on their normal diet for 5 days and then went onto a caffeine-free diet, consuming either their usual caffeine intake in 2 divided doses or placebo for 2 days. 52% experienced headache during the placebo period compared to 2% on normal diet or 6% with caffeine capsule substitution. Caffeine withdrawal negatively affected mood as measured by POMS (Profile of Mood States) with an increase in tiredness and anxiety along with a decrease in vigor. Depression scores also rose (Beck depression inventory score). The study illustrated that abrupt cessation of caffeine resulted in a clinically important withdrawal syndrome in normal adults. It is noteworthy that the prevalence of headache in the general population is 27% (Hughes J. 1992), yet only 6% coffee drinkers and 6% tea drinkers associate headache with omission of caffeine intake (Rogers P. et al 1995).

The time course for these symptoms to develop is short. Overnight abstinence is part of the usual pattern of caffeine consumption and it is usual for withdrawal symptoms to develop (Richardson N.et al 1995). Lane extended this time period to show that symptoms were not just related to awakening and that missing a mid-morning caffeinated beverage resulted in loss of energy (Lane J. 1998). Four hours of caffeine deprivation can cause dysphoria (Phillips-Bute B. et al 1997), although at 90 minutes there are no symptoms of caffeine withdrawal (Richardson N.et al 1995).

The symptoms increase for 24-48 hours and then begin to improve. Usually they have subsided by day 7 (Griffiths R.et al 1988b). High daily intake of caffeine can lead to a prolongation of the symptoms for up to 10 days or more (Griffiths R. et al 1986b). This may be explained by the fact that the rate of caffeine metabolism may vary by 13 fold throughout the population (Denaro C. et al 1991). It has been shown that caffeine consumption is directly related to the symptoms of caffeine withdrawal (Evans S. et al 1999).

Griffiths et al demonstrated that caffeine withdrawal could occur when subjects were maintained on as little as 100mg caffeine per day (Griffiths R.et al 1990). Evans and Griffiths showed that whether identical caffeine intake was in single or multiple doses the effects of withdrawal were indistinguishable. They also demonstrated that 3 days of caffeine consumption (300mg/ day) was the minimum requirement before symptoms of withdrawal occurred (Evans S.et al 1999).

Much work has been published on the subjective effects of caffeine withdrawal, less work has considered performance. Lane and Philips-Bute failed to show psychomotor impairment with 4-18 hours respectively of caffeine deprivation (Phillips-Bute B.et al 1997; Lane J. 1998). However at 24 hours impairments have been reported in a variety of tasks e.g. finger tapping (Silverman K.et al 1992a).

The above summarises the parameters whereby caffeine withdrawal occurs in an otherwise healthy population. It can be so disruptive to life that in 1994 the American Psychiatric Society included it in the DSM IV, although only as a 'proposed', rather

than an official category (American Psychiatric Association. 1994). James views this with a sense of irony as the body of research behind this phenomenon is beyond contention (James J. 1997b).

11. Caffeine Tolerance

The question that tolerance develops to all or some of the acute effects of caffeine has continued over several decades and remains highly controversial (Nehlig A. 1998; James J. 1997a). There follows a review of the literature on the development of tolerance to the cardiovascular and mood effects of caffeine, those on behaviour and performance having been previously discussed. The mechanism underlying these effects is not known. It is thought to be mediated centrally rather than by an increase in caffeine metabolism as there is little change in plasma caffeine level with daily injections of caffeine into rats (Lau C. et al 1994). Centrally, the number of adenosine A_1 receptors is increased following long term caffeine administration in rats (Fredholm B. 1982). This effect appears to be due to up regulation as opposed to increased gene transcription (Johansson B. et al 1993). This is not the complete explanation however. Mukhopadhyay and Poddar showed that GABAergic activity is decreased in the cerebral cortex in rodents not tolerant to the locomotor effects of caffeine which is increased to baseline levels as tolerance develops (Mukhopadhyay S. et al 1998): they demonstrated that this was also caffeine dose dependent.

Tolerance to Cardiovascular Effects of Caffeine

The most cited reference in favour of tolerance to the haemodynamic effects of caffeine developing is by Robertson et al (Robertson D. et al 1981). In this and another study (Robertson D.et al 1978) the authors demonstrated that in young men blood pressure and hormonal responses (renin and catecholamines) initially increased with a caffeine challenge (250 mg) but that habituation quickly developed over 3 days with continued ingestion of 750 mg caffeine per day; less than 10% of the American population consume this much caffeine (Gilbert R. 1984). Subsequently analysis of this work has been critical, not least because of the statistics used but other aspects of the methodology, which include small subject numbers, abnormal salt loading prior to testing and the aforementioned high doses of caffeine. However other researchers

have demonstrated tolerance to the cardiovascular effects of caffeine as well (Ammon H. et al 1983, Izzo J. et al 1983). These studies suggested that habitual use of caffeine leads to complete or near complete tolerance within days (>1 day). They did not however, mirror usual, staggered caffeine consumption but the studies are characterised by a prior period of prolonged abstinence followed by a high dose of caffeine administered once or twice daily. Thus subsequent studies which have considered usual patterns of consumption, have demonstrated the pressor effects of further doses of caffeine during the morning albeit with a smaller response than the first cup of coffee (Lane J. et al 1989; Goldstein I. et al 1990). Shi et al demonstrated that the tolerance phenomenon depended on amount of caffeine consumed, schedule of consumption and elimination half life (Shi J. et al 1993). They estimated using a parametric pharmacokinetic-pharmacodynamic model for caffeine that it would take about 20 hours or five drug half-lives for the effects of caffeine tolerance to wear off. Given that caffeine consumption is associated with 10 - 12 hours of overnight abstinence with more caffeine consumed in the morning than afternoon hours, it can be demonstrated that overnight abstinence is enough for sensitivity to be recovered and that at best tolerance is partial.

Further evidence for the continuing effects of caffeine on blood pressure are studies involving 24 hour ambulatory blood pressure monitoring. Most of these studies have shown an increase in systolic and diastolic blood pressure when caffeine is consumed compared to placebo, usually shortly after ingestion (Jeong D.et al 1990; James J. 1994; Superko H. et al 1994; Green P.et al 1996). 3 other 24 hour ambulatory studies failed to show such a strong relationship (MacDonald T. et al 1991; Myers M. et al 1991; Eggertsen R. et al 1993). Interestingly the differences in data analysis may be causal for this anomaly, as the former studies averaged blood pressure reading over 0.25 to 3 hours as opposed to the equivocal studies which averaged data over 4 to 24 hours. The haemodynamic effects of caffeine are diminished by 3 hours, potentially obscuring an effect of caffeine when a longer time interval was used in analysis. In Green and Suls study of 24 hour ambulatory blood pressure readings, subjects were allowed to consume caffeine/ placebo as per their usual patterns of consumption. The authors demonstrated a rise in systolic and diastolic blood pressures with caffeine

consumption (3.6. and 5.6 mmHg respectively). They were able to show that for systolic but not diastolic blood pressure, the rise was dose dependent (Green P.et al 1996). James et al showed that compared with the effects of caffeine ingestion after a period of abstinence, habitual caffeine consumption only decreased the peak pressor response by 25% (James J. 1994), suggesting that tolerance once again is partial not complete.

Finally the most important but also most controversial question: *Is there an association between long term caffeine consumption and hypertension?* Of the recent studies, most show an inverse relationship e.g. (Gyntelberg F. et al 1995; Lewis C. et al 1993) and some show no relationship (Lancaster T. et al 1994), with only one showing a positive relationship (Burke V. et al 1992). James argues convincingly that these epidemiological studies fail to identify a positive relationship between caffeine and elevated blood pressure because in measuring blood pressure in fasted individuals (as the majority of the studies did) means that the caffeine levels in the subjects' bodies would be low, thus potentially hiding the relationship (James J. 1997a). In this article he suggests that far from there being no relationship, there would be a 9-14% reduction in coronary heart disease and a 17 to 24 % decrease in stroke if the population as a whole ceased to consume caffeine because caffeine consumption is actually associated with a rise in population blood pressure of 2-4 mmHg using the 24 hour blood pressure data described above (Jeong D.et al 1990; James J. 1994; Superko H.et al 1994).

Few studies have considered the possibility of tolerance developing to caffeine's effects on CBF. No difference was demonstrated between the resting CBF in the high (6 cups coffee/ day) and light users (3 cups coffee/ day) (Mathew R.et al 1983), leading the authors to suggest long term effects of caffeine on CBF i.e. tolerance developing. However this is not valid as the caffeine consumption of the 'light' users is only relative. However, further evidence for this is that on caffeine withdrawal in habitual users, CBF rises (Couturier E. et al 1997). This study used the technique of transcranial Doppler sonography to measure blood velocity in the cerebral arteries (anterior and posterior circulations). It has been demonstrated that changes in blood velocity are

proportional develop to changes in total CBF (Sorteberg W. 1992; Debrah K.et al 1996).

Overall the situation is not clear as Debrah et al concluded that tolerance did not develop to this effect in their study (Debrah K. et al 1995). Whilst they showed that the acute rise in mean arterial blood pressure was lost in the caffeine replete state (9 young, healthy adults had consumed 250mg caffeine once daily for six days), the decrease in middle cerebral artery blood velocity was not affected by prior continued caffeine ingestion. The authors suggest that there is dissociation between the central and peripheral effects of tolerance to continued caffeine consumption.

Tolerance to the Effects of Caffeine on Mood

The positive effects of caffeine on mood have been described above. There is less information regarding the question of tolerance developing with continued use. Warburton carried out an experiment which was designed to be relevant to every day caffeine consumption. Subjects were given 70 or 150 mg caffeine after one hour of caffeine abstinence. At both doses subjects felt significantly more clear headed, happy and calm with a decrease in tension (Warburton D. 1995). However, evidence for the development of tolerance in these parameters can be found in two field studies by Höfer and Bättig (Höfer I. et al 1994a; Höfer I. et al 1994b). In these subjective wakefulness increased above preabstinence baseline levels when caffeine consumption was resumed. Similarly Green and Suls did not demonstrate improved mood with continued caffeine consumption compared to placebo (Green P.et al 1996).

With regard to the negative effects of caffeine, these in habitual users relate to the effects of caffeine withdrawal and it has been demonstrated that caffeine acts as a negative reinforcer (Rogers P.et al 1995) i.e. consumption is continued to avoid symptoms of withdrawal. As this occurs on a regular (daily) basis with enforced overnight abstinence, tolerance would not be expected to develop. Negative changes in mood with caffeine consumption are observed with high doses of caffeine or in non-users. The symptoms include anxiety, tension, restlessness and sleeplessness. The differences in responses to users and non users may be due to tolerance and habituation.

Section III REVIEW OF THE BODY OF RESEARCH TO DATE REGARDING CAFFEINE AND HYPOGLYCAEMIA

The Best Defense against Hypoglycaemia is to recognise it. Is Caffeine useful?

The following section reviews the current body of evidence regarding the influence of caffeine on the perception of and physiological response to hypoglycemia. Many of the aspects have been previously described in detail. Important points are repeated here for clarity and emphasis. It is my hope that this section brings together the hypothesis behind this thesis, thus enabling the reader to move onto the experimental sections with an understanding of the line of investigations.

The diagnosis of diabetes brings changes to an individual's lifestyle which may be more dramatic than with most other chronic diseases. Many of the 'simple' pleasures of life are at best limited (e.g. avoidance of certain pleasurable foods) or at worst become a potentially mortal sin (e.g. smoking). Alcohol has to be consumed with caution because of its acute effect on the perception of hypoglycaemia (Kerr D. et al 1990), chocolate becomes a rare treat and sexual intercourse a pleasant memory if the complications of diabetes develop. These are but a few basic aspects of daily living which the majority of the population take for granted. Even the simple pleasure of consuming the most commonly used drug in the world, 1, 3, 7-trimethylxanthine or caffeine has implications for the person with diabetes.

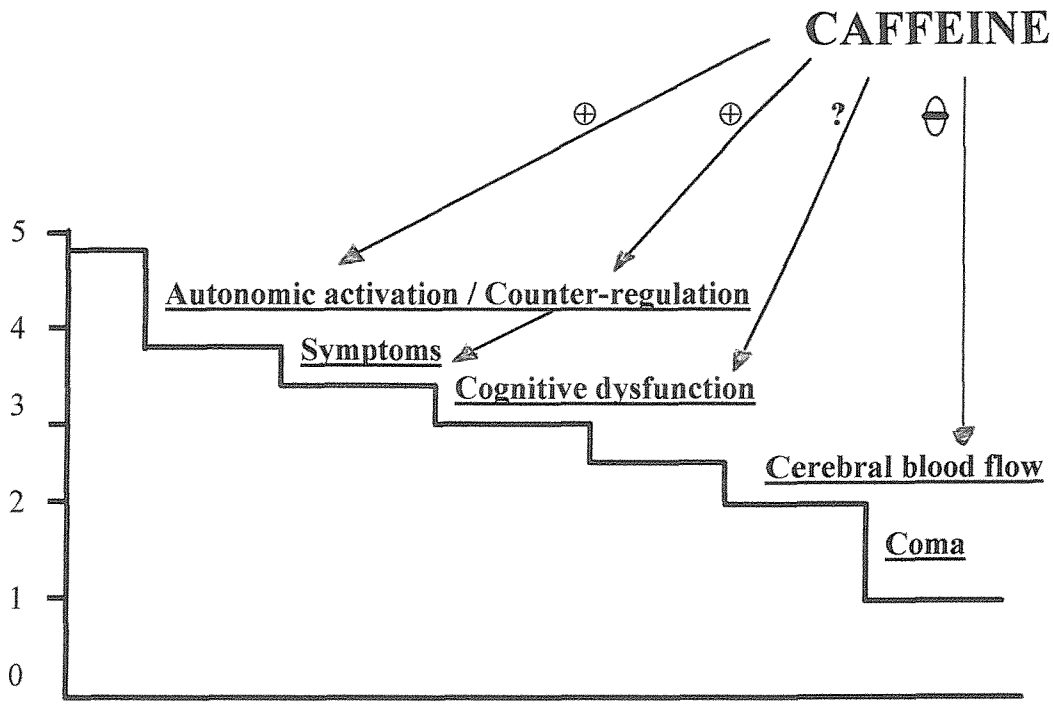
Caffeine, a basic component of coffee, tea and over the counter remedies for coughs and colds, is also added during the manufacturing process to a number of non-alcoholic soft drinks (Institute 1983). Thus it is consumed in many different forms throughout the world but is consistently taken by 80% of adults in most populations. Given the ubiquitous presence of caffeine, there is a general perception that at worst there are no negative consequences for health. In fact, acute ingestion of modest amounts is recognised to raise blood pressure, alter cognitive performance and reduce cerebral blood flow (Mathew R.et al 1985b; Nehlig A.et al 1992). These effects may be particularly relevant for patients with diabetes, especially those at risk of hypoglycaemia as a consequence of treatment with insulin or sulphonylureas.

'Hypoglycaemia is a biochemical term and the value that defines it depends upon the nature of the sample' (Marks V. 1986). In clinical practice hypoglycaemia is usually defined as the blood glucose level at which detectable physiological changes occur which, if not corrected, leads to the development of characteristic warning symptoms and impairment of higher cerebral function. The level of blood glucose that defines hypoglycaemia varies but is usually taken to be less than 3.0 mmol/l. For individuals at risk of hypoglycaemia, the important consequences of caffeine ingestion relate to a direct effect of uncoupling the relationship between cerebral blood flow and glucose utilisation within the brain. As the brain cannot manufacture glucose, nor store significant amounts, it is exquisitely dependent on a continuous supply of this substrate from the peripheral circulation to maintain normal function. For more than 60 years, it has been recognised that ingestion of caffeine acutely reduces cerebral blood flow (Gibbs F.et al 1935) even with amounts similar to those found in 1-2 cups of tea or coffee (Mathew R.et al 1985a). In addition caffeine augments brain glucose utilisation due to increased firing of cortical neurons and release of local neurotransmitters (Nehlig A. et al 1984). Therefore, caffeine ingestion may increase an individual's sensitivity to neuroglycopenia through the combined influence of reducing substrate delivery whilst simultaneously increasing brain glucose metabolism. These physiological changes appear to be mediated by antagonism of adenosine receptors (Fredholm B. 1980; Daly J. 1993) and may be prolonged because of caffeine's long half-life (Pfeifer R.et al 1988).

As blood glucose levels fall below normal the trigger for release of counterregulatory hormones and the development of warning symptoms depends upon cerebral glucose availability and the ability of the brain to maintain metabolism (Boyle P.et al 1994). Thus it may be anticipated from the above that caffeine can alter the usual physiological responses to hypoglycaemia (Figure III.1). Under laboratory conditions, acute ingestion of caffeine (in doses equivalent to that in 2-3 cups of drip-brewed coffee) markedly enhances the symptomatic and sympathoadrenal responses to hypoglycaemia in healthy volunteers and patients with type-1 diabetes (Kerr D.et al 1993a; Debrah K.et al 1996). Debra et al used a hyperinsulinaemic clamp to compare the effects of 250mg caffeine and matched placebo on the physiological responses to

stepped hypoglycaemia. Within 30 minutes of caffeine administration, there was a 15-20% reduction in middle cerebral artery velocity; a surrogate measure CBF (Sorteberg W. 1992). With the combination of caffeine and mild hypoglycaemia (3.8mmol/l), adrenaline levels were more than twice that seen with placebo. When blood glucose was clamped at 2.8mmol/l, adrenaline, growth hormone and cortisol responses were also higher following caffeine administration (but not noradrenaline) compared to placebo. At this blood glucose level patients reported more intense symptoms of hypoglycaemia (autonomic and neuroglycopenic) with caffeine ingestion .

Figure III.1 The usual hierarchy of physiological responses to hypoglycaemia and the influence of caffeine.



As a corollary, increasing cerebral blood flow by 30% at the onset of acute hypoglycaemia (using a bolus injection of acetazolamide) attenuated both the counterregulatory hormonal response to and perception of hypoglycaemia without altering the normal cognitive responses to acute blood glucose lowering (Thomas M.et al 1997). Caffeine has been shown to block the increased cerebral blood flow induced by hypoglycaemia in rats (Horinaka N. et al 1997). In this study animals who had been

rendered hypoglycaemic (blood glucose 2.0 mmol/l) showed a 51% rise in CBF. This rise was reduced in the presence of 10 mg/kg caffeine and abolished by 20 mg/kg caffeine iv.

Caffeine alone stimulates catecholamine release from the adrenal medulla, with plasma adrenaline production being more sensitive to caffeine than noradrenaline (Robertson D.et al 1978). This influence of caffeine on catecholamine release may be relevant for patients with type-1 diabetes who have lost the usual glucagon response to a low blood glucose level. In such individuals 'catecholamines are in the very first line of defence against hypoglycaemia' (Bolli G. 1998).

Caffeine may also be a useful adjuvant therapy for patients in whom intensive insulin regimens are prescribed who run the risk of suffering from an increased risk of severe hypoglycaemia (DCCT 1993). Within the DCCT 31% of severe hypoglycaemic episodes occurred without warning symptoms and in 51% the symptoms were insufficient to avert the problem. For some patients, fear of hypoglycaemia may cause them to eschew all attempts at improving glycaemic control.

As the counterregulatoy hormones have been shown to increase during hypoglycaemia in the presence of caffeine, the rate of glucose recovery theoretically could be enhanced. A related compound theophylline (1,3 dimethylxanthine) has been shown to augment glucose recovery, when given intravenously prior to the induction of hypoglycaemia. By inhibiting phosphodiesterase, theophylline increases cyclic adenosine monophosphate (cAMP) levels, the intracellular messenger of glucagon and adrenaline. Enhanced glucose recovery was correlated to the concentration of cAMP but not levels of counterregulatory hormones (Hvidberg A. et al 1994). Interestingly this effect was attenuated with long term oral supplementation prior to hypoglycaemia induction (Hvidberg A. et al 1998).

Intellectual performance is affected both by hypoglycaemia and caffeine ingestion. Perceived wisdom suggests that caffeine can improve intellectual performance by increasing alertness and improving concentration, whilst hypoglycaemia has the

opposite effect (Pramming S. et al 1986). Although there is a large literature of formal studies examining the effect of caffeine on a variety of intellectual tasks, the results are often conflicting and inconclusive. In a review of caffeine's effects, Nehlig et al concluded 'it is very difficult to form a definite opinion on caffeine's effects on the central nervous system. Interpretation of the data is highly subjective and the effects vary greatly from one subject to another. A number of positive effects of caffeine on cognitive performances are related to an increase in arousal and a suppression of boredom in repetitive tasks rather than a direct effect on cognitive performances' (Nehlig A.et al 1992). In healthy volunteers subjected to modest hypoglycaemia (90 minutes at 2.8 mmol/l), caffeine ingestion was associated with greater deterioration in cognitive performance as assessed by P300 auditory evoked responses (Kerr D.et al 1993a). In contrast patients with type-1 diabetes appear to be protected from any adverse effect of caffeine during lowering of blood glucose (Debrah K.et al 1996). These discrepancies may be partly explained by the demonstration that cerebral glucose metabolism can be preserved during prolonged hypoglycaemia in healthy volunteers (Boyle P.et al 1994). The effects of caffeine on intellectual function may be less marked in diabetic patients whose cerebral glucose metabolism has had to 'adapt' as a consequence of previous hypoglycaemic episodes. Thus for individuals at risk of hypoglycaemia the effects of caffeine on brain glucose metabolism may be more important than its influence on substrate delivery.

Others have argued that the proposed 'beneficial' effects of caffeine are in fact a manifestation of treating acute caffeine withdrawal in experimental volunteers who have fasted from the previous evening (James J. 1994). The clinical manifestations of acute withdrawal, namely headache, lethargy and mood disturbance including depression and anxiety are often unrecognised but can be severe enough to interfere with normal daily activities (Silverman K.et al 1992a). Daily consumption of caffeine can, in itself, be related to an increase in anxiety and tension. Other negative aspects of caffeine consumption include exacerbation of panic attacks (Hughes R. 1996) decreased hand steadiness (James J. 1997b), poor quality of sleep (Landolt H.-P.et al 1995). Rarely caffeine can become a drug of abuse and induce the syndrome of

'caffeine intoxication', (American Psychiatric Association 1994). This is characterised by restlessness, insomnia, rambling flow of thought and tachycardia.

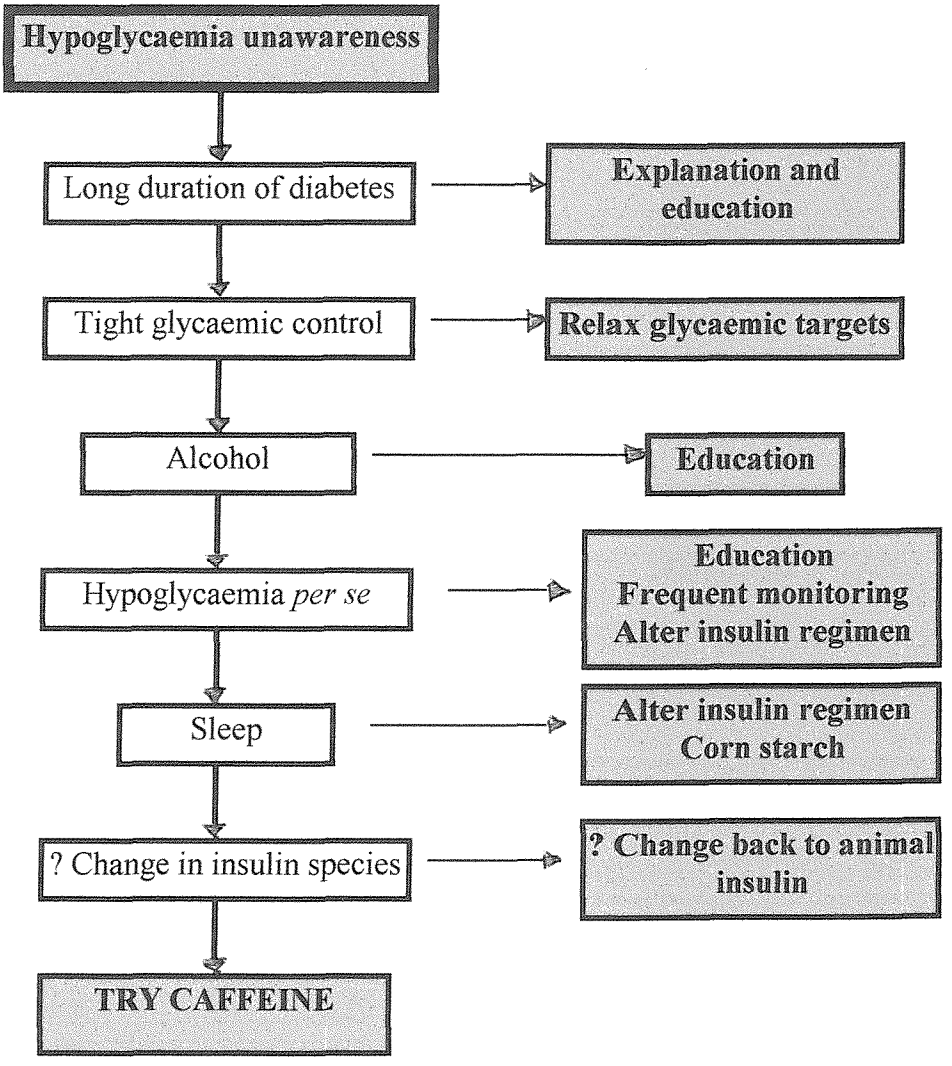
Numerous studies have examined coffee as an independent risk factor for ischaemic heart disease. Whereas case control studies have tended to find an association, many cohort studies and meta-analysis have not (Greenland S. 1993; Kawachi I. et al 1994). There have been no studies specifically examining the cardiovascular consequences of ingesting caffeine in patients with diabetes or its effects on the rate of silent myocardial ischaemia. Caffeine toxicity has been associated with many types of cardiac arrhythmias. This effect appears to be dose dependent because most studies have indicated a neutral effect of ingesting a modest amounts of caffeine on cardiac rhythm (Myers M. et al 1990).

Another widely held belief about caffeine is that individuals can become tolerant to its effects. Initial studies suggested that the peripheral consequences of caffeine ingestion e.g. a rise in blood pressure are lost with sustained use (Robertson D. et al 1981; Robertson D. et al 1984). Others have reported conflicting results (Green P. et al 1996). In people who are susceptible to hypertension the effect of caffeine on blood pressure may be augmented and prolonged compared to normotensive controls (Pincomb G. et al 1996). Within the brain, Debra et al has shown that its action on reducing cerebral blood flow does not diminish completely with recurrent dosing (Debrah K. et al 1995). Furthermore the development of tolerance may be weakened by the very nature of caffeine use – in some individuals overnight abstention may be long enough to allow the pressor response to caffeine to return. Tolerance to caffeine may develop via a number of different mechanisms including an increase in adenosine receptors (Kaplan G. et al 1992) and possibly an increase in neuronal GABAergic activity (Mukhopadhyay S.et al 1998).

In summary, ingestion of modest amounts of caffeine enhances the intensity of warning symptoms and increases levels of counter-regulatory hormones at least under laboratory conditions. Thus caffeine could become part of the strategy used to deal with the problem of hypoglycaemia unawareness (Figure III.2). Caffeine has been described as the 'opium of the people' (Horton R. 1998) but controversy continues as

to the purported health risks and benefits of regular caffeine use. The view has been expressed that 'if caffeine is not bad for you it should be' (Grady D. 1986). Suspicions remain as to the motives of the multinational multibillion-dollar caffeine industry, which adds a substance of no (or very little) health benefit during the manufacture of a wide variety of food and drinks. Nevertheless, for patients with diabetes at risk of hypoglycaemia, caffeine may be beneficial. Unlike most other therapies it is inexpensive, safe and remarkably popular with its consumers.

Figure III.2 Hypoglycaemia Unawareness: the problems and suggested treatments



Section IV METHODS

1. Study Details

Ethical Permission

The hospital ethics committee granted permission for each of the studies carried out at the Royal Bournemouth Hospital, Bournemouth (studies 2- 6). The Defense Evaluation and Research Association (DERA) granted ethical permission for study 1.

Written, informed consent was obtained from all subjects, following detailed explanations about the experimental procedures.

Subjects

The details of selection criteria for the subjects are explained in the individual study chapters. Prior to enrolment for study 1, the general level of mental ability was assessed on each individual using the National Adult Reading Test (NART) (Nelson H. et al 1991) . This assesses the premorbid IQ, i.e. the peak level of mental ability attainable by a subject prior to cognitive deterioration. In this task the subject was asked to read aloud 50 irregular, english words of progressing difficulty (e.g. ache, depot). The number pronounced correctly equaled the score. The NART has been validated against the Wechsler Adult Intelligence Scale (Wechsler D. et al 1981) and has been shown to correlated closely with this test in subjects without reason for cognitive decline.

Schedule

The studies were all performed between August 1996 and September 1998 at the Royal Bournemouth Hospital, Bournemouth, Dorset and DERA- Queen's Gate, Farnbourough, Hants.

2. Laboratory Studies of Hypoglycaemia

Experimental Procedure

The subjects were admitted to DERA research room in study 2 or to the Bournemouth Diabetes and Endocrine Centre research department in study 4: see the individual study chapters for precise details of time and fasting instructions. A Teflon cannula was inserted into the ante- cubital fossa of the non- dominant arm. This was used to infuse

insulin (Actrapid, Novo Nordisk, Copenhagen, Denmark) via a pump. Simultaneously an infusion of 20% dextrose at a variable rate was infused.

A second cannula was inserted retrograde into a vein on the back of the non-dominant hand. This hand was heated using a Plexiglas box to 60°C and the cannula used to take arterialised blood samples. These were taken at 3- 5 minute intervals and measured at the bedside (Yellow Springs Instrument 2300 Stat, Yellow Springs, Ohio, USA).

The process of warming the hand causes arterio-venous shunting to occur here. Arterialised venous blood has been shown to approximate closely with true arterial blood glucose concentrations (Liu D. et al 1992). Thus the concentration of substrate delivered to the tissues can be accurately measured and the problem of venous blood glucose variability during hypolgycaemia minimised.

Hyperinsulinaemic Glucose Clamp Technique

In studies 2 and 4 hypoglycaemia was induced and maintained using a modified, manual hyperinsulinaemic glucose clamp (De Fronzo R.et al 1979). This method uses a fixed rate insulin infusion and a variable rate 20% dextrose infusion. Insulin was infused at 6 mU/Kg/min starting at time 0, it was then decreased to 4 mU/Kg/min at 4 minutes and finally to the maintenance rate of 2 mU/Kg/min at 7 minutes. This initial loading with insulin serves to saturate the insulin receptors thus facilitating subsequent manipulation of blood glucose by the experimenters.

An infusion of 20% dextrose was commenced 5 minutes after the insulin infusion, at an initial rate of 2 mg/Kg/hour. The subsequent rate of dextrose infusion was determined by the arterialised blood glucose concentrations measured by the bedside every 3- 5minutes (see below).

In both studies a period of stable euglycaemia was first established to enable baseline measurements to be taken. Hypoglycaemia was induced by halving the rate of dextrose infusion used for the maintenance of euglycaemia. The rate was then adjusted as required, to maintain stable hypoglycaemia, at the predetermined level. The time

allowed for the change from euglycaemia to hypoglycaemia was 20 minutes. The blood glucose was then held steady for 10 minutes prior to a set of measurements being made. The predetermined glucose concentrations for both studies were 4.5mmol/l and 2.5mmol/l for euglycaemia and hypoglycaemia respectively.

This is the one-step euglycaemia- hypoglycaemia clamp. It allows the investigator to provide a reproducible fixed level of hypoglycaemia for a predetermined time and identical stimulus with which to compare subjects. The subjects were not informed of their blood glucose concentrations throughout the glucose clamp. During a euglucaemic clamp, the blood glucose was maintained throughout at 4.5mmol/l. In study 2 insulin was stopped after $^{+}$180 minutes with euglycaemia restored over 20 minutes. Measurements of blood glucose continued through a final test battery studying the effects of caffeine on the recovery period.

The hyperinsulinaemic glucose clamp produces plasma insulin levels that are supraphysiological. This may have an influence on the outcome of experimental studies employing this technique. However, as both studies 2 and 4 are of a cross over design, this variable is controlled for. The variability of the threshold for cognitive dysfunction between individuals causes a methodological problem too (Gonder-Frederick L. et al 1994). There is less variability in this parameter though than others, such as the threshold for counterregulatory hormones production. In choosing 2.5mmol/ l as the blood glucose concentration during hypoglycaemia, the threshold for cognitive dysfunction is reached or exceeded for the majority of subjects, especially healthy volunteers. One more important reservation of this technique is that it is not wholly analogous to the 'real life' experience of hypoglycaemia. Therefore interpretation of the results must be made with caution. Despite these uncertainties the hyperinsulinaemic glucose clamp is the only method available for reliably maintaining the blood glucose at predetermined concentrations for enough time to allow the administration of a battery of tests.

3. Physiological Measurements during euglycaemia and hypoglycaemia studies

Heart Rate and Blood Pressure

Heart rate and blood pressure were measured using an automated device (Dinamap, Critikon, UK) During a set of measurements, three readings for these parameters would be made and the mean average calculated for use in further statistical analysis. During the hypoglycaemia studies, electro-cardiac monitoring was mandatory

Middle Cerebral Artery Blood Velocity

The velocity of blood flow in the middle cerebral artery (V_{MCA}) is directly proportional to total cerebral blood flow (CBF) (Sorteberg W. 1992). The technique has been validated consistently in multiple studies (e.g. Bishop B. et al 1986, Deborah K. et al 1996). An assumption is made that the change in diameter of the blood vessels is small. Transcranial Doppler measurement of blood flow in the middle cerebral artery provides a quick, reproducible method whereby changes in CBF can be monitored over a short period of time. Left and right V_{MCA} were measured in all studies using this technique (SciMed, Bristol, UK, studies 1, 3, 4 and 5 and Nicolet Biomedical, Warwick, UK in study 2). Three consecutive readings were taken each time on each side with the maximum velocity recorded for analysis.

4. Full Blood and Plasma measurements during euglycaemia and hypoglycaemia studies

Glucose

Whole blood measurements of glucose were made at the bedside (Yellow Springs, Ohio, USA). During the hypoglycaemic clamp studies these were made every three to five minutes. During the euglycaemic studies (3+5) these were made every 30 minutes. In study 1, patients recorded their capillary blood glucose readings using One Touch Profile Meters (Lifescan, Johnson and Johnson Co., High Wycombe, UK)

Caffeine

Caffeine capsules used throughout the studies described in this thesis contained 200mg caffeine with lactose filler. Plasma caffeine was measured by enzyme multiplication

immunoassay (EMIT®; Behring Diagnostics, Milton Keynes, UK) on an Olympus AU560 autoanalyser (Olympus Optical, Eastleigh, UK).

Catecholamines

Plasma adrenaline and noradrenaline were measured by HPLC using electrochemical detection (ESA Inc., North Chelmsford, MA, USA) (Meineke I. et al 1989).

Growth Hormone

Plasma growth hormone was measured using a two site immunoradiometric assay, standardised against the first international reference preparation of growth hormone (MRC 66/217).

5. Hypoglycaemia symptoms assessment

A validated symptom questionnaire was completed at the beginning and end of each stage of the clamp procedure (Hepburn D. et al 1992; Deary I. et al 1993a) to assess the intensity of hypoglycaemic symptoms. The questionnaire divides 11 of the most common symptoms of hypoglycaemia into 3 groups:

Neuroglycopenic: confusion, drowsiness, odd behaviour, speech difficulty inco-ordination

Neurogenic: hunger, palpitations, sweating, shaking

Other: nausea, headache

Each symptom is graded from 1 (not present) to 7 (very intense). The total score for each sub group is calculated. In study 1, a similar hypoglycaemia study was used, having been adapted to local needs (Clarke W.et al 1995).

6. Mood assessment in euglycaemia studies

The UWIST mood adjective check list (Matthews G.et al 1990). 24 adjectives which describe three aspects of mood are graded 1- 4 by the subject throughout the study, at specific time points. Thus levels of energy, hedonsim and tense arousal can be measured. Higher scores for the first two are positive, being associated with happiness and high energy levels respectively. In the third aspect a high score relates to increased tension. The maximum score for each aspect of mood is 32 and the minimum is 8.

7. Cognitive function tests used in euglycaemia and/or hypoglycaemia studies

During the individual studies, all the cognitive function testing took place under the same lighting conditions, in a sound attenuated room.

Four choice reaction timer test

This is a psychomotor performance test (Wilkinson R. et al 1975). The subject is presented with a square divided equally into four. As each of these quarters lights up randomly in turn, the subject has to move the light on by pressing a corresponding button on the control panel. Accuracy and speed of reaction are recorded over 5 minutes.

Visual Perception Threshold Tests

Visual change detection (VCD) assesses the speed of early visual processing by measuring the brain's ability to identify the locus of change in a stimulus array (McCrimmon R.et al 1996). The stimulus display consists of an array of 49 rectangles on a computer monitor screen to which, after a variable interval, a single identical rectangle is added. The subject's task is to identify this addition. The different time intervals between the presentation of the array and the onset of the change are 14, 28, 42, 56, 70 and 84 ms. The whole test involves 10 trials of the 6 different stimulus duration, unless otherwise stated in individual methods section. A total accuracy score is obtained.

Visual movement detection (VMD) resembles the VCD test in all respects, except that the target rectangle, rather than appearing after the rest of the array, appears with the array (McCrimmon R.et al 1996). After a variable interval it moves horizontally by a distance identical to its width (3mm). This creates the subjective sensation of sudden movement. The test is generated in the same format as the VCD test. The interval between the onset of the array and the target rectangle appearing to move are also identical (i.e. 14-84ms). A random block of 60 presentations (10 trials of 6 different stimulus duration) is also employed in this test as with VCD test and the total number of correct responses is obtained.

8. Statistical methods

The details of statistical analysis carried out the results of each study are described in each of the relevant chapters. All statistical procedures were carried out on Microsoft Excel (Microsoft Corp., Seattle, USA) and Systat (SPSS Inc., Chicago, USA) programmes.

Section V CLINICAL STUDY

Study 1 Influence of Caffeine on the frequency and perception of hypoglycaemia in free-living patients with type 1 diabetes mellitus

Patients with type 1 diabetes rarely achieve an ideal standard of glycaemic control, largely due to the imprecision of insulin therapy, which confers a risk of post absorptive hypoglycaemia (DCCT 1993). The best defence against hypoglycaemia is the ability to recognise warning symptoms and to take appropriate action (i.e. consume carbohydrate in a relatively pure form). Unfortunately, up to 25% of patients with long standing type 1 diabetes have a severely compromised ability to detect falling blood glucose levels, putting these individuals at risk of sudden and severe neuroglycopenia (Hepburn D.et al 1990).

In healthy volunteers and patients with type 1 diabetes, acute ingestion of modest amounts of caffeine (250-400mg, equivalent to 2-4 cups of drip-brewed coffee) (Debry G. 1994), markedly enhances the intensity of warning symptoms and the usual hormonal counter-regulatory response to 'clamped' hypoglycaemia, under laboratory conditions (Kerr D.et al 1993a, Debrah K.et al 1996). However although the perception of hypoglycaemia is augmented by prior caffeine ingestion, it is unclear whether low blood glucose levels will become associated with warning symptoms if caffeine is used on a daily basis.

The aim of this study was to examine the influence of caffeine on the frequency and perception of hypoglycaemia in 'free-living' patients with type 1 diabetes during everyday activities and outside the laboratory environment. The supplement dose of caffeine was almost equivalent to average consumption of caffeine in the United Kingdom (444mg/ day) (James J. 1997b).

Methods

Subjects

39 non-smoking patients with type 1 diabetes (Table V.1.1) gave written consent for the study. They were recruited on the basis of stable glycaemic control (average HbA_{1c} $\leq$ 10%), absence of complications (except background retinopathy) and were on no

other medication apart from insulin as a basal bolus regimen with 4 injections each day. The study was carried out between October 1996 and September 1998. 5 patients failed to complete more than 2 phases of the study and their data have been excluded from further analysis.

Experimental procedure

This was a prospective, randomised, placebo-controlled double blind study consisting of 4 continuous phases (Figure V.1.1). Throughout phase A (baseline, 2 months duration) patients continued with their usual diet, during which 7 day food diaries were used to calculate average daily caffeine consumption. For phases B (2 months), C and D (3 months each) they were provided with decaffeinated or caffeine-free products (tea, coffee and carbonated soft drinks); thus establishing a low caffeine diet (<15mg per day) until the completion of the study. In addition, during phases C and D patients were randomised to capsules containing either 200 mg of caffeine twice daily or matched placebo with cross-over at 3 months. Patients were contacted directly at least once a week. Compliance was maintained by encouraging the patients to report any dietary discrepancy, in addition to regular checks of study medication including counting capsules.

Four capillary blood glucose levels were collected each day (pre-meal and before bed). Patients were also instructed to take additional measurements during suspected episodes of hypoglycaemia. The hypoglycaemic events recorded at the end of a phase were divided into symptomatic and biochemical hypoglycaemic events. The former were defined as the development of characteristic warning symptoms with a confirmatory capillary blood glucose level (< 3.5 mmol/l). The intensities of associated warning symptoms were recorded using a validated symptom questionnaire (Clarke W.et al 1995). Nine symptoms were assessed on a scale of 1 to 6. Symptoms were compared using total scores (i.e. including all symptoms) as previous work has shown that caffeine affects both neuroglycopenic and neurogenic symptoms (Debrah K.et al 1996). If possible, confirmatory data were obtained from relatives/carers. Biochemical, asymptomatic hypoglycaemic events were identified by routine testing alone.

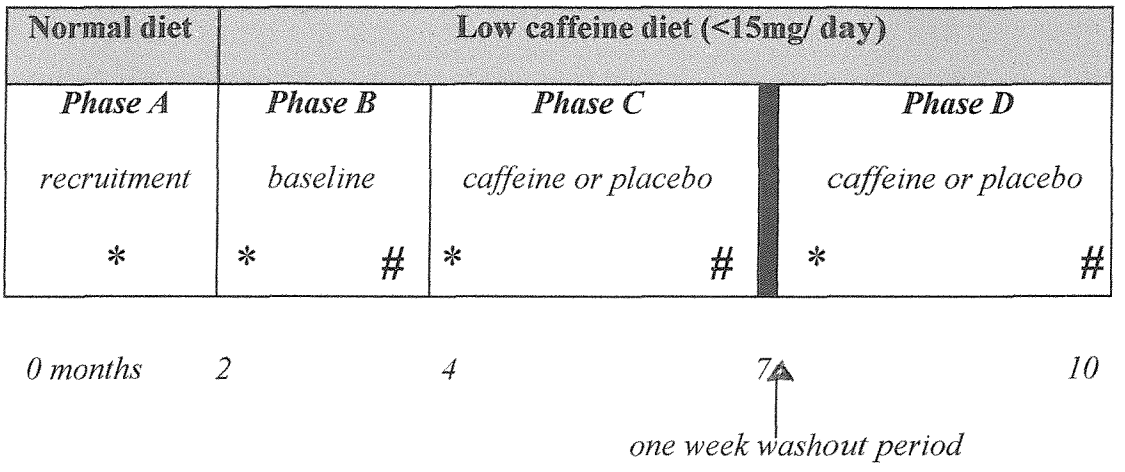
All visits to the Research Unit were scheduled before 1030 hrs, after breakfast and insulin. During phase A patients familiarised themselves with cognitive function testing. Thereafter visits were made two days from the start and during the last seven days of each phase. During phases C and D they took a caffeine/ placebo capsule one hour before the study time. The visits lasted 1 hour. Height, weight and body mass index were measured at the start, with the following measurements made after 15 minutes resting supine:

- Capillary blood glucose, HbA1c (Behring Diagnostics, Milton Keynes, UK) and plasma lipid levels Olympus AU860 autoanalyser (Olympus Optical, Eastleigh, UK).
- Plasma caffeine level.
- Heart rate and sitting blood pressure.
- Middle cerebral artery velocity.
- Cognitive function using a 4-choice reaction time test.

Statistical Analysis

Sample size calculation was based on the effect of caffeine on changes in symptom scores reported previously (Kerr D.et al 1993a). Primary outcomes of interest, highlighted during the planning stage, were frequency of hypoglycemia and effects on intensity of warning symptoms. This two period, placebo/caffeine crossover design was analysed using paired t-tests assuming no carry-over effect (Armitage P. et al 1987). Results are presented as means with standard error or as a difference between the two treatment phases with 95% confidence intervals. A significance level of 5% was used.

Figure V.1.1 The 10 month study schedule.



* = attendance at the research unit 2 days into phase, for clinical measurements

= attendance at the research unit in last week of phase, for clinical measurements

Results

Patient details are shown in Table V.1.1. Following introduction of a low caffeine diet, average caffeine levels were <0.4 ± 0.1mmol/l, with caffeine supplementation levels averaged 2.4 ± 0.3mmol/l 90 minutes after breakfast ($p< 0.0001$). Throughout all phases of the study, HbA1c, plasma lipids and body weight were unaffected by caffeine status.

During phases C and D symptomatic episodes of hypoglycaemia were more frequent with caffeine (1.3 vs. 0.9 episodes per week; 0.4 [0.2 to 0.9], difference [95%CI], $p<0.03$) (Figure V.1.2), associated with more intense warning symptoms (29 vs. 26 for total symptom scores; 3 [0.3 to 5.4], $p<0.05$) (Figure V.1.3). In contrast the number of asymptomatic, biochemical episodes (0.6 ± 0.2 and 0.7 ± 0.2 episodes per week) and the average blood glucose level recorded during a symptomatic event (2.8 ± 0.1 and 2.7 ± 0.1 mmol/l) were unaffected by the prevailing caffeine status. There were 13 episodes of severe hypoglycaemia affecting 4 individuals on placebo compared to 6 episodes among 3 patients whilst they were taking caffeine supplementation ($p = 0.3$). The influence of caffeine on the frequency of hypoglycaemia was strongly influenced by gender with men being more sensitive to caffeine than women ($p<0.02$).

Throughout, diastolic blood pressure and heart rate were not influenced by caffeine status. Among men systolic blood pressure was also unchanged by caffeine (Table V.1.2). For women, re-introduction of caffeine caused a modest pressor response (115 vs. 110 mmHg; 5 [3 to 8] mmHg, $p<0.01$). At all stages V_{MCA} was higher in women than men (Figure V.1.4). With re-introduction of caffeine into the diet V_{MCA} fell ($p<0.001$) although this effect was sustained only in men. 4-choice reaction time improved slightly in both men and women with caffeine supplementation (from 0.55 to 0.54 s; -0.01 [-0.02 to –0.01] s, $p<0.05$).

Figure V.1.2 Total number of symptomatic and biochemical hypoglycaemic events during caffeine and placebo phases of the trial (phases C and D).

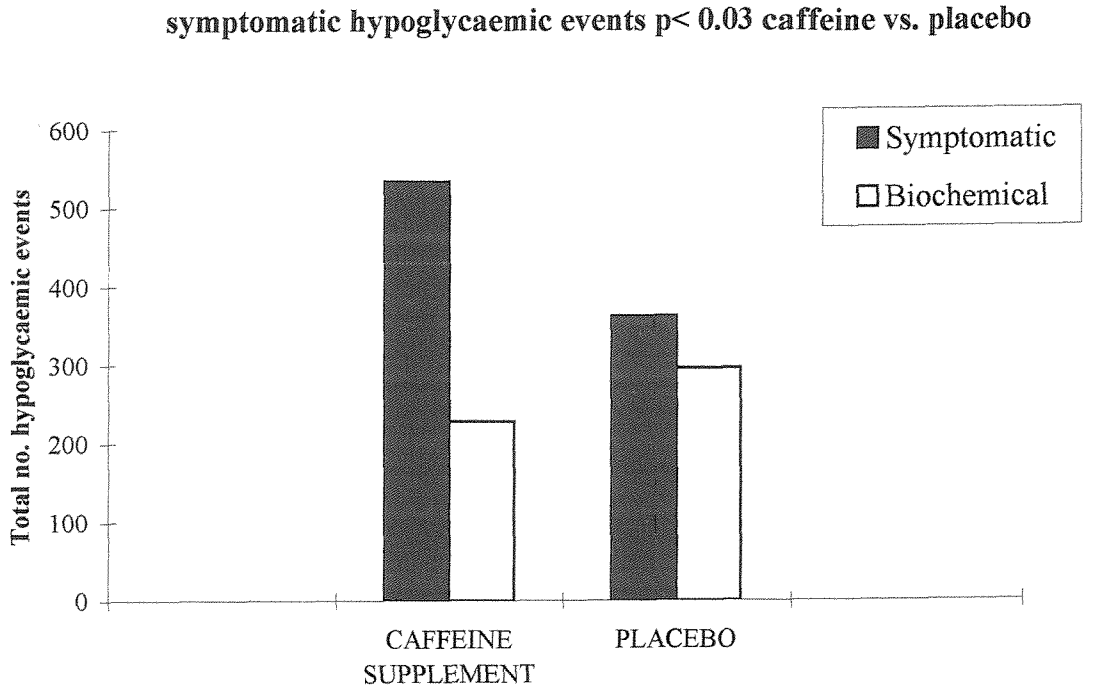


Figure V.1.3 Influence of caffeine on intensity of hypoglycaemic warning symptoms during the caffeine and placebo phases of the trial (phases C and D).

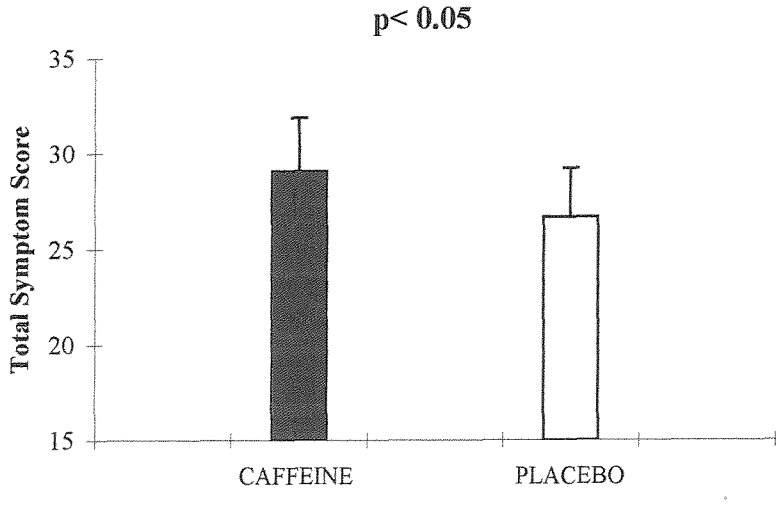
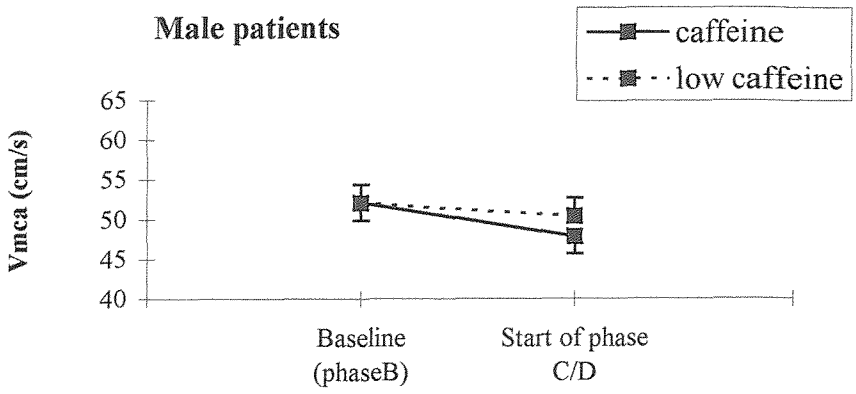


Figure V.1.4 Change in middle cerebral artery blood velocity during caffeine or placebo supplementation compared to baseline



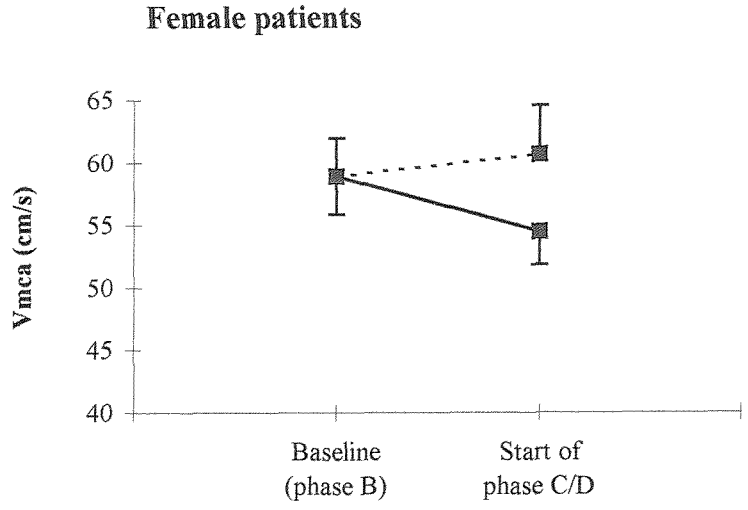


Table V.1.1 Patient details (mean ± se).

	SUBJECTS
Male : Female	22 : 12
Age (years)	38 ± 2
Daily caffeine consumption (mg/ day)	395 ± 37
Duration of diabetes mellitus (years)	15 ± 1.7
$HbA1_c$ at recruitment (%)	7.9 ± 0.2
BMI at recruitment (Kg/m^2)	25.4 ± 0.5

Table V.1.2 Changes in blood pressure and heart rate throughout the study, showing the mean difference between the caffeine and placebo supplementation (mean ± se).

		Base	Average Caff	Average Low Caff	Difference mean [95% CI]
Syst (mmHg)	A	124 ± 3	124 ± 3	121 ± 3	3 [1,4]*
	M	130 ± 4	130 ± 4	128 ± 4	2 [-1,2]
	F	113 ± 4	115 ± 4	110 ± 4	5 [2,7]*
Diastol (mmHg)	A	68 ± 2	69 ± 2	68 ± 2	1 [0,2]
	M	69 ± 3	69 ± 2	68 ± 2	1 [-2,2]
	F	66 ± 3	67 ± 3	68 ± 2	1 [-1,2]
Heart rate	A	71 ± 1	72 ±2	69 ±2	0 [-2,2]
	M	67 ± 2	67 ±3	67 ±2	1 [-3,4]
	F	76 ± 2	72 ±3	73 ±3	-1 [-4,2]

KEY

* = $p < 0.05$ caffeine vs. low caffeine status

A = all subjects M = male subjects F = female subjects

Discussion

In clinical practice hypoglycemia is usually defined as the blood glucose level at which, if not corrected, leads to the development of characteristic warning symptoms and impairment of higher cerebral function. In this study 'free-living' type 1 diabetic patients reported a 44% increase in the number of mild/ moderate hypoglycaemic episodes associated with more intense warning symptoms when caffeine was included within their diet. This was not associated with any adverse effect on diabetic control or plasma lipid levels. Whilst there were fewer severe hypoglycaemic episodes during caffeine use, these were too infrequent for useful statistical comparison.

These findings support previous laboratory based work where acute ingestion of caffeine (in doses equivalent to that in 2-4 cups of drip-brewed coffee) markedly enhanced the symptomatic and sympathoadrenal responses to 'clamped' hypoglycaemia in healthy volunteers and patients with type 1 diabetes (Kerr D.et al 1993a; Debrah K.et al 1996). These effects of caffeine may relate to uncoupling of brain blood flow and glucose utilisation, via antagonism of adenosine receptors (Nehlig A.et al 1992). Ingestion of caffeine acutely reduces brain blood flow, whilst simultaneously augmenting brain glucose utilisation by enhancing firing of cortical neurones (Grome J.et al 1986). Therefore, caffeine ingestion may increase an individual's sensitivity to neuroglycopenia through the combined influence of reducing substrate delivery whilst simultaneously increasing brain glucose metabolism. The reduction in V_{MCA} following caffeine ingestion has been reported previously (Kerr D.et al 1993a) and correlates with changes in cerebral blood flow as measured by Xenon inhalation (Kerr D. et al 1993a). Thus although not directly measured, changes in Vmca are likely to represent alterations in substrate delivery.

Caffeine supplementation did not alter HbA_{1c} or plasma lipid levels. The variable effects of caffeine on glucose tolerance reported previously may be partly explained by the prevailing caffeine status of enrolled subjects (Wachman A. et al 1970, Deakins M. 1939). Giving a single dose of 200mg to a caffeine-naïve individual will modestly elevate blood glucose levels 3- 4 hours after a glucose load without any effect on fasting levels or insulin concentration (Piziol A. et al 1998). Others have reported a strong positive correlation between coffee consumption and cholesterol level (Thelle

D. et al 1983), although this discrepancy may be partly explained by the method of preparing coffee (Urgert R. et al 1997). Under normal circumstances, habitual caffeine consumption causes a smaller pressor response to caffeine than placebo but the effect is not eliminated (James J. 1994). The rise in blood pressure seen here was comparatively modest (systolic only), but could be clinically significant, especially in those whom are already at increased risk of ischaemic heart disease.

The effect of caffeine on hypoglycaemia frequency was influenced by gender with men being more affected than women. In general low blood glucose levels are better tolerated by women (Draelos M.et al 1995) aand detected earlier (Gonder-Frederick L. et al 1994). The effect of caffeine on memory tasks is also influenced by gender (Arnold M.et al 1987). Here reaction time improved slightly with regular caffeine use in all subjects. A review of the effect of caffeine on intellectual performance concluded that the purported positive effects of caffeine on cognition is due to increased arousal and suppression of boredom with repetitive tasks rather than a direct effect on cognitive performances (Nehlig A.et al 1992).

In summary, ingestion of modest amounts of caffeine enhances the intensity of hypoglycaemic warning symptoms in patients with type 1 diabetes without altering their standard of glycaemic control. As a consequence the number of mild/ moderate hypoglycaemic episodes are increased. Although this may be seen as problematic, the enhancement of early warning symptoms allows an individual to be alerted to impending hypoglycaemia early enough to be able to do something about it. Alternatively, given that caffeine did not reduce the number of asymptomatic events and symptomatic episodes were more frequent with caffeine, this could be considered as increasing the risk for severe hypoglycaemia (Kovatchev B.et al 1998) but fortunately this was not seen here.

Section VI LABORATORY STUDIES OF CAFFEINE AND HYPOGLYCAEMIA IN HEALTHY VOLUNTEERS

Study 2 Influence of caffeine on symptomatic, hormonal and cognitive responses during and following recovery for hypoglycaemia

Although caffeine is recognised as having psychostimulant effects, its use is now so widespread (Barone J.et al 1996) that it is essentially viewed as a socially acceptable drug. The effects of caffeine on intellectual performance, blood pressure and lipids have been extensively investigated and may be relevant to people with type 1 diabetes mellitus who are at increased risk of macrovascular disease.

In addition, acute ingestion of modest amounts of caffeine augments the hormonal and symptomatic responses to hypoglycaemia in both healthy volunteers and patients with type 1 diabetes mellitus, (Kerr D.et al 1993a; Debrah K.et al 1996). However both of these studies involved subjects withdrawing from caffeine for 72 hours prior to testing. Thus the response obtained may represent relief from the syndrome of caffeine withdrawal rather than acute effect of caffeine per se (James J. 1994; Rogers P.et al 1998). Studying the response to a caffeine challenge after overnight deprivation mimics usual patterns of caffeine consumption.

The brain is almost unique, in that it cannot manufacture nor store significant amounts of the substrate required for normal function (i.e. glucose) and thus is dependent upon a continuous supply from the peripheral circulation for normal function. Although the brain can utilise alternative agents such as ketone bodies, this is likely to occur under extreme conditions only, such as prolonged starvation (Owen O. et al 1967). Previous studies have suggested that global cerebral blood flow (CBF) does not increase during hypoglycaemia (to increase substrate delivery) until peripheral glucose levels have fallen to less than 2.2 mmol/l (Tallroth G.et al 1992; Powers W.et al 1996). However, one study has suggested that following recovery from hypoglycaemia CBF increased, even though the blood glucose level achieved during hypoglycaemia did not cross this threshold (Eckert B.et al 1998). This suggests that the duration of any hypoglycaemic stimulus is likely to be important in determining physiological and cognitive responses.

The study reported here also investigated the effect of visual function during hypoglycaemia with the added effect of caffeine. There are reported specific effects of hypoglycaemia on visual function. P300 amplitude was decreased during a visual search task conducted with a blood sugar of 2.5 mmol/l, whereas auditory P300 amplitude was unaffected by such a reduction (Lindgren M.et al 1996). Other changes include deterioration in the early stages of visual information processing, deterioration in static contrast and impairment of colour vision (McCrimmon R.et al 1996). P100 (the first major positivity recorded over the occipital cortex following stimulation with a reversing checkerboard pattern) may be prolonged during hypoglycaemia (Harrad R.et al 1985). Both type 1 diabetic patients and controls were studied, the changes were significant in both groups separately and as a whole. However a similar study reported no significant changes in visual sensation with blood glucose decreased to 2.38 mmol/l (Tamburrano G.et al 1988).

Previous studies have also given conflicting results as to the influence of caffeine on electrophysiological function during hypoglycaemia. An increase in latency of the auditory oddball P300 (blood glucose 2.8 mmol/l) after caffeine ingestion was found, with no similar change in latency observed in the placebo condition (Kerr D.et al 1993a). This study was conducted on healthy volunteers, whereas no such effect was demonstrated in patients with type 1 diabetes (Debrah K.et al 1996). The P300 provides little information about the specific cognitive processes affected by hypoglycaemia or by caffeine. To investigate the effects of caffeine further, it is necessary to examine its effects on more specific cognitive processes.

Thus the aim of this study was twofold. Firstly it was to examine the influence of acute caffeine ingestion on the symptomatic, hormonal and cognitive responses both during and upon recovery from hypoglycaemia in subjects who had only abstained from caffeine overnight. Secondly, assessment of the visual system could provide a sensitive measure of the effects of caffeine, if indeed caffeine has an effect over and above that of hypoglycaemia. The current study used tests of visual information processing (McCrimmon R.et al 1996) to examine the simultaneous effects of caffeine and reduced blood glucose in combination with pattern reversal evoked potentials.

Methods

Subjects

16 healthy, non-diabetic subjects (8 male, aged 21 to 37 years) gave written, informed consent for the study. All subjects consumed caffeine on a daily basis and none had any relevant previous medical history, or were taking any regular medication. They were right handed except for one male subject and of above average intelligence as assessed by the National Adult Reading Test (Nelson H.et al 1991). Each subject was informed that they would be required to attend the department on four separate occasions. The first visit was to familiarise subjects with the tests and order of testing that would be used during the experimental condition. This helped to minimise practise and learning effects. The results from this session were discarded. On the subsequent three occasions subjects underwent a hyperinsulinaemic glucose clamp. Each study was performed at least seven days apart to avoid any carry-over effect.

Experimental procedure

Subjects were studied at the Defence Evaluation and Research Agency, Farnborough, UK. They were admitted at 09.00, having had a light breakfast at 07.00 but abstained from caffeine consumption from 21.00, the previous evening. Cannulae were inserted in preparation for the glucose clamp procedure as described in the methods section.

The three glucose clamps took place in a counterbalanced manner. Initially euglycaemia was maintained for 90 minutes (4.5 mmol/l) at the end of which 200 mg of caffeine or matched placebo was ingested (Figure VI.2.1). Thereafter blood glucose was either lowered to 2.5 mmol/l over 20 minutes and held there for a further 70 minutes in two of the conditions (hypoglycaemia with caffeine, H+C or hypoglycaemia with placebo, H+P). In the third condition 200 mg caffeine was given and euglycaemia maintained throughout the study (E+C study). At $^{+}$180 minutes in all conditions the insulin infusion was turned off and the glucose infusion was increased as necessary to restore euglycaemia over 20 minutes. Blood sugar was then held at 4.5 mmol/l until the end of the experiment (70 minutes later). Subjects were not informed of their blood glucose level at anytime during the laboratory sessions.

For electrophysiological recording, electrodes were fixed to the scalp according to the International 10-20 system (Jasper H. 1958). A further chain of five electrodes was positioned 5cm above the inion, extending 10cm to the left and right of the midline. Activity at all sites was referred to linked earlobes. Electrodes were also positioned around the eyes to monitor eye movements. A ground electrode was located mid-forehead.

At $^{+}20$ (euglycaemia), $^{+}120$ (hypoglycaemia/ euglycaemia) and $^{+}210$ (euglycaemia) minutes the following measurements were made:

- Left and right middle cerebral artery blood velocity (V_{MCA}),
- Blood pressure and heart rate,
- Plasma counterregulatory hormones and caffeine levels,
- Hypoglycaemic symptom questionnaire

The above tests were repeated after the following psychometric tests had been performed.

Tests of visual function

Visual change detection The different time intervals between the presentation of the array and the onset of the change are 14, 28, 42, 56 and 70 ms. The whole test involves 10 trials of the 5 different stimulus duration.

Visual movement detection The interval between the onset of the array and the target rectangle appearing to move are identical to VCD (i.e. 14-70ms). A random block of 50 presentations (10 trials of 5 different stimulus duration) is also employed in this test.

Pattern reversal visual evoked potentials (VEP)

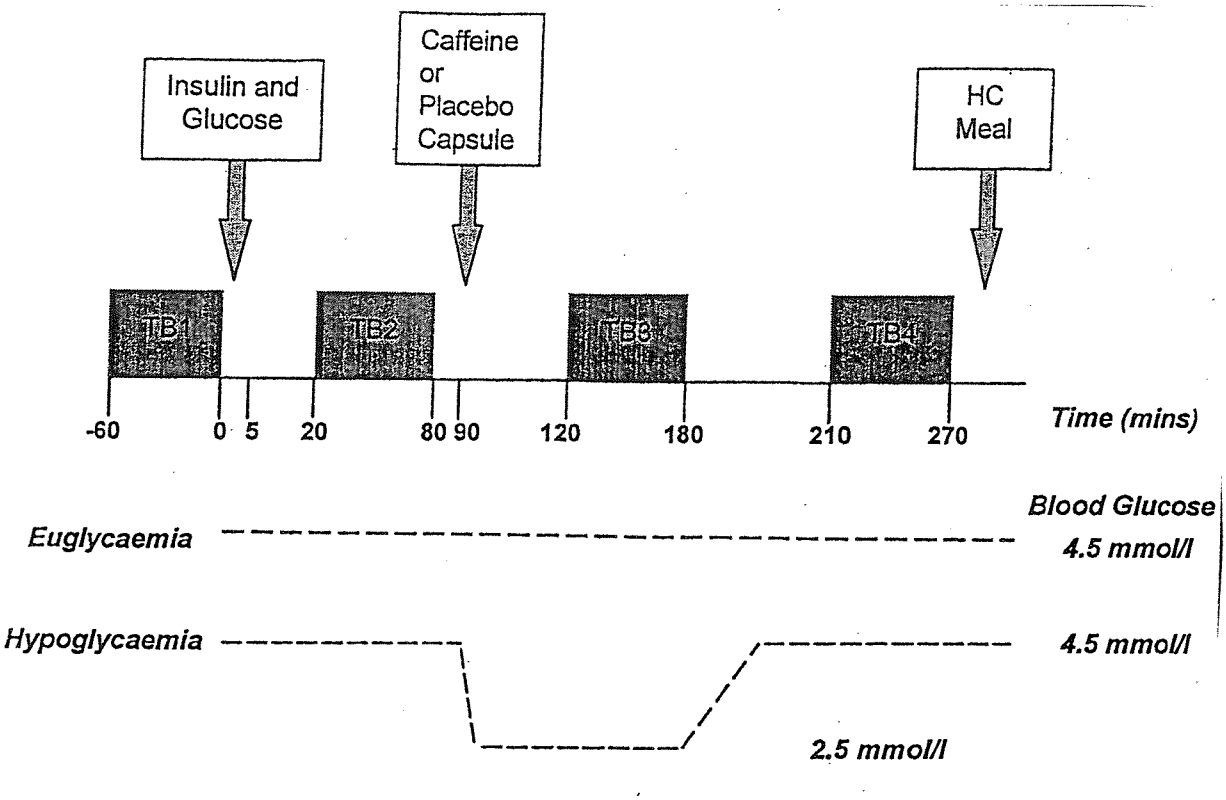
The subject was seated 1m from a visual display screen which displayed a black and white, full-field checkerboard which subtended 16° at the eye. Individual checks subtended 50° visual angle and the screen was viewed binocularly. The checkerboard reversed twice per second and averages were computed from responses to two sets of 100 reversals during each test battery. The sampling rate was 2000 samples per second

across a 200msec epoch. The high pass filter was set to 1 Hz and the low pass filter to 500 Hz. Baseline measurement of VEP were made prior to the start of the insulin infusion.

Statistical Analysis

Overall differences between serial measurements were examined by repeated measures ANOVA with factors condition (E+C, H+P and H+C) and time. In addition pairwise comparisons were also carried out. Comparison of euglycaemia and H+C examined the direct effects of hypoglycaemia. Whilst the effects of caffeine during hypoglycaemia were examined by comparing H+P and H+C. The Greenhouse-Geisser correction was used where appropriate. Data are shown as mean ± se.

Figure VI.2.1 Schematic representation of the study.



TEST BATTERY= BP, HR, V_{MCA} symptom score, VCD,VMD, BP, HR, symptom score, V_{MCA}

Results

Achieved blood glucose and plasma caffeine levels are shown in Tables VI.2.1 and VI.2.2 respectively. Following the ingestion of 200 mg caffeine, there was a reduction in V_{MCA} in both the caffeine conditions, although this was more marked in the H+C condition (Figure VI.2.2) ($F= 3.7$, $p< 0.001$ all conditions across time; $F= 3.5$, $p< 0.005$ H+C vs. P+C; $F = 3.5$, $p< 0.005$ H+C vs. E+C). However, there was also an interaction between V_{MCA} and gender. Although both sexes were similarly affected by caffeine ingestion during hypoglycaemia, V_{MCA} rose above baseline only in females during H+P ($F= 5.9$, $p< 0.02$, H+C vs. H+P). As expected, systolic pressure rose and diastolic pressure fell during hypoglycaemia, no effects of caffeine was demonstrated (Figure VI.2.3). Heart rate was unchanged throughout the study.

With the exception of noradrenaline, counteregulatory hormone levels were similar prior to the ingestion of caffeine or placebo (Figure VI.2.4). During hypoglycaemia, adrenaline production was greater in H+C ($F = 30.0$, $p< 0.001$ all conditions across time; $F= 4.98$, $p< 0.005$, H+C vs. H+P across time). In contrast noradrenaline rose by a similar amount during hypoglycaemia, when the initial rise prior to hypoglycaemia was corrected for. Growth hormone (GH) responses were significantly different between the two hypoglycaemic conditions, with sustained higher levels of GH during recovery in H+C ($F= 8.6$, $p< 0.0001$ all conditions across time; $F= 4.88$, $p< 0.005$ H+C vs. H+P across time) (Figure VI.2.4). The adrenaline responses during hypoglycaemia were consistently greater in males ($F= 7.97$, $p< 0.005$).

During euglycaemia, symptom scores were unaffected by caffeine (Figure VI.2.5) whereas total symptoms were more intense during hypoglycaemia and recovery following caffeine ingestion (total symptom score H+C vs. H+P $F= 4.2$, $p< 0.05$). This was most marked with neurogenic symptoms ($F=6.33$, $p< 0.02$). Overall total symptoms were more intense in females than males (H+C vs. H+P, $F= 4.96$, $p< 0.03$).

Change in intellectual performance (VCD and VMD) is shown in during hypoglycaemia (Figure VI.2.6). In VCD neither absolute nor difference scores changed across the conditions (Table VI.2.3). There was no difference in the absolute scores for

VMD across the conditions. However the change in VMD scores showed an effect of condition ($F= 4.1$, $p< 0.03$), such that the direction of change in performance was different across conditions. There was an improvement in performance during euglycaemia but not during either hypoglycaemic condition. There was no effect of caffeine between the two hypoglycaemic conditions but only H+C prevented an improvement in performance similar to that seen in the euglycaemic condition ($F= 5.7$, $p< 0.05$).

There was an interaction during the visual information processing tasks between performance and gender with females scoring more highly than males ($F = 24.36$, $p= 0.001$). This difference was greatest for VMD scores. Further analysis showed that there was a trend towards poorer performance in VMD for males as a result of hypoglycaemia ($F= 3.48$, $p= 0.059$).

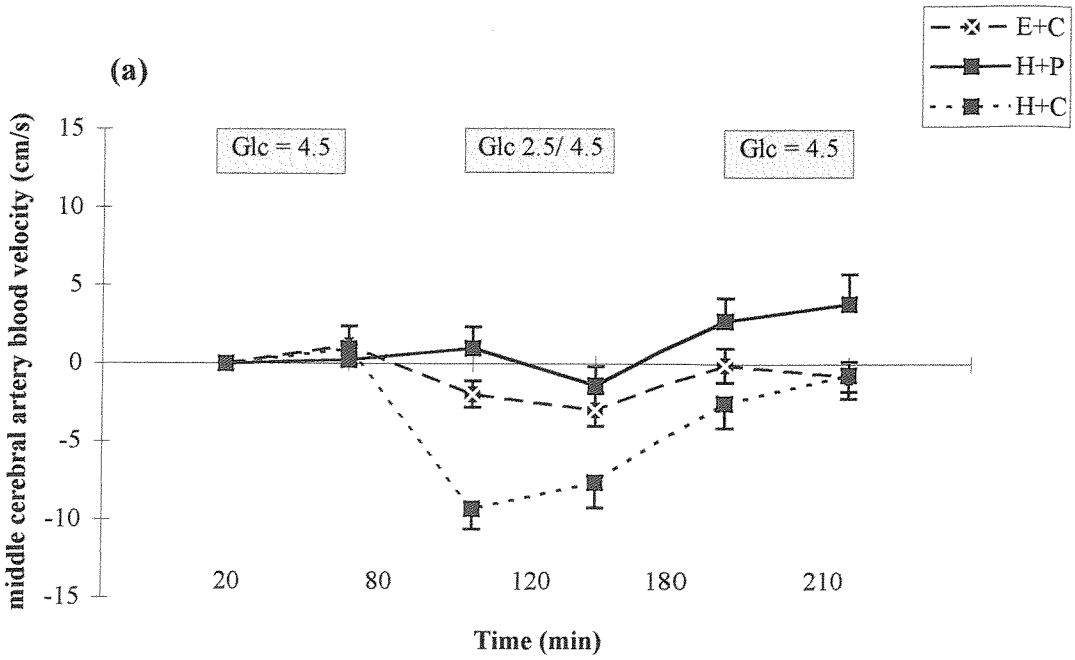
Increase in latency for P100 was observed in both hypoglycaemic conditions ($F = 3.55$, $p< 0.046$), there was no specific effect of caffeine (Figure VI.2.7, Table VI.2.4). Considerable inter-subject variability in the latency of P100 was noted during the third test battery, at which point hypoglycaemia had been induced. Subjects were subsequently divided into 2 groups by a median split based on the change in P100 latency from TB1 (initial baseline state) to TB3 (hypoglycaemia) during H+P condition. The mean latency increase was 0.5ms for Group 1 and 5.6ms for Group 2 (Figure VI.2.8). There was no difference between these groups in terms of either gender or age. A group by condition interaction was also observed for P100 amplitude between E+C and H+C ($F = 8.06$, $p< 0.02$). P100 latency showed a group by condition by time effect between E+C vs. H+C ($F= 4.43$, $p< 0.02$) and a group by condition effect between H+P vs. H+C ($F = 7.19$, $p< 0.03$).

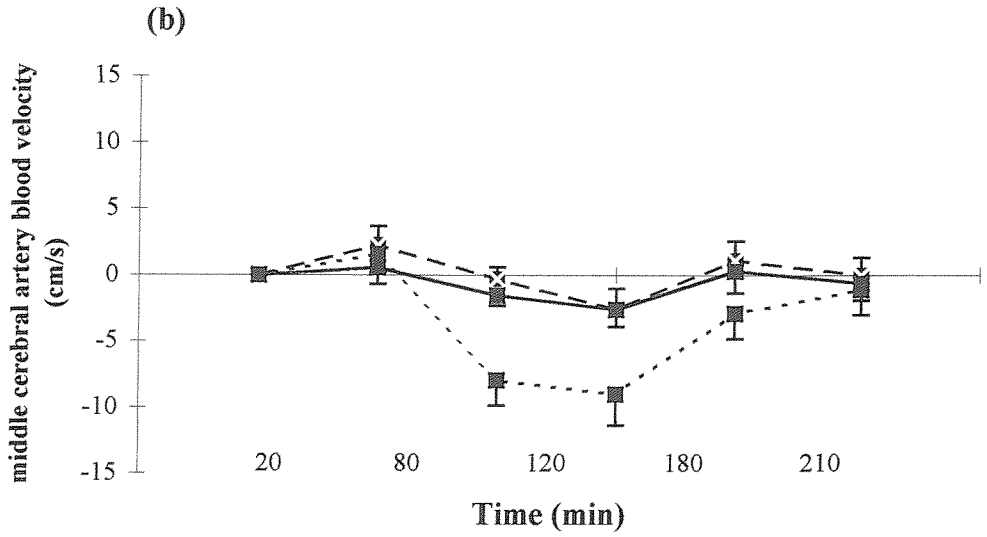
The subjects who showed the largest P100 latency increase during hypoglycaemia (Group 2) were also the subjects who showed the most significant deterioration in performance of the VMD task ($F= 8.98$, $p< 0.02$, Figure VI.2.9). This deterioration was not reversed during the recovery period.

A comparison of TB2 to TB4 (controlled euglycaemia to recovery) showed that hypoglycaemia produced an increase in P100 latency ($F= 6.26$, $p< 0.01$). In addition P100 amplitude was decreased in H+C vs. H+P ($F= 3.52$, $p< 0.05$). A median split was also carried out based on the P100 latency change between TB2 (controlled euglycaemia) and TB3 (hypoglycaemia) during the H+P condition. The mean delay in latency was 0.4ms Group 1 and 5.7ms Group 2 (Figure VI.2.10): there were no differences between these groups in terms of gender or age. There were no changes in P100 latency between groups in the analysis of condition pairs. A summary of the results of VEP analyses is shown in Table VI.2.5.

VEP data from TB1 to TB2 were compared in order to investigate the differences between the analyses of TB1 to TB4 and the analyses of TB2 to TB4. The correlation between the change in blood glucose concentrations and the change in VEP component latency was computed between TB1 and TB2. A negative correlation was found between the change in blood glucose concentration and P100 latency for the caffeine condition across all subjects ($r= -0.54$, $p< 0.05$). No correlation was found between the changes in blood glucose, change in VEP component latency and subsequent allocation to median group.

Figure VI.2.2 Change in middle cerebral artery blood velocity during the three study conditions (a) all subjects (b) males (c) females: mean ± se





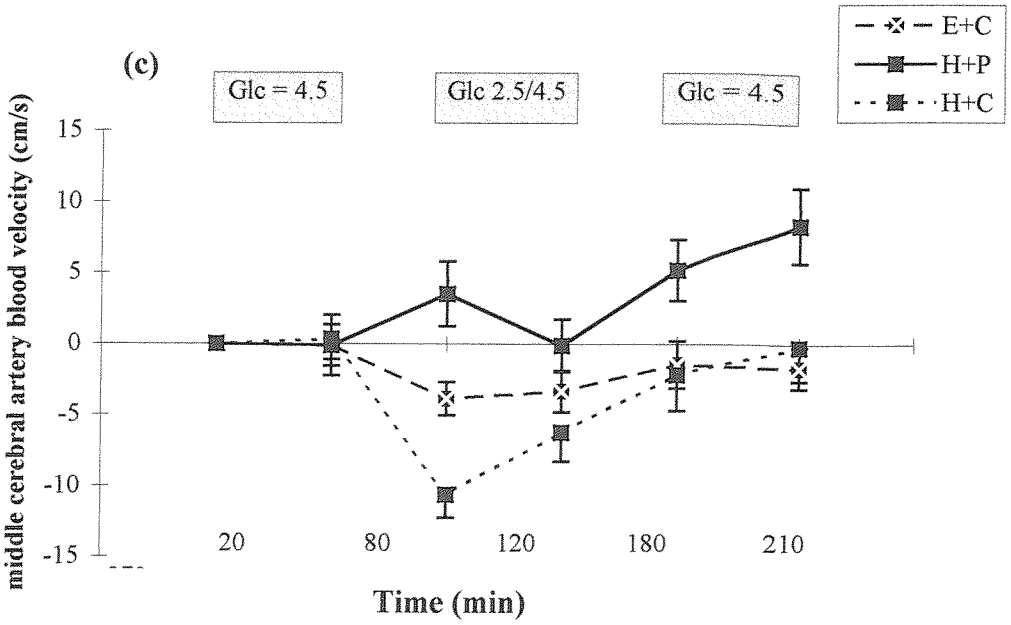
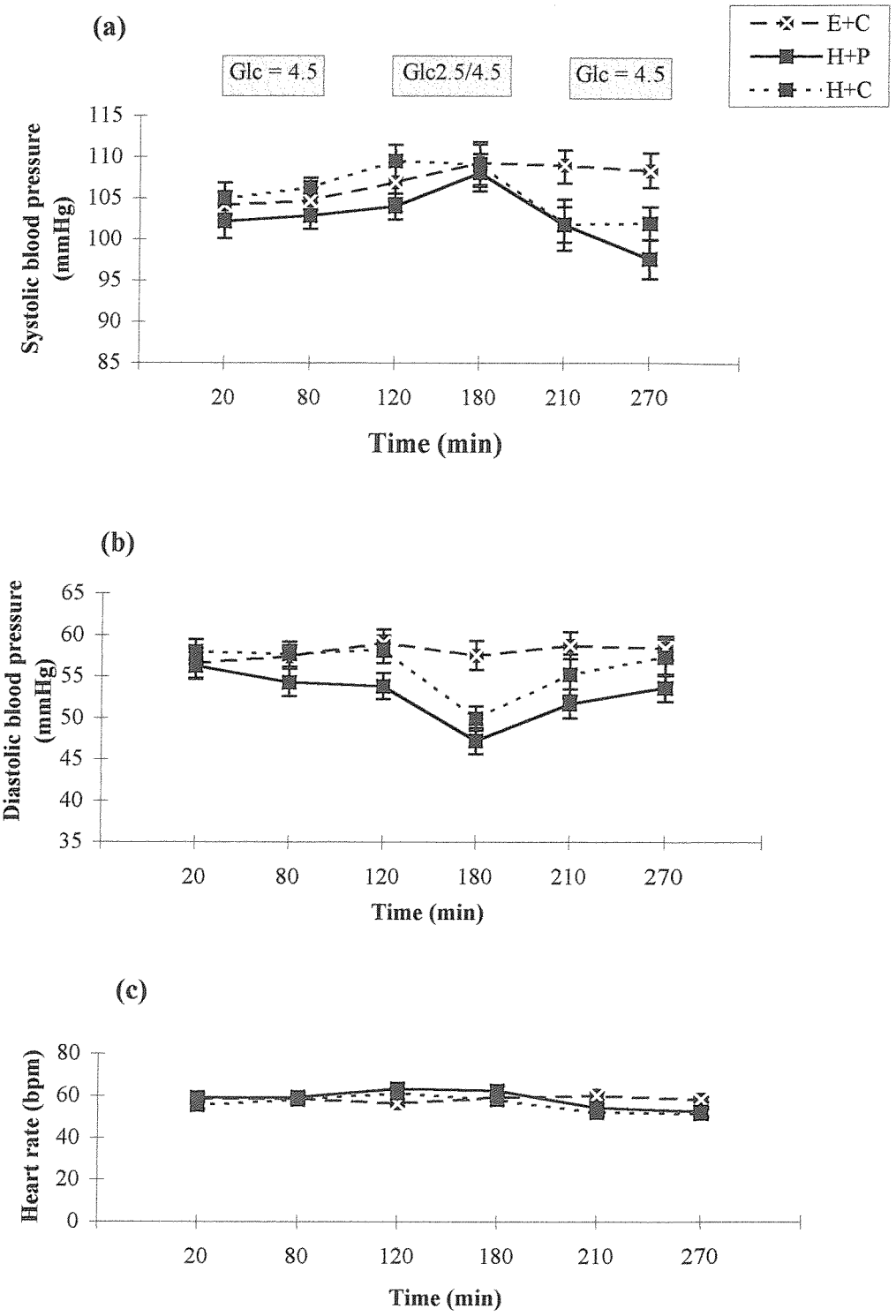

(c)
Glc = 4.5
Glc 2.5/4.5
Glc = 4.5
E+C
H+P
H+C
middle cerebral artery blood velocity (cm/s)
15
10
5
0
-5
-10
-15
20
80
120
180
210
Time (min)

Figure VI.2.3 Cardiovascular responses during the three study conditions (a)systolic blood pressure (b) diastolic blood pressure (c) heart rate: mean ± se



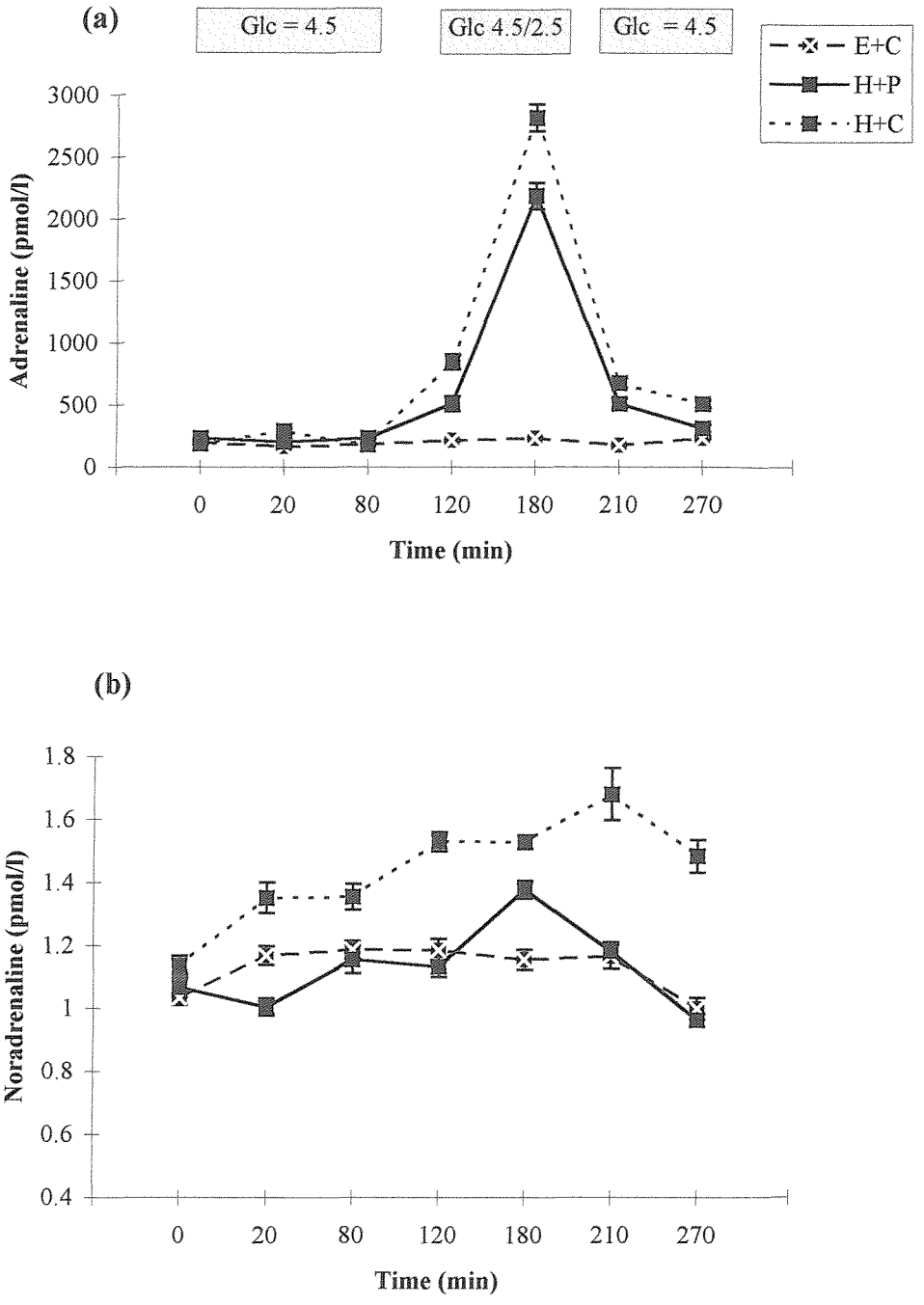


Figure VI.2.4 Counterregulatory hormone concentrations during the three study conditions (a) adrenaline, (b) noradrenaline (c) growth hormone: mean ± se

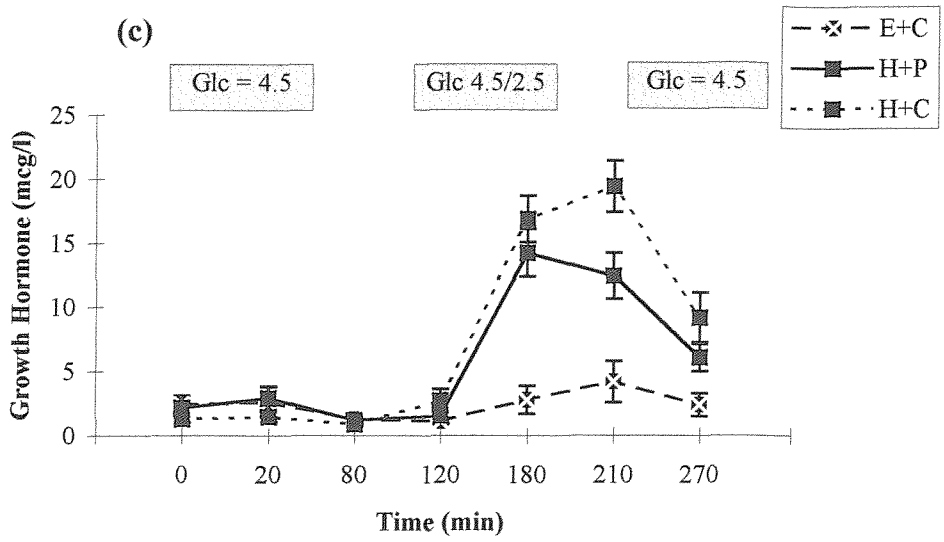
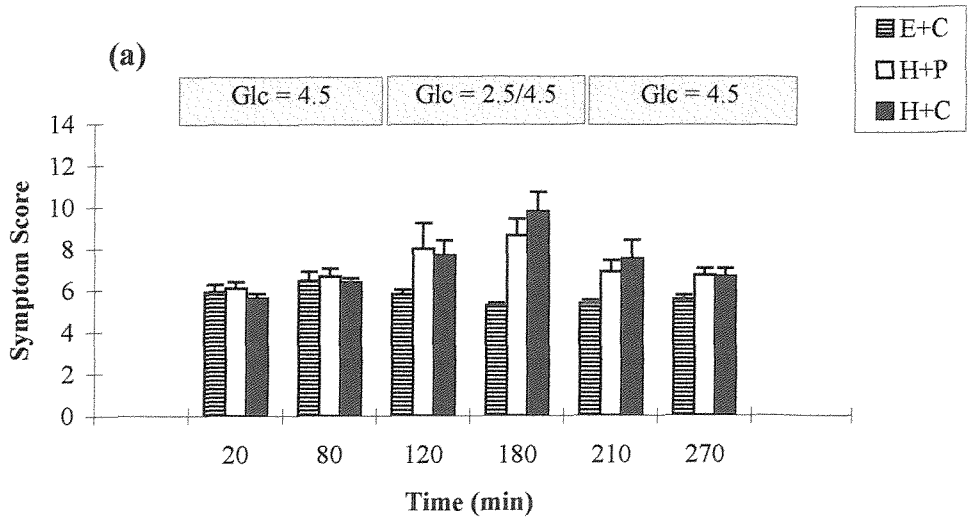
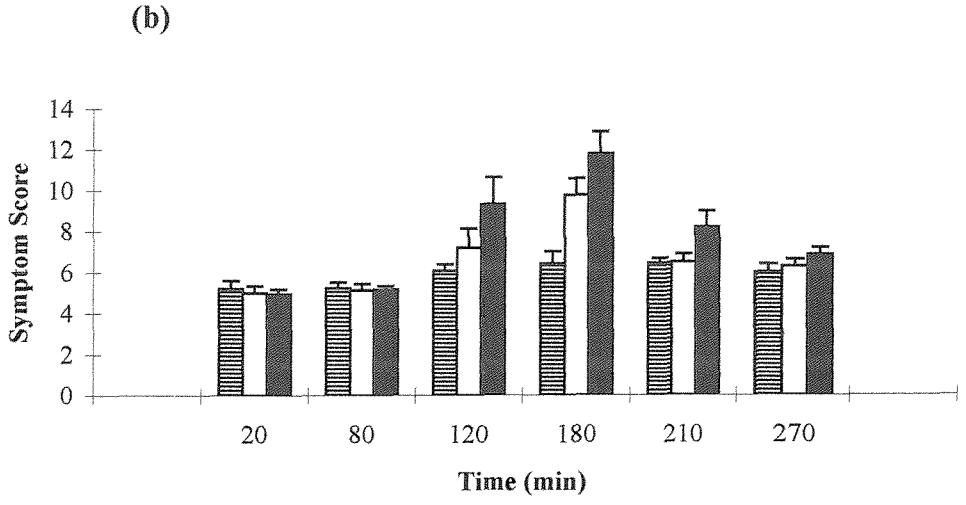

(c)
Glc = 4.5
Glc 4.5/2.5
Glc = 4.5
E+C
H+P
H+C
Growth Hormone (mcg/l)
25
20
15
10
5
0
0
20
80
120
180
210
270
Time (min)

Figure VI.2.5 Symptoms during the three study conditions

(a) neuroglycopenic, (b) neurogenic, (c) other symptoms: mean ± se





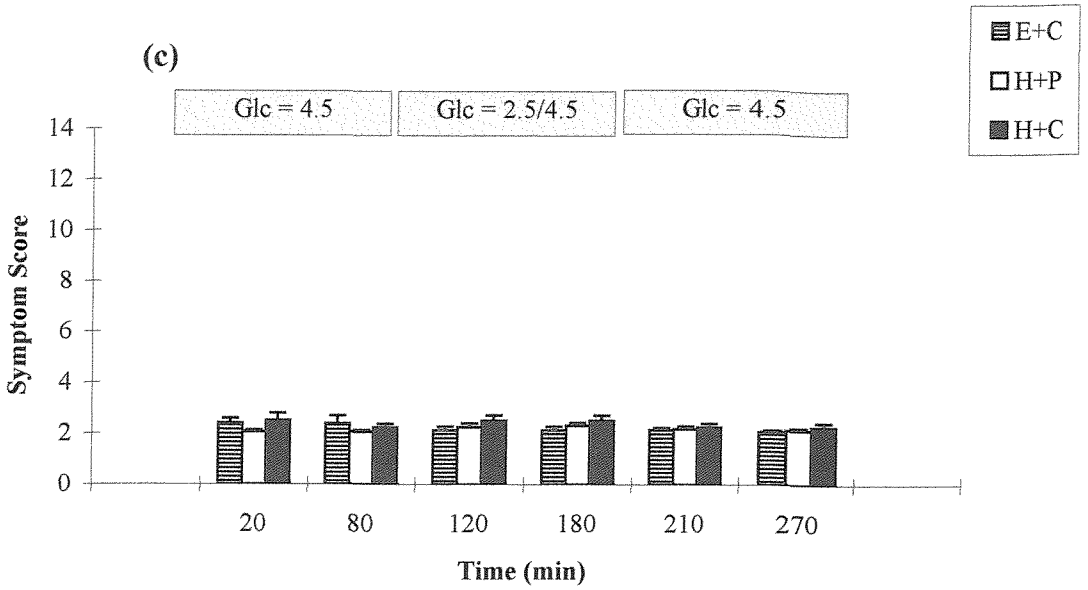
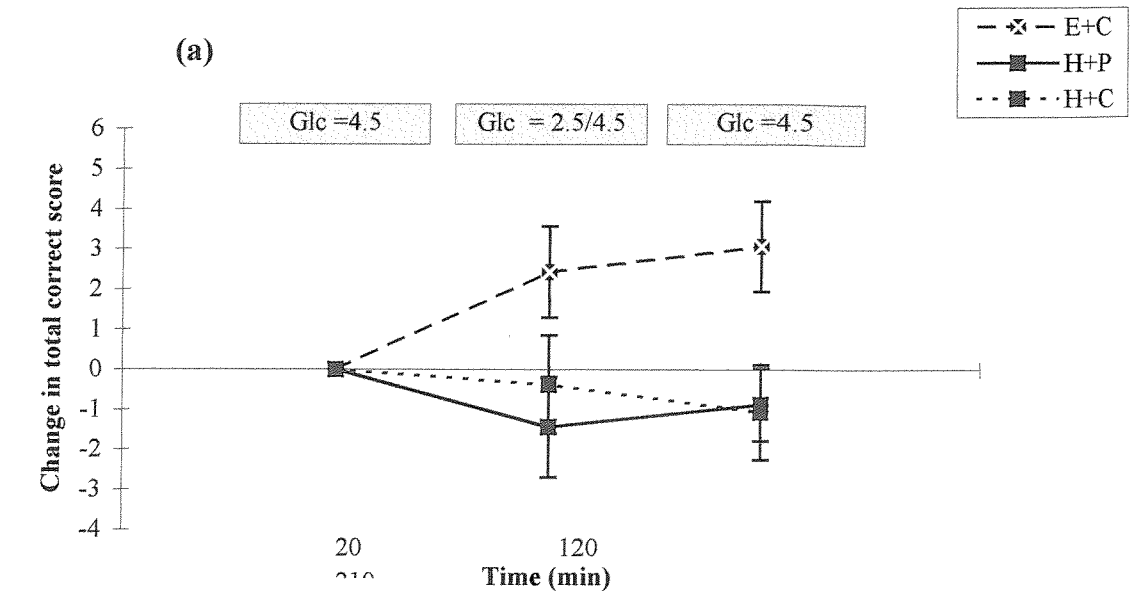

(c)
Glc = 4.5
Glc = 2.5/4.5
Glc = 4.5
E+C
H+P
H+C
Symptom Score
14
12
10
8
6
4
2
0
20
80
120
180
210
270
Time (min)

Figure VI.2.6 Change from baseline in visual perception tests (a) VCD (b) VMD: mean ± se



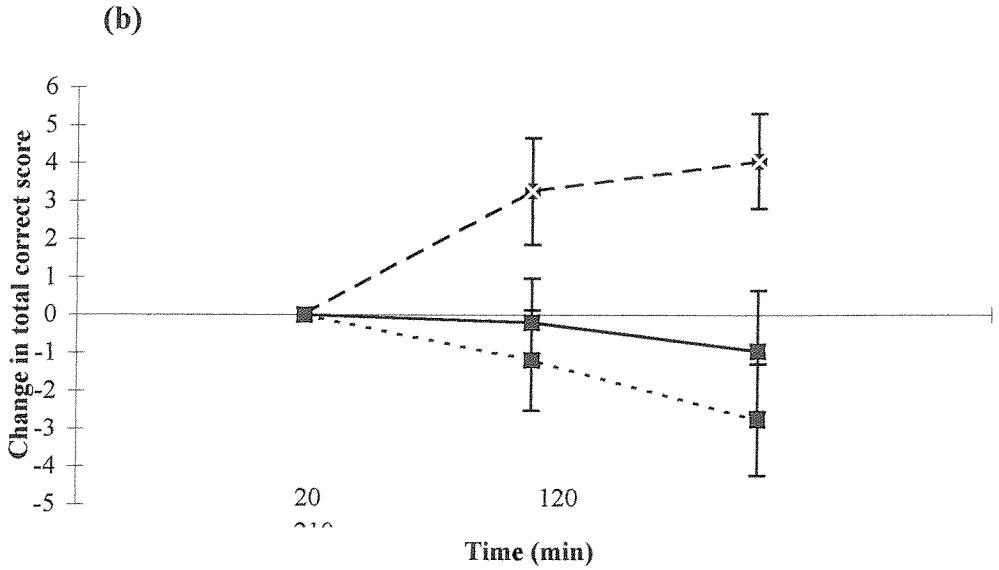


Figure VI.2.7 Group averaged VEPs at Oz, comparing runs 2,3 and 4 to run 1

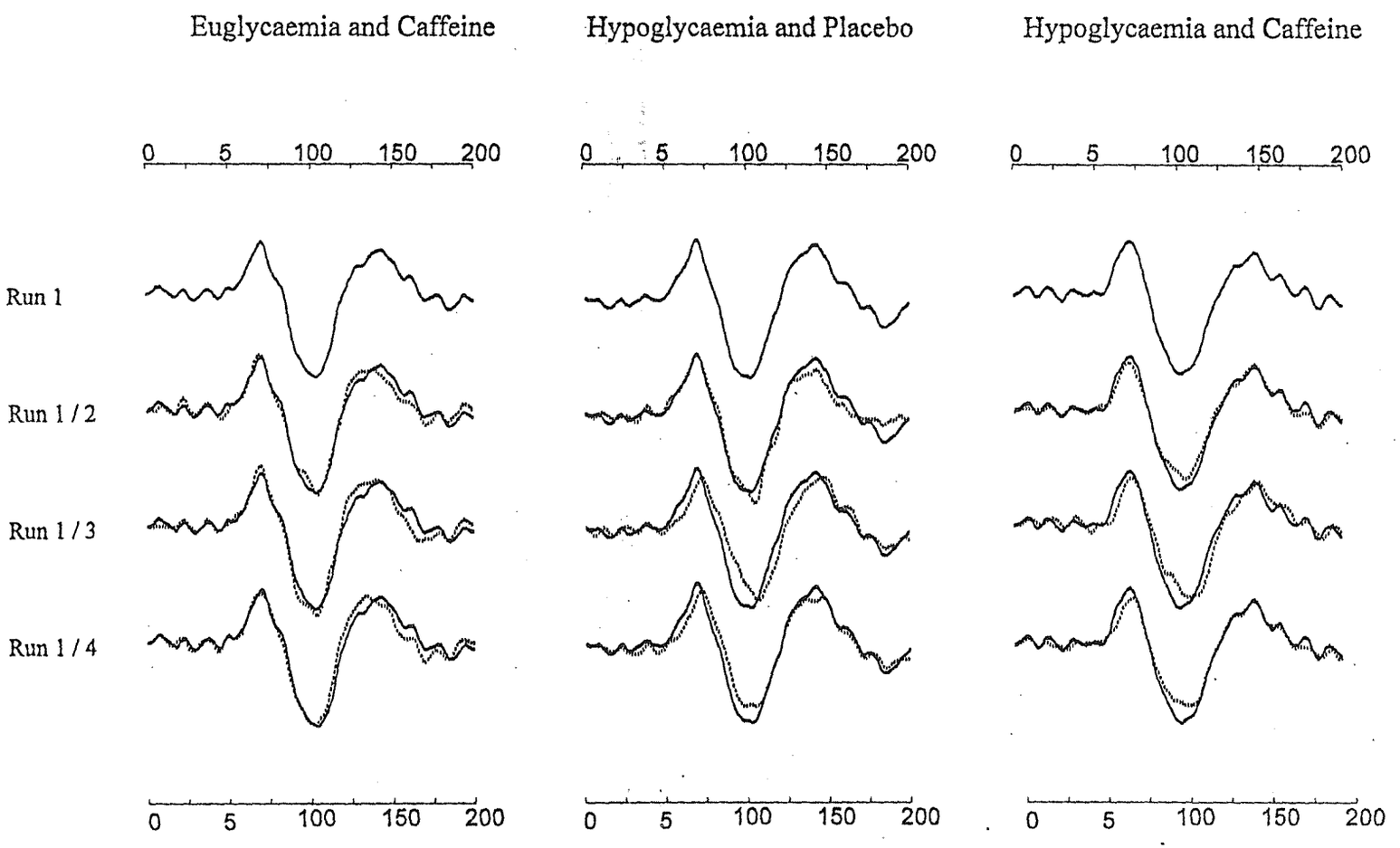


Figure VI.2.8a Comparison of VEPs from rest at Oz, for Group 1 (based on change in P100 latency between runs 1 and 3 in H+P condition).

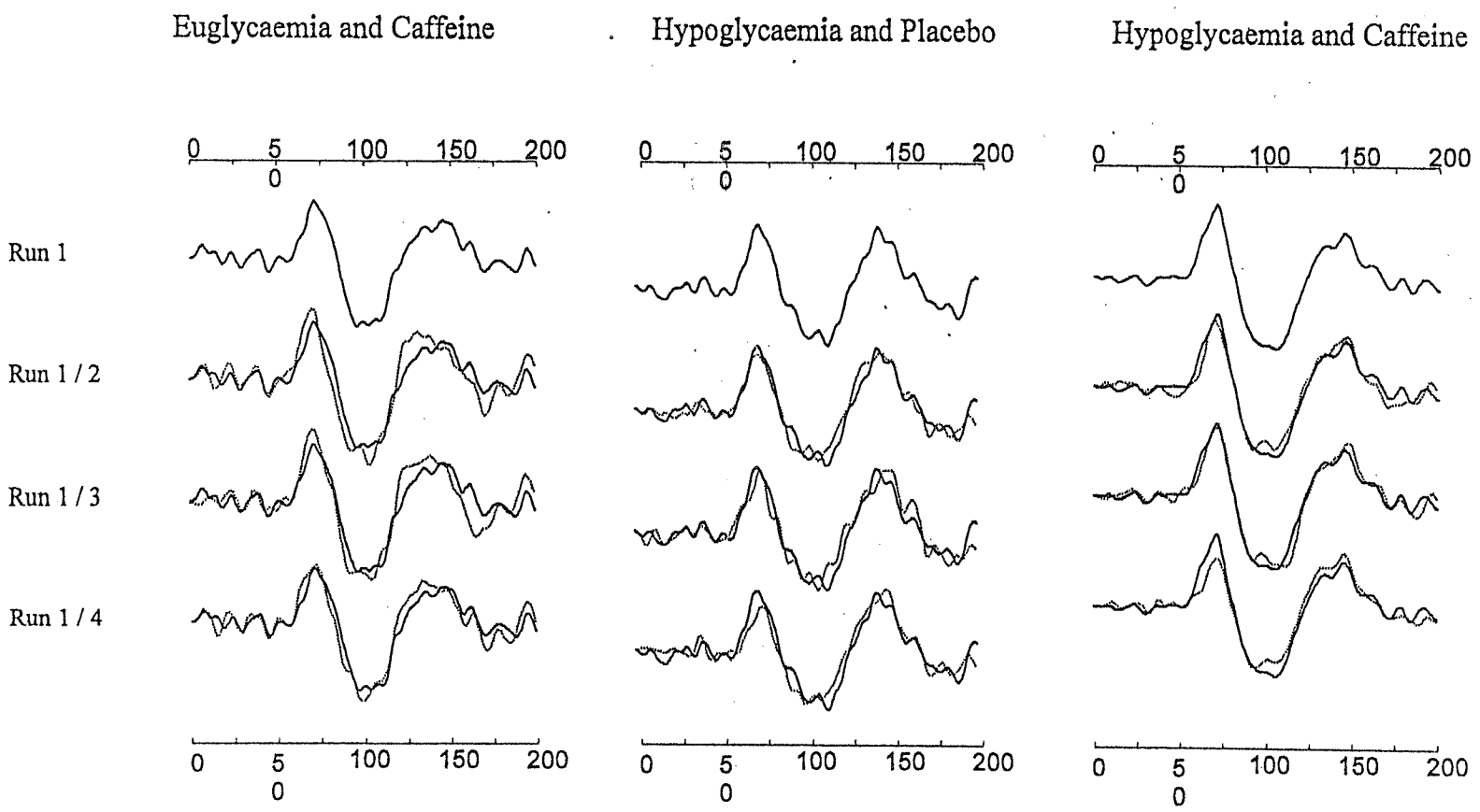


Figure VI.2.8b Comparison of VEPs from rest at Oz, for Group 2 (based on change in P100 latency between runs 1 and 3 in H+P).

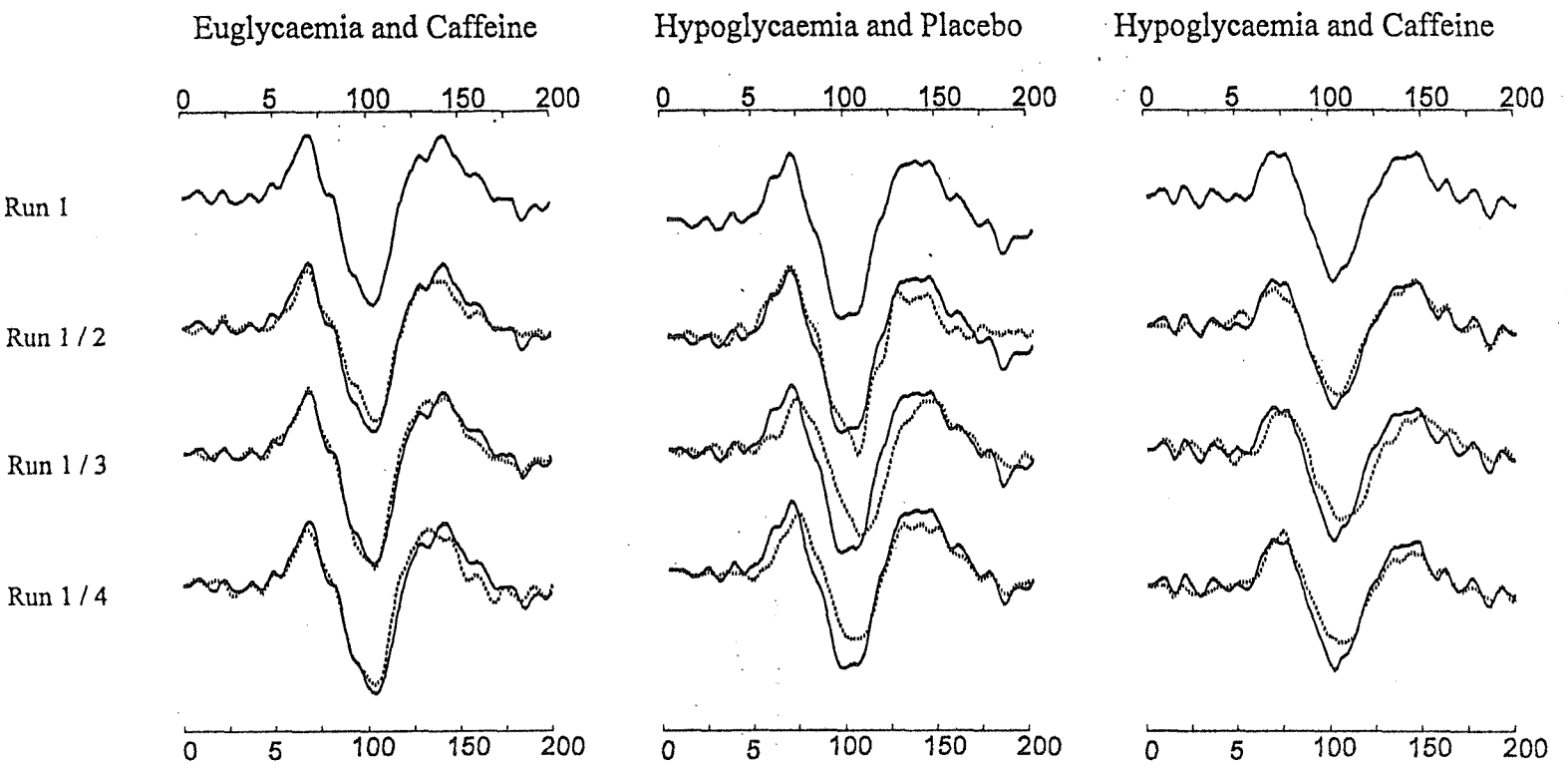
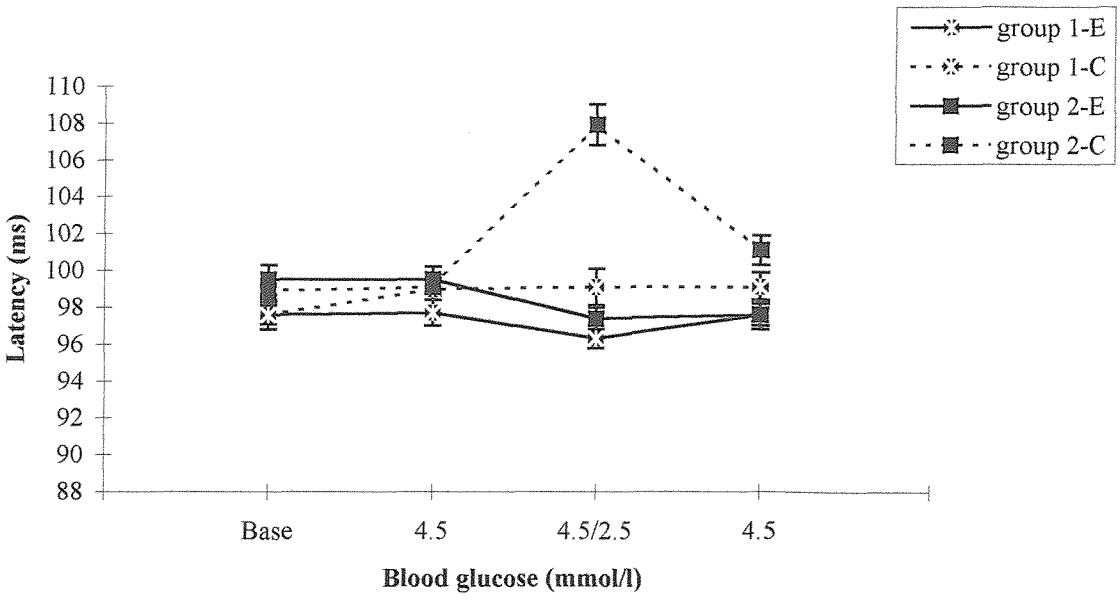


Figure VI.2.9 Change in P100 latency between median groups (based on change in P100 latency between runs 1 and 3 in H+P)



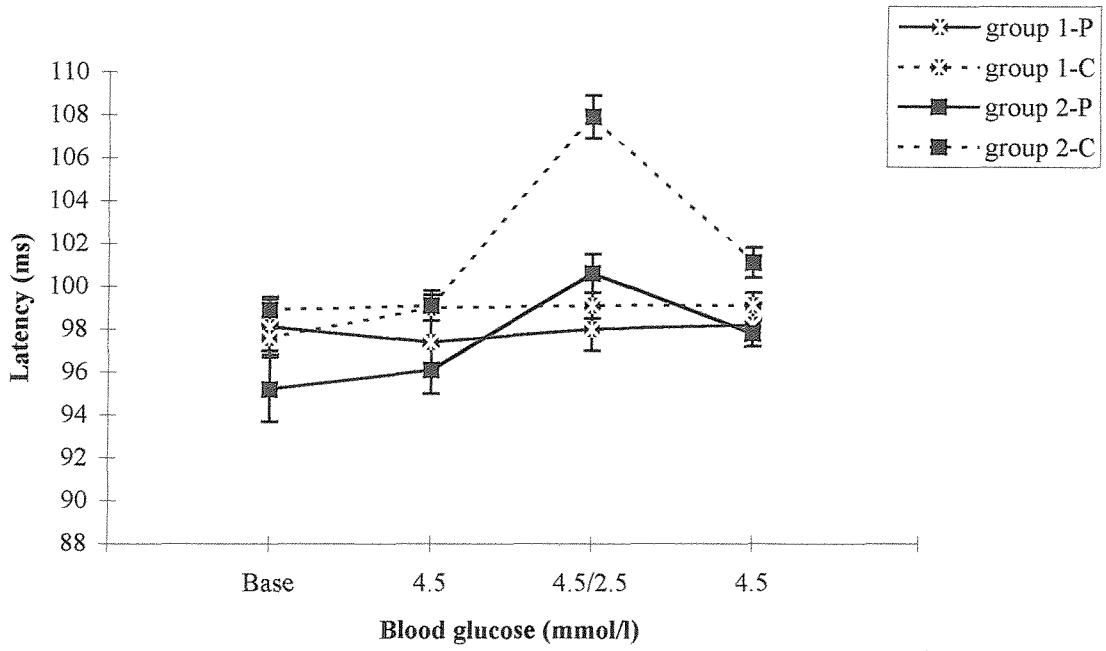


Figure VI.2.10 Change in visual movement detection between median groups (based on change in P100 latency between runs 1 and 3 in H+P)

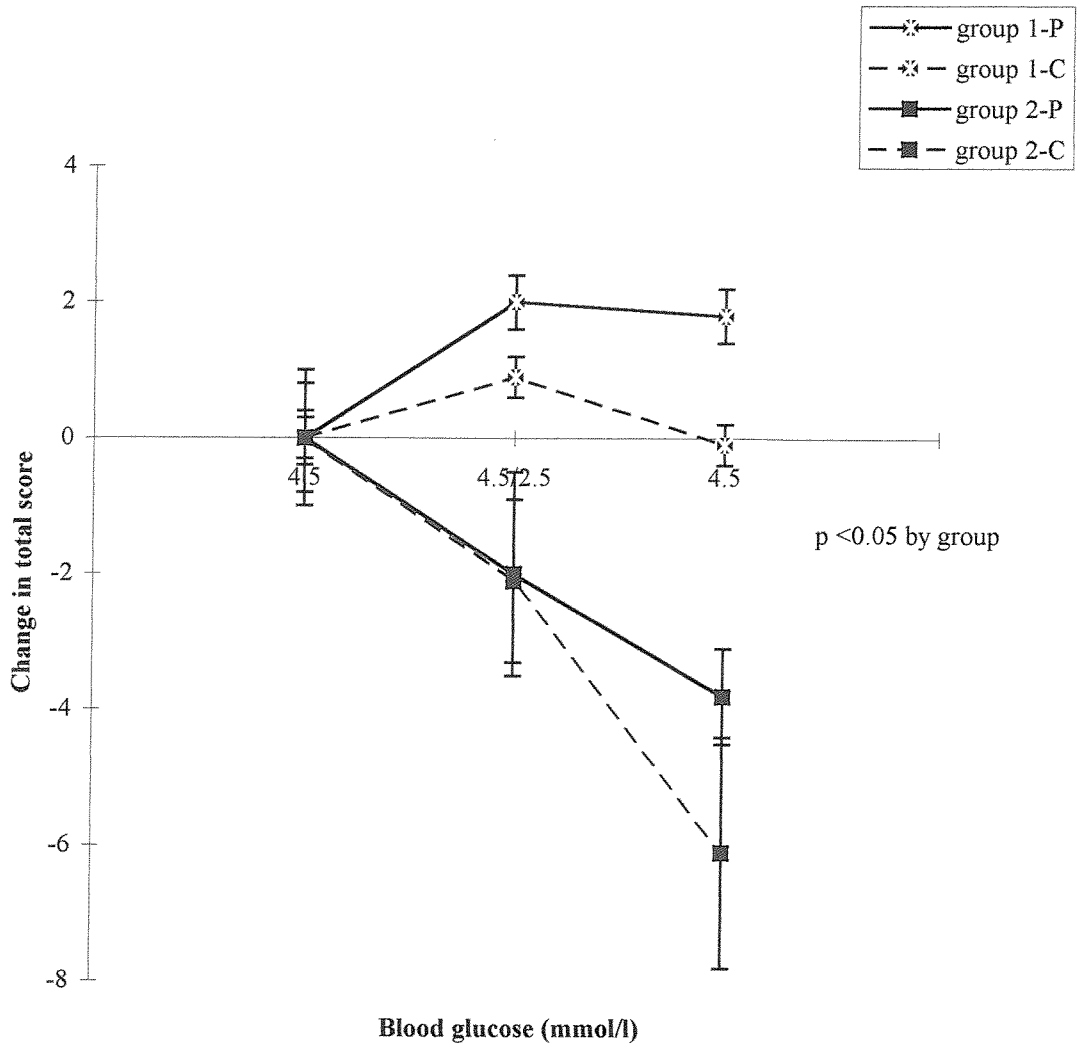


Figure VI.2.11 Group averaged VEPS at Oz, comparing runs 3 and 4 with run 2

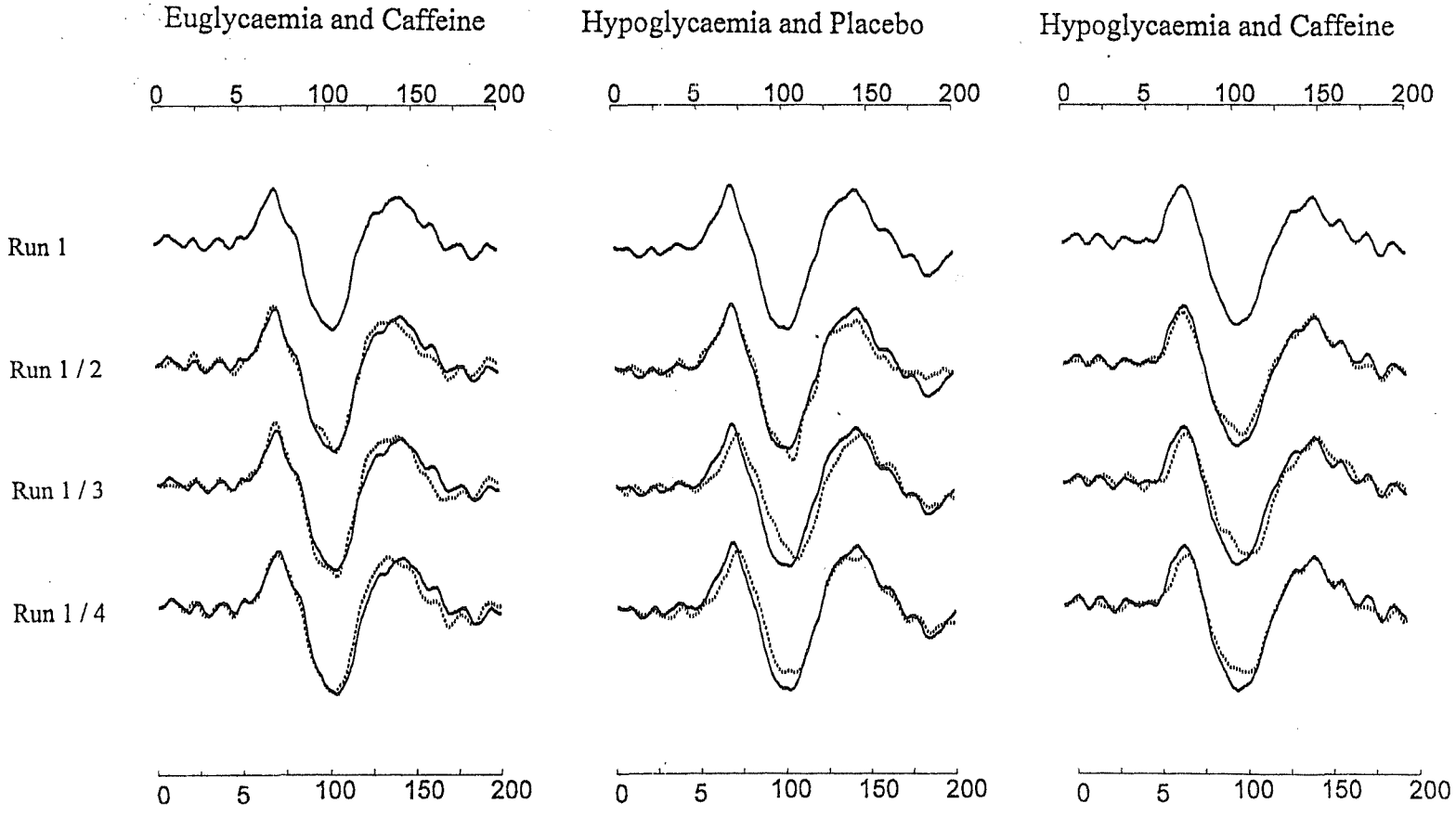


Table VI.2.1 Achieved blood glucose concentrations (mmol/l)

	Baseline (mean ± se)	Euglycaemia	Hypoglycaemia/ Euglycaemia	Euglycaemia (insulin stopped)
E+C	4.46 ± 0.36	4.57 ± 0.02	4.50 ± 0.02	4.50 ± 0.02
H+P	4.60 ± 0.56	4.6 ± 0.03	2.78 ± 0.02	4.39 ±0.04
H+C	4.45 ± 0.36	4.54 ± 0.02	2.82 ± 0.03	4.31 ± 0.09

Table VI.2.2 Average caffeine levels (mmol/l)

	Baseline	+20 min	+80 min	+120 min	+180 min	+210 min	+270 min
E+C	0.6 ± 0.2	0.6 ± 0.2	0.5 ± 0.1	2.6 ± 0.3	2.7 ± 0.3	2.5 ± 0.3	2.1 ± 0.3
H+P	0.7 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
H+C	0.8 ± 0.4	0.8 ± 0.4	0.7 ± 0.4	2.8 ± 0.3	2.5 ± 0.4	2.2 ± 0.4	2.0 ± 0.5

Table VI.2.3 Mean scores for performance (number correct out of 50) during the information processing tests

	Absolute VCD Score mean ± se			Difference in VCD from Eug mean ± se		Absolute VCD Score mean ± se			Difference in VCD from Eug mean ± se	
	Eug	Eug/ Hypo	Eug	Eug/ Hypo	Eug	Eug	Eug/ Hypo	Eug	Eug/ Hypo	Eug
E+C	28 ± 4.7	31± 4.2	30 ± 3.9	2.4± 4.3	3.1± 4.2	41 ± 5.9	44 ± 2.7	45 ± 3.6	3.3± 5.5	4.1± 4.8
H+P	30± 6.5	28 ± 5.0	29± 6.3	-1.6±4.8	-1.2±3.9	41 ± 3.7	41 ± 4.8	40 ± 6.4	-0.2±4.6	-0.9±6.4
H+C	30± 3.8	30± 4.2	29± 5.6	-0.4±4.9	-1.1±4.7	43± 3.4	42± 4.8	40± 7.0	-1.2±5.3	-2.8±5.9

Table VI.2.4 VEP latencies TB1 - TB4 and latency changes

	P100 LATENCY (msec, mean ± se)					
	TB1	TB2	TB3	TB4	TB3-1	TB3-2
E+C	98.1 ± 0.9	98.0± 1.1	96.1± 0.7	96.9 ± 0.8	-1.9 ± 0.7	-1.8 ± 0.7
H+P	96.6 ± 0.9	97.2 ± 0.9	99.1 ± 1.2	98.7 ± 0.9	2.6 ± 1.3	2.8 ± 1.0
H+C	97.1 ± 1.0	98.31 ± 1.4	102.8 ± 1.8	99.4 ± 1.1	5.7 ± 1.6	4.5 ± 1.7

Table VI.2.5 Summary of significant P100 results

		TB1 to 4		TB2 to 4	
		P100 Latency	P100 Amplitude	P100 Latency	P100 Amplitude
All conditions	All subjects	* (condition x time)	-	** (condition x time)	-
	Median groups	* (gp x condition x time)	* (gp x condition x time)	* (gp x condition x time)	* (gp x condition)
Hypo-glycaemia (E+C vs. H+C)	All subjects	*** (condition x time)	-	** (condition x time)	-
	Median groups	* (gp x condition x time)	* (gp x condition)	-	-
Caffeine (H+P vs. H+C)	All subjects	-	-	-	* (condition x time)
	Median groups	* (gp x condition)	-	-	-

- no significant difference
* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Discussion

The present study shows that caffeine, after overnight caffeine abstention, can influence the perception of and hormonal counterregulatory responses to hypoglycaemia with prolonged effects even when euglycaemia has been restored. The combination of caffeine and hypoglycaemia was associated with a significant decrease in V_{MCA} with no increase above baseline after hypoglycaemia was corrected; this was more marked in females. In addition warning symptoms during hypoglycaemia were more intense with caffeine, an effect which lasted into the recovery period. Adrenaline and growth hormone responses during hypoglycaemia were augmented by caffeine, compared with placebo with the latter hormonal response continuing into recovery as well. Whilst cognitive function was similarly affected during hypoglycaemia, improvement in VMD was significantly prevented with caffeine compared to the euglycaemia study. Overall males showed a trend to perform less well than females during hypoglycaemia.

The data confirm that hypoglycaemia has a significant effect on visual function, as measured by the increase in P100 latency from both rest and controlled euglycaemia to hypoglycaemia. In addition this study shows that in those subjects who are most sensitive to a reduction in blood glucose concentration, caffeine ingestion produces a further increase in P100 latency. For those subjects, there is a significant inverse relationship between latency and deterioration in performance of a visual information processing task during hypoglycaemia.

Although tolerance to the effects of caffeine may occur, usual patterns of consumption (more in the morning with overnight abstinence) means that this phenomenon is incomplete (Shi J.et al 1993). In this study subjects were asked to abstain from caffeine consumption for 14 hours only, in contrast to more prolonged abstinence of previous studies (Kerr D.et al 1993a; Debrah K.et al 1996). Responses to hypoglycaemia were still increased by the ingestion of caffeine, which has important implications in the use of caffeine to promote hypoglycaemia awareness.

Interestingly, caffeine consumption in habitual consumers, under rest conditions is not associated with increased levels of catecholamines (Spindel E. et al 1984; Del Rio G. et al 1996). However, during a stressful situation, prior caffeine ingestion can increase adrenaline production (Van Soeren M.et al 1993). Noradrenaline has consistently been found to be less affected by caffeine ingestion (Van Soeren M.et al 1993; Van Soeren M.et al 1996). These findings were replicated here using the stress of hypoglycaemia as a stimulus.

With respect to other counterregulatory hormones, *in vitro* experiments have shown that caffeine increases the production of growth hormone in the presence of GHRH (Herrington J. et al 1994; Brown O. et al 1999). This may be mediated via intracellular calcium release, although the concentrations of caffeine achieved in culture have been high (up to 40 mmol/l (Herrington J.et al 1994)). The prolonged growth hormone response is interesting. Growth hormone begins to rise at around 20 minutes after the onset of hypoglycaemia, induced by a bolus dose of insulin (Garber A.et al 1976). The level remains elevated, after restoration of normal catecholamine levels but this effect is exaggerated with prior caffeine consumption. The mechanism for this is unclear but may be due to the increased neuroglycopenia induced by caffeine consumption.

The half-life of caffeine is 3 to7 hours (Rall T. 1990). This is reflected in that V_{MCA} decreased for 100 minutes and rose in the recovery period but not above baseline. Although the accepted threshold for CBF to increase in hypoglycaemia is 2.2 mmol/l (Tallroth G.et al 1992; Powers W.et al 1993), this was not achieved here but there was a significant rise in V_{MCA} recorded in the recovery period, as previously found by Eckert et al (Eckert B.et al 1998). Previous studies had only demonstrated raised CBF for a short duration in recovery from hypoglycaemia (Tallroth G.et al 1992). In restoring normal body function this may be of critical importance.

This late rise in V_{MCA} has not been examined in detail. It would appear that the hypoglycaemic event starts a metabolic or vascular reaction which is slowly restored. Prolonged hyperinsulinaemia has been shown to increase CBF (Kerr D.et al 1991). In the present study, insulin infusion was stopped at the end of hypoglycaemia and the

next V_{MCA} reading was made approximately 35 minutes later. Serum insulin levels were not measured here, however at the second V_{MCA} measurement during recovery the effect of insulin would be negligible. Thus even if there was a stimulatory effect of insulin on CBF, it is not likely that the increase in V_{MCA} was due to insulin.
Symptoms of hypoglycaemia are experienced more intensely with caffeine compared to placebo in recovery, despite catecholamine levels decreasing dramatically. This is of clinical importance in that subjects would potentially continue to treat hypoglycaemia even though glucose levels have returned to normal. Thus glucose disequilibrium could be extended beyond usual recovery time from hypoglycaemia. Restoration of cognitive impairment and symptoms of hypoglycaemia depend on the level and duration of hypoglycaemia but recover between 10 and 90 minutes after restoration of blood glucose (Blackman J.et al 1990; Blackman J.et al 1992; Gonder-Frederick L.et al 1994; Lindgren M.et al 1996).

Caffeine had no effect on cognition during hypoglycaemia, which given the compromise in supply of glucose to the brain with caffeine is surprising. However, Ruijter et al showed that whilst caffeine only maintained reaction time over an extended period of testing (10 minutes), this represented the suppression of fatigue and boredom, 'supporting other evidence for more pronounced effects of caffeine in suboptimal conditions' (Ruijter J. et al 2000). Thus although caffeine causes a decrease in V_{MCA}, its psychostimulant effects are enough not to compromise cognition further during hypoglycaemia.

Although not significantly different performance in VMD was preserved better in females than males during hypoglycaemia. It has previously been demonstrated that hypoglycaemia is better tolerated by women than men (Draelos M.et al 1995). Women performed better in the tests of visual information processing with a tendency towards better preservation of performance in the VMD test in females than males. This contrasts with the finding of higher total symptom scores during hypoglycaemia in females but less adrenaline production. Following hypoglycaemia alone V_{MCA} rose above baseline in females only, suggesting a faster recovery from hypoglycaemia.

From this study VEP (P100) latency is a sensitive indicator of glycaemic status. It has identified that P100 latency increase not only from baseline euglycaemia to hypoglycaemia (Harrad R.et al 1985) but also from controlled euglycaemia to hypoglycaemia. It is evident from the data that there were large, individual differences in P100 latency during hypoglycaemia. The median splits which were carried out showed significant group differences for P100 latency between conditions, indicating that some individuals were more sensitive to visual function change, as a result of hypoglycaemia. The comparison with the subjects' own baseline glycaemic level produced a greater number of changes in P100 than the comparison with the controlled euglycaemic condition. In those subjects who were most sensitive to the effects of hypoglycaemia in the baseline condition, effects of caffeine ingestion were observed over and above those of hypoglycaemia. In these subjects caffeine produced a further significant increase in the P100 latency.

Essentially the effects of caffeine are demonstrable during hypoglycaemia after overnight abstention and some are prolonged such that the restoration of normality is postponed. Physiologically this can be measured with the increase in GH at this time, as well as the failure of V_{MCA} (CBF) to rise above baseline. Symptoms of hypoglycaemia remain high in this recovery period after caffeine too. However, caffeine ingestion combined with hypoglycaemia is not associated with an increased negative effect on cognition, as measured by the visual inspection threshold tests; suggesting that the stimulant action of caffeine outweighs the decrease in blood glucose supply. In addition gender differences were identified, which included less adrenaline production in females but higher total symptom score of hypoglycaemia following caffeine ingestion combined with a trend towards better preservation of cognition during hypoglycaemia albeit in one test only. The results also suggest that those subjects who are visually sensitive to the effects of hypoglycaemia may experience further deterioration following caffeine ingestion.

Clinically this study is relevant in that the effects of caffeine on the responses to hypoglycaemia are present after overnight abstention only in healthy volunteers. However for the first time the effects of caffeine in the recovery period have been

studied and these remain, such that recovery is compromised with the prolonging of symptom generation and no increase in V_{MCA}, a surrogate marker of CBF. Fundamentally caffeine continues to aid the recognition of hypoglycaemia but may delay recovery because of its long duration of action.

Study 3 Does the Brain become tolerant to Sustained Caffeine Use? Implications for Treatment of Hypoglycaemia Unawareness.

Due to the enormous consumption of caffeine world-wide, its effects on performance and physiology have been extensively investigated. However the debate about the benefits and drawbacks of caffeine ingestion remains unresolved (James J. 1997b; Nehlig A. 1998). Numerous placebo controlled studies have been published showing improvement in a variety of cognitive function tests including simple reaction time (Azcona O.et al 1995), digit symbol substitution (File S.et al 1982) and logical reasoning (Suenaga N.et al 1997). Although a smaller number of studies have shown no effect of caffeine stimulus recognition and memory (Rush C. et al 1994a; Rush C. et al 1994b), the balance of evidence is in favour of a psychostimulant action of caffeine.

The relevance of these studies to everyday life is questionable because in the majority, subjects were required to abstain from caffeine overnight or for more than 24 hours and the amount of caffeine administered was relatively high, up to 600 mg in a single dose. The problem with caffeine deprivation prior to testing, means that symptoms of caffeine withdrawal can be invoked (James J. 1994). Thus it is not possible to describe the net effects of caffeine with these studies as caffeine withdrawal has been shown to decrease performance in both humans (e.g. finger tapping (Silverman K.et al 1992a)) and animals (Griffiths R.et al 1988b).

There is evidence that acute ingestion of caffeine has a pressor effect. It is widely held that tolerance develops to this with sustained use (Robertson D.et al 1981) but this is disputed (James J. 1994). Furthermore other circulatory effects can be measured e.g. decrease in cerebral (Mathew R.et al 1985b) and forearm blood flow (Daniels J.et al 1998), which are potentially better markers of the action of caffeine on the cardiovascular system. Debrah et al demonstrated a dissociation between central (cerebral blood flow) and peripheral (blood pressure) effects of caffeine with tolerance developing in the latter only (Debrah K.et al 1995).

Earlier studies which claimed to show tolerance to the pressor effects of caffeine (Robertson D.et al 1981; Ammon H.et al 1983; Izzo J.et al 1983; Höfer I.et al 1994) have not mirrored 'normal' staggered caffeine consumption but are characterised by a prior period of prolonged abstinence followed by a high dose of caffeine administered once or twice daily. Subsequent studies which have considered more usual patterns of consumption, have reported the pressor effects of further doses of caffeine during the morning albeit with a smaller response than the first cup of coffee (Lane J.et al 1989; Goldstein I.et al 1990). Shi et al demonstrated that the tolerance phenomenon depended on the amount of caffeine consumed, schedule of consumption and elimination half life (Shi J.et al 1993). They estimated, using a parametric pharmacokinetic-pharmacodynamic model for caffeine that it would take about 20 hours or five drug half-lives for the effects of caffeine tolerance to wear off. Given that caffeine consumption is associated with 10 - 12 hours of overnight abstinence with more caffeine consumed in the morning than afternoon hours, it can be demonstrated that overnight abstinence is enough for sensitivity to be recovered and that at best tolerance is partial.

Caffeine has been shown to influence the physiological (decreased middle cerebral artery blood velocity (V_{MCA}) (Kerr D.et al 1993a; Debrah K.et al 1996), counterregulatory hormone (Kerr D.et al 1993a; Debrah K.et al 1996)) and psychological responses (increased symptoms (Kerr D.et al 1993a; Debrah K.et al 1996), decreased cognitive function (Kerr D.et al 1993a)) to hypoglycaemia. These studies were conducted in healthy volunteers and type 1 diabetic patients who had withdrawn from caffeine for 72 hours prior to testing. As part of a further investigation into these effects on hypoglycaemic responses, the aim of this study was to investigate the tolerance phenomenon in similar parameters, during euglycaemia in healthy volunteers.

Methods

Subjects

14 healthy, left-hemisphere dominant, regular caffeine consumers (180-500mg per day, 7 males and aged 23- 38 years) gave written, informed consent for the study, after approval was obtained from the local hospital ethics committee. None of the subjects had any relevant previous medical history, nor were they taking any regular medication or smoking. Each subject was informed that they would be required to attend the department on three separate occasions. The first session served to familiarise the subjects with the study protocol. The following two studies were identical except for the dietary preparation. They were performed at least two weeks apart to avoid any carry-over effect.

Experimental procedure

Prior to the second and third visits, subjects consumed a caffeine-free diet for seven days. This was supplemented, in a double-blinded, randomised, cross-over design with either 200mg caffeine capsules bd. or matched placebo. The order was counter-balanced across participants. The final capsule was taken on the morning of the study, one hour before attending the research unit. Thus, the subjects were either caffeine-replete (C-replete) or caffeine-naïve (C-naïve) at the start of each study (Figure VI.3.1).

On the morning of a study, subjects were admitted at 09.00, having fasted overnight. At this time, subjects completed a questionnaire scoring the strength of 32 symptoms relating to caffeine-withdrawal rated on a scale of 0 to 3 (Silverman K.et al 1992a). A retrograde cannula was inserted After insertion of the cannula, subjects rested supine for twenty minutes before the experimental protocol began. Baseline measurements were taken of:

(i) Heart rate and blood pressure
(ii) V_{MCA}
(iii) Mood using the UWIST mood score
(iv) 4-choice reaction time (4CRT)
(v) Visual information processing tests
- *Visual change detection*
- *Visual movement detection*

(vi) Plasma caffeine levels.

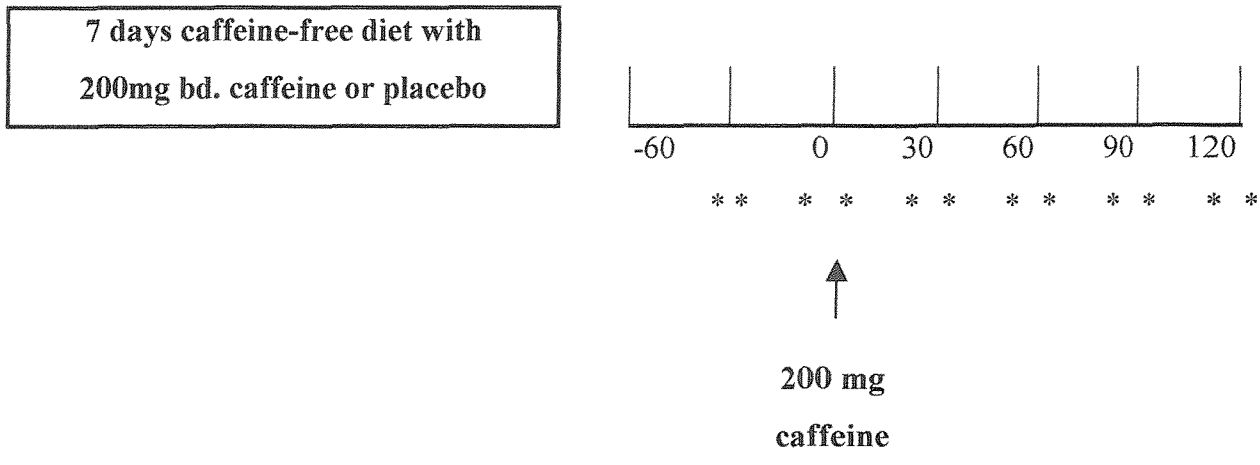
Thereafter, subjects consumed a cup of decaffeinated coffee with 200mg caffeine added. All measurements were repeated in the same position, at 30 min intervals for the next 2 hours with blood glucose levels measured every 15 min. Blood sugar was maintained above 4.0 mmol/l throughout the studies thus avoiding the confounding effect of hypoglycaemia.

Statistical analysis

Overall differences between serial measurements were examined by summary measures (Matthews J. et al 1993). Area under the curve (AUC) by the trapezoid method and maximum response were calculated for the responses of each individual. Group means were compared by paired Student's t-tests. Where data were not normally distributed comparisons were made after logarithmic transformation. Results are expressed as individual means with point estimate of differences between means and 95%CI. Otherwise data are shown as mean ± se.

Figure VI.3.1: Schematic representation of the study. Each subject completed 2 studies, which were arranged in a counterbalanced manner.

* * = TEST BATTERY= BP, HR, V_{MCA}, 4CRT, VCD, VMD, UWIST mood score



Results

Baseline and maximum caffeine concentrations for the two experimental conditions are shown in Table VI.3.1 with average blood glucose readings for the duration of the studies. Caffeine withdrawal questionnaires showed no statistical difference in total scores after caffeine abstinence or supplementation for 7 days (26.9 ± 2.5 C-naïve vs. 27.8 ± 2.3 C-replete, p =0.34).

Haemodynamics

Baseline V_{MCA}, heart rate and blood pressure were similar in both studies. After the caffeine challenge, V_{MCA} decreased significantly in the both conditions, although this was more marked during the C-naïve condition (-8.0 [-10.0, -6.1] cm/s C-naïve vs. -4.9 [-6.8, -2.9] cm/s C-replete, p<0.02) (mean difference [95%CI]). The decrease in V_{MCA} was sustained for the duration of the study (Figure VI.3.2).

In the C-replete state, the caffeine challenge caused a significant rise in systolic blood pressure at 30 minutes but this was not significantly different from the rise in C-naïve study ($^{+}$8.7 [4.2, 13.1] mmHg C-naïve vs. $^{+}$4.5 [0.8, 8.3] mmHg C-replete, p = 0.13). Although there were no differences in baseline blood pressures (systolic and diastolic) AUC for change in systolic blood pressure against time was significantly different (p< 0.04) (Figure VI.3.3). In contrast the initial rise in diastolic blood pressure was greater in the C-naïve condition ($^{+}$5.5 [2.3, 8.6] mmHg C-naïve vs. $^{+}$1.1 [-0.8, 3.0] mmHg C-replete, p< 0.005) but this difference was not statistically sustained for the study duration (AUC C-naïve vs. C-replete p= 0.055) (Figure VI.3.3). Heart rate was unaffected by prevailing caffeine status.

Symptom score

Tense mood was most affected by caffeine status (Figure VI.3.4). Baseline measurements were significantly different (11.6 ± 0.6 C-naïve vs.16.3 ± 1.6 C-replete, p< 0.01) as were AUC for mood score against time graphs (1302 ± 54 score, min C-naïve vs. 1717 ± 127 score, min, p< 0.02). Caffeine consumption in the C-replete state was associated with a greater decrease in tense mood (-1.1 [-2.6, -0.34] C-naïve vs. -4.7 [-7.5, -1.9] C-replete, p< 0.02). Whilst caffeine consumption improved energetic mood

in both conditions this effect was not significantly different between conditions ($p = 0.24$) (Figure VI.3.4). Hedonic mood increased significantly from baseline during C-naïve only (increase in hedonic mood score $^{+}2.9 \pm 1.2$ above baseline, $p< 0.05$), although the increase observed was not significantly different between conditions ($p = 0.51$) (Figure VI.3.4).

Psychometric tests

VCD and VMD did not improve with caffeine consumption in either condition (Table VI.3.2). 4 Choice reaction time improved similarly, following caffeine ingestion in both C-naïve and C-replete conditions (Figure VI.3.5).

Figure VI.3.2: Change in middle cerebral artery blood velocity in C-replete and C-naïve conditions following a caffeine challenge (mean ± se)

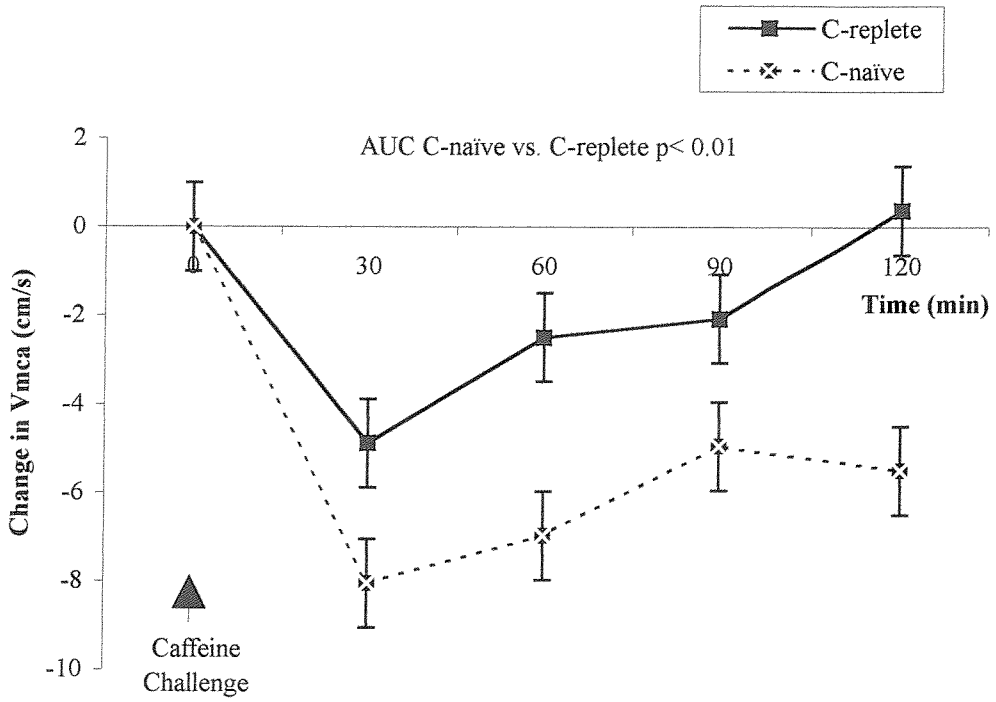
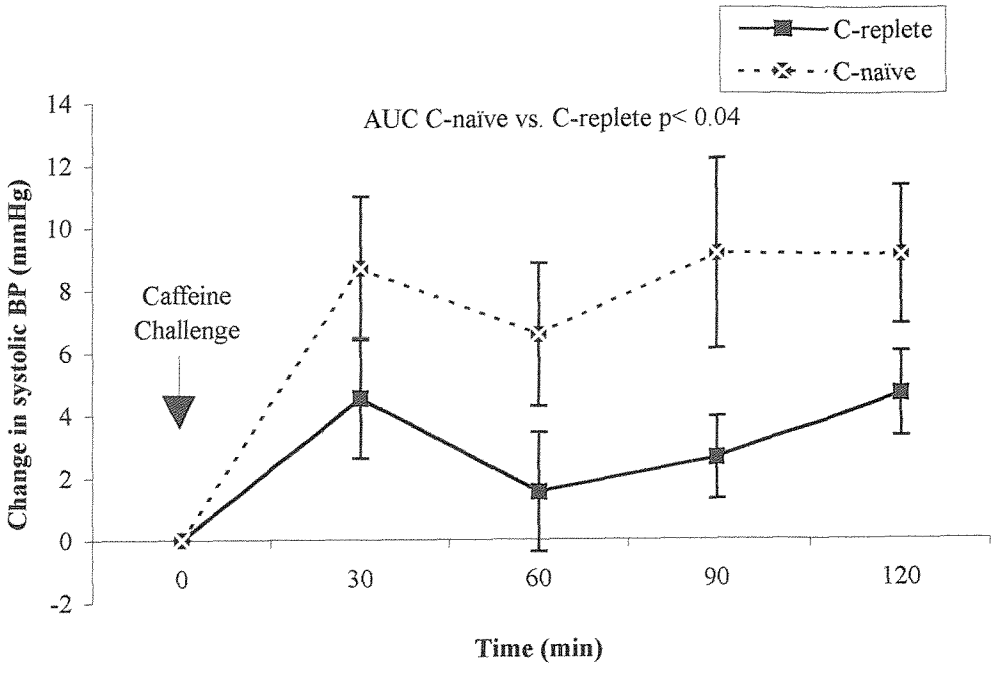
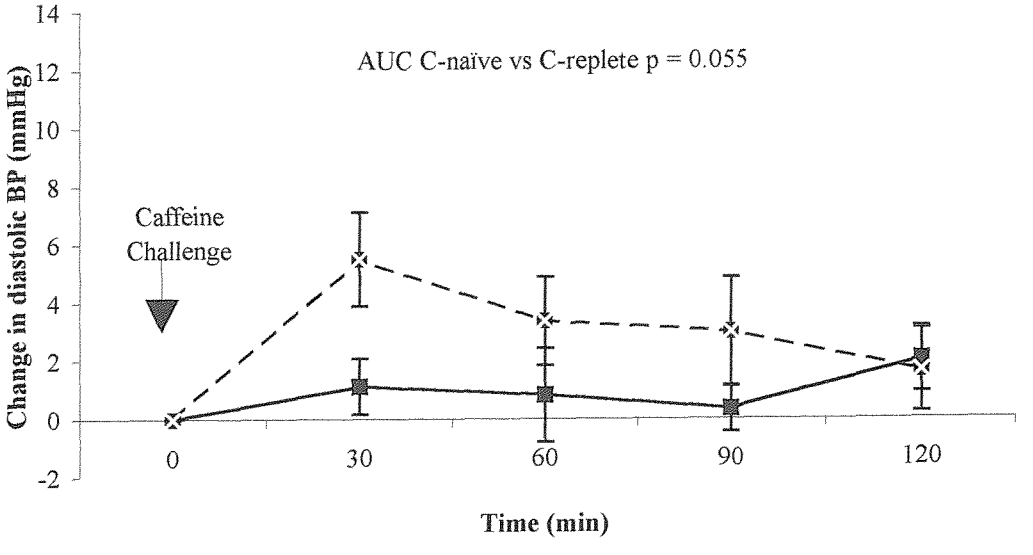


Figure VI.3.3: Change in blood pressure in C-replete and C-naïve conditions following a caffeine challenge (a) systolic, (b) diastolic (mean ± se)





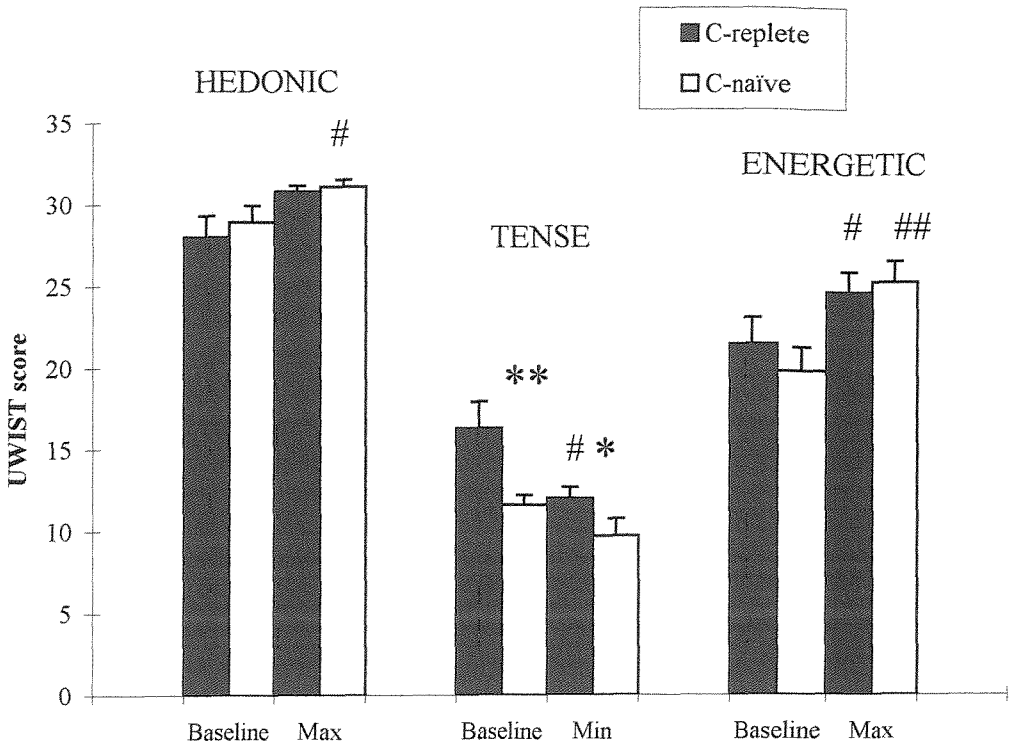


Figure VI.3.4: Mood scores in C-replete and C-naive conditions and following caffeine challenge (mean ± se)

* = C-naive vs. C-replete $p < 0.02$

** = C-replete vs. C-naïve $p < 0.01$

\# = Max/ Min vs. Baseline $p < 0.05$

\#\# = Max/ Min vs. Baseline $p < 0.005$

Figure VI.3.5: 4-choice reaction time results in C-replete and C-naive conditions and following caffeine challenge (mean ± se)

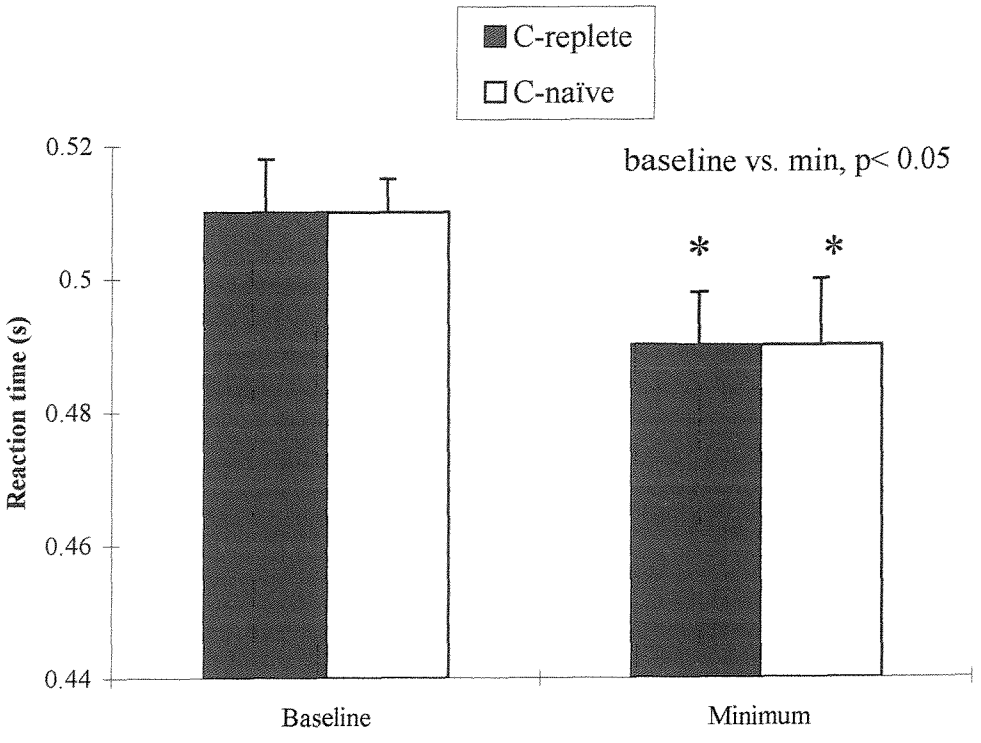


Table VI.3.1: Mean plasma caffeine and blood glucose levels during C-replete and C-naïve studies.

$^{\alpha}$ = p< 0.001 C-naïve vs. C-replete

$^{\beta}$ = p< 0.02 C-naïve vs. C-replete

Condition	Baseline Caffeine (mmol/l)	Maximum Caffeine (mmol/l)	Average Blood Glucose (mmol/l)
C-replete	2.33 ± 0.62 $^{\alpha}$	5.41 ± 1.45 $^{\beta}$	4.50 ± 0.1
C-naive	0.17 ± 0.05 $^{\alpha}$	4.08 ± 1.18$^{\beta}$	4.09 ± 0.08

Table VI.3.2: Total Scores of VCD and VMD tests during C-replete and C-naïve studies (mean ± se).

	Baseline	60 min	120 min
VCD C-replete	42.7 ± 1.6	43.6 ± 1.3	43.6 ± 1.0
C-naive	42.5 ± 1.7	44.9 ± 1.2	44.8 ± 1.4
VMD C-replete	54.8 ± 1.2	54.9 ± 0.9	55.1 ± 0.6
C-naive	54.4 ± 1.4	53.2 ± 1.6	55.6 ± 1.1

Discussion

Whilst the development of tolerance to some effects of caffeine has been previously demonstrated (Colton T. et al 1968), it remains a controversial area. In this study, during euglycaemia, V_{MCA} and blood pressure (systolic and diastolic) responses to acute caffeine ingestion were attenuated with sustained caffeine use compared to a caffeine-naïve state. However tolerance was incomplete as significant changes from baseline were still recorded in V_{MCA} and systolic blood pressure. Thus vascular responses were demonstrated with a subsequent dose of caffeine in the fully C-replete state as average British caffeine consumption is 359 mg/ day (Scott N.et al 1989) to 444mg/ day (James J. 19997b). Interestingly daily caffeine consumption was associated with an increase in tense mood which resolved after further caffeine consumption. Only this aspect of mood that was associated with a significantly different response to the caffeine challenge between the two conditions. Development of (partial) tolerance to the haemodynamic changes is in contrast to the effect on psychomotor function. Here prior caffeine ingestion was not associated with different performance in all the tests, compared with the C-naïve state. Finally the differences recorded are not explained by the treatment of caffeine withdrawal symptoms as these were at a similar level at the beginning of both C-replete and C-naïve studies.

Mood is influenced by different patterns of caffeine consumption. It has been demonstrated with free-living and laboratory studies that caffeine consumption is associated with increased anxiety, tension, restlessness, nervousness and anger (Lane J. et al 1987; France C. et al 1989; Green P.et al 1996). The UWIST mood score has not previously been used in caffeine studies but the results here would concur with other scores used. The marked reduction in tense mood following caffeine ingestion during C-replete could represent the treatment of caffeine withdrawal symptoms. These can develop over a short period of time (Lane J. 1997; Lane J. et al 1998) . It may seem somewhat illogical that people chose to consume a substance which increases negative feelings. Green and Suls have suggested that consumption continues because of a perceived benefit such as increased productivity (Green P.et al 1996). Whilst hedonic mood did not improve significantly from baseline during C-replete unlike energetic mood, the size of these increases were not significantly different between the two

conditions. This would suggest that tolerance to caffeine's more arousing effects did not develop although this is in contrast to Green and Suls study (Green P.et al 1996). Increases in positive feelings are a usual feature of caffeine studies (Griffiths R. et al 1989; Silverman K. et al 1992b), although no dose-response relationship has been demonstrated (Quinlan P. et al 2000). Older people show increased positive effects of caffeine on mood compared to younger subjects with younger people expressing more anger with caffeine consumption (Arciero P. et al 1998). With respect to mood, tolerance has been largely overlooked by investigators in this field, although Zwyghuizen-Doorenbos et al suggested that instead of tolerance developing Pavlovian conditioning to the alerting effects of caffeine may also occur (Zwyghuizen-Doorenbos A. et al 1990).

This study adds to the body of previous work that tolerance to the haemodynamic effects of caffeine consumption is present but partial. The pressor response may be reinstated by a brief period of abstinence (Lane J.et al 1989), which may be as short as 3 hours (Sung B.et al 1994). In the conditions of this experiment partial tolerance was demonstrated to the response in V_{MCA} with regular caffeine ingestion. Earlier, Mathew et al demonstrated that cerebral blood flow decreases with caffeine ingestion (Mathew R.et al 1985a; Mathew R.et al 1985b). In the latter study subjects withheld from caffeine consumption for a minimum of 2 hours only, with an 18% decrease in CBF associated in caffeine consumption still demonstrated (Mathew R.et al 1985b). This effect was still measurable 90 minutes after the caffeine challenge.

4-choice reaction time improved in both conditions to the same degree but no improvement was seen in either condition with the visual perception threshold tests. The Health and Lifestyle Survey provided an opportunity to examine the issue of caffeine consumption on cognition and tolerance (Jarvis M. 1993). In this study 9003 British adults completed a number of performance tests (including choice reaction), as well as information on caffeine intake. Overall caffeine consumption showed a dose-response relationship with improved cognitive performance with no evidence for the phenomenon of tolerance to caffeine.

Caffeine has been shown to narrow attention and thus increase selectivity of information processing (Anderson K.et al 1982; Lorist M.et al 1994a). In practice however selectivity may operate at many levels and it has subsequently been demonstrated that the stimulatory visual effects of caffeine are limited to situations with only a few visual inputs (Kenemans J. et al 1998). The multiple elements of the visual information processing tests may exceed this limit.

In conclusion, whilst the history of exposure to caffeine will influence the effect of a dose, tolerance to this drug's vascular effects is incomplete both centrally and peripherally. In contrast tolerance was not demonstrated in performance (4 CRT) nor improvements in positive aspects of mood. This has implications for the effects of caffeine on physiological responses to hypoglycaemia in that the augmentation of these responses may not be lost with continued caffeine use (investigated separately). Differences in mood can be attributed to caffeine consumption and interestingly, there are negative effects associated with regular caffeine consumption.

Study 4 Dissociation of augmented physiological, hormonal and cognitive responses to hypoglycaemia with sustained caffeine use.

Caffeine is the most ubiquitous psychoactive drug with world-wide consumption averaging 76mg/person/day (Gilbert R. 1984), rising to 206 mg/person/day in the United States and more than 350mg/person/day in the UK and Scandinavia (Barone J.et al 1996). In everyday life the consumption of caffeine present in tea, coffee, soft drinks and other foodstuffs produces effects that are difficult to detect or so subtle as to go unnoticed. However, when continuous caffeine use is stopped abruptly, characteristic symptoms (e.g. headache, lethargy and anxiety) appear quickly, as a consequence of physical dependence (Silverman K.et al 1992a).

After 24-48 hours abstinence, acute caffeine ingestion is associated with an approximate 15% reduction in brain blood flow, a rise in blood pressure and release of adrenomedullary adrenaline (Nehlig A.et al 1992). However with sustained use, the effects of caffeine on blood pressure and catecholamine release, but not brain blood flow, are decreased suggesting dissociation between the development of central and peripheral tolerance to regular caffeine ingestion (Debrah K.et al 1995).

Among healthy volunteers and patients with type 1 diabetes who use caffeine regularly, prior ingestion of modest amounts of caffeine markedly augments the symptomatic and hormonal counter-regulatory responses to hypoglycaemia (Kerr D.et al 1993a; Debrah K.et al 1996). For example, compared to individuals who have not been exposed to caffeine, ingestion of 250 - 400 mg caffeine at the onset of hypoglycaemia is associated with a more than two fold greater rise in plasma epinephrine levels (Kerr D.et al 1993a; Debrah K.et al 1996). However, these studies were performed in caffeine users who had abstained from the drug for at least 72 hours. The observed effects could have represented relief from the syndrome of caffeine withdrawal rather than acute effect of caffeine per se (James J. 1994).

The aim of this study was to determine whether the acute effects of caffeine ingestion on the perception of and physiological responses to hypoglycaemia are attenuated with controlled sustained caffeine use.

Methods

Subjects

11 healthy, left-hemisphere dominant, regular caffeine consumers (180-500mg per day, 5 males and aged 24- 36 years) gave written, informed consent for the study. On two visits to the laboratory, subjects underwent identical glucose clamp procedures. For seven days prior to each clamp, subjects consumed a caffeine-free diet, supplemented, in a double-blinded, randomised, cross-over design with either 200mg of caffeine bd. or matched placebo. The final capsule was taken on the morning of the clamp study, one hour before attending the research unit. Thus the subjects were either caffeine-replete (C-replete) or caffeine-naïve (C-naïve) at the start of each glucose clamp study.

Experimental procedure

On the morning of a study, subjects were admitted at 09.00, having fasted overnight. Cannulae were inserted in preparation for the glucose clamp procedure as described in the methods section. During the two glucose clamp sessions, euglycaemia was maintained for 80 minutes (4.5 mmol/l) **at the end of which a capsule containing 200 mg of caffeine was ingested**. Thereafter blood glucose was lowered to 2.5 mmol/l over 20 minutes and held there for a further 80 minutes. Subjects were not informed of their blood glucose level at anytime during the laboratory sessions (Figure VI.4.1). At $^{+}20$ (euglycaemia) and $^{+}120$ (hypoglycaemia) minutes the test battery began and took 60 minutes to complete. The individual tests were administered in the order described below:

- Plasma caffeine and catecholamine levels.
- Heart rate and blood pressure
- Brain blood flow using a transcranial Doppler technique to assess middle cerebral artery blood velocity (V_{MCA}).
- Hypoglycaemia symptom questionnaire was completed at each stage

These tests were repeated after the following psychometric tests had been performed.

Psychometric tests of general cognitive function

Trail making type B (Reitan R. et al 1974) is a divided attention task. The subject has to connect correctly an alternating series of numbers (1-13) with letters (A-L) as quickly as possible.

Digit symbol task (Wechsler D.et al 1981) is a coding performance test. 1-9 digits are represented by a specific symbol. Subjects have one minute in which to write down as many corresponding symbols for each digit, in a given array of numbers.

Four choice reaction time test (Wilkinson R.et al 1975)

Psychometric tests of the hemispheres

Semantic processing and line orientation tests were used to discriminate between verbal-logical (left hemisphere) and spatial (right hemisphere) processing. Controlled word association is a test of executive function involving the frontal lobes in particular.

Semantic processing (Baddeley A. 1981) assesses ability to utilise stored information (semantic memory). This test contains 50 subject-verb questions of which half are sensible (e.g. Do caterpillars crawl?) and half are nonsensical (e.g. Do dishes yodel?). The time taken to respond to these sentences is recorded. Different statements are used each time the test was administered to the same subject.

Line orientation (Benton A. et al 1975) examines the ability to estimate the angular relationships between line segments by visually matching angled line pairs to 11 numbered radii forming a semi-circle. The number of correct matchings is recorded, as is the time taken to complete the test.

Controlled oral word association (Benton A. et al 1978) is an oral fluency test consisting of three word naming trials. The subject says as many words as possible beginning with the given letter in one minute. In each set of three letters words

beginning with the first letter have a relatively higher frequency, the second letter has a lower frequency and the third letter still lower.

Visual information processing tests

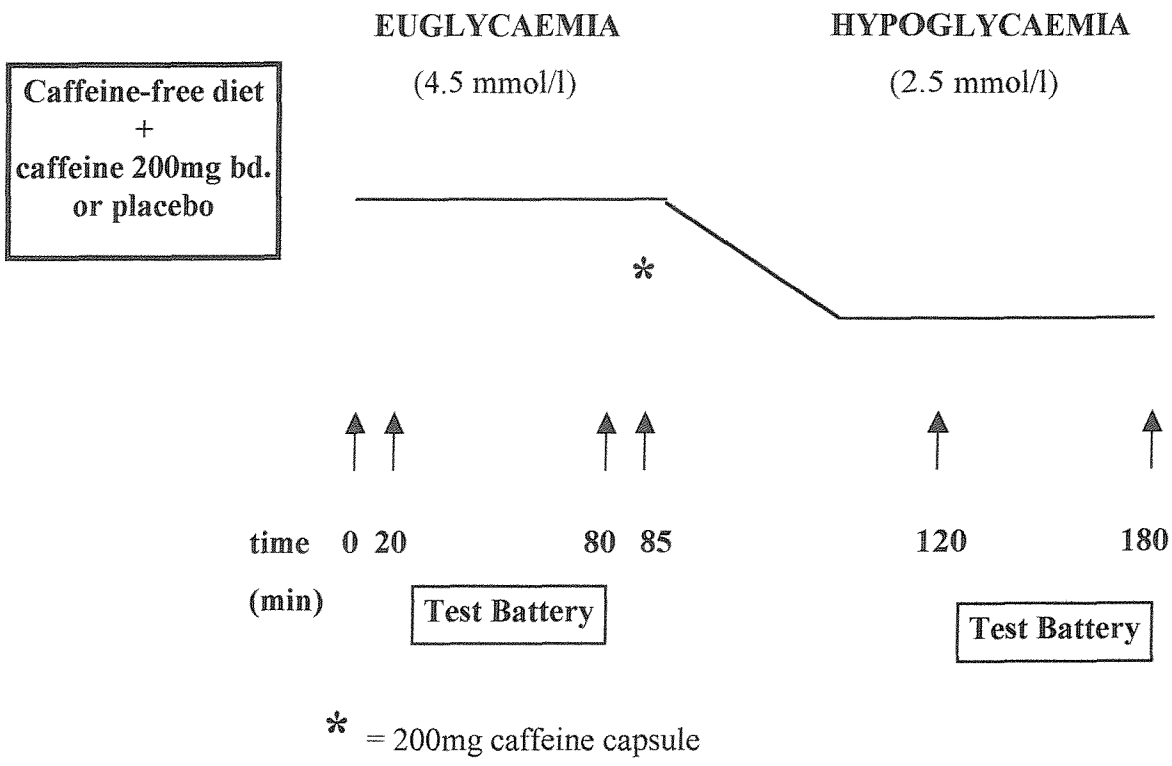
Visual change detection (VCD) The different time intervals between the presentation of the array and the onset of the change were 14, 28, 42, 56, 70 and 84 ms. The whole test involved 10 trials of the 6 different stimulus duration.

Visual movement detection (VMD) The interval between the onset of the array and the target rectangle appearing to move were identical to VCD (i.e. 14-84ms).

Statistical analysis

Overall differences between serial measurements were examined by summary measures (Matthews J.et al 1993). Area under the curve (AUC) by the trapezoid method and maximum response were calculated for the responses of each individual. Group means were compared by paired Student's t-tests. Where data were not normally distributed comparisons were made after logarithmic transformation. Results are expressed as individual means with point estimate of differences between means and 95%CI. Otherwise data are shown as mean ± se.

Figure VI.4.1 Schematic representation of the study. Each subject completed 2 studies, which were arranged in a counterbalanced manner.



* = 200mg caffeine capsule

TEST BATTERY= BP, HR, V_{MCA}, Cognitive function
(as ordered in the text), BP, HR, V_{MCA}

Results

Blood glucose levels were indistinguishable in C-naïve and C-replete conditions as demonstrated by achieved average blood glucose levels- 4.49 ± 0.01 mmol/l (mean ± se) and 2.61 ± 0.01 mmol/l C-naïve vs. 4.57 ± 0.01 mmol/l and 2.62 ± 0.01 mmol/l C-replete. At the start of the C-naive studies caffeine levels were 0.17 ± 0.03 mmol/l vs. 2.2 ± 0.3 mmol/l for the C-replete studies ($p<0.001$). Peak caffeine levels were reached at 120 minutes in 21/22 studies (2.79 ± 0.2 mmol/l (C-naïve) vs. 4.39 ± 0.5 mmol/l (C-replete), $p< 0.01$).

Euglycemia V_{MCA}, heart rate and blood pressure were similar in both studies. After the caffeine challenge, V_{MCA} fell significantly only in the C-naïve condition (-5.1 [-7.3, -3.0] cm/s C-naïve (mean [95%CI for the difference]), p euglycaemia vs. V_{MCA} at 120 minutes < 0.001) compared to -1.9 [-4.0, 0.2] cm/s C-replete, p = 0.1: delta C-naïve vs. delta C-replete p < 0.04). The decrease in V_{MCA} was sustained for the duration of the study (Figure VI.4.2) although AUC were not significantly different (p= 0.17).

When blood glucose was lowered to 2.5mmol/l, caffeine status did not affect the rise in systolic, or the fall in diastolic blood pressure (Table VI.4.1). Heart rate was unaffected by prevailing blood glucose and caffeine status.

Baseline values for catecholamines were similar at the start and did not alter significantly during the euglycaemic phase of either study. During hypoglycaemia the hormonal counter-regulatory response was not significantly affected by prevailing caffeine status (Figure VI.4.3).

Overall the symptoms experienced in each condition were similar except for neuroglycopenic symptoms which were more intense in the C-naïve condition ($p< 0.04$). However the pattern of responses generated differed in that the C-naïve condition was associated with an increase in symptoms after approximately 30 minutes of hypoglycaemia. However by the end of the hypoglycaemia phase (after 80 minutes), symptoms were experienced more intensely in the C-replete condition (Figure VI.4.4).

Global cognitive performance (trail making type B, digit symbol substitution and 4 choice reaction time) and information processing deteriorated to the same extent during hypoglycaemia, in both studies (Table VI.4.2). Verbal tests were more adversely affected in the C-replete state ($p<0.05$ vs. C-naïve) (Figure VI.4.5).

Figure VI 4.2 Change in middle cerebral artery blood velocity during the two study conditions from euglycaemia to hypoglycaemia: (mean ± se).

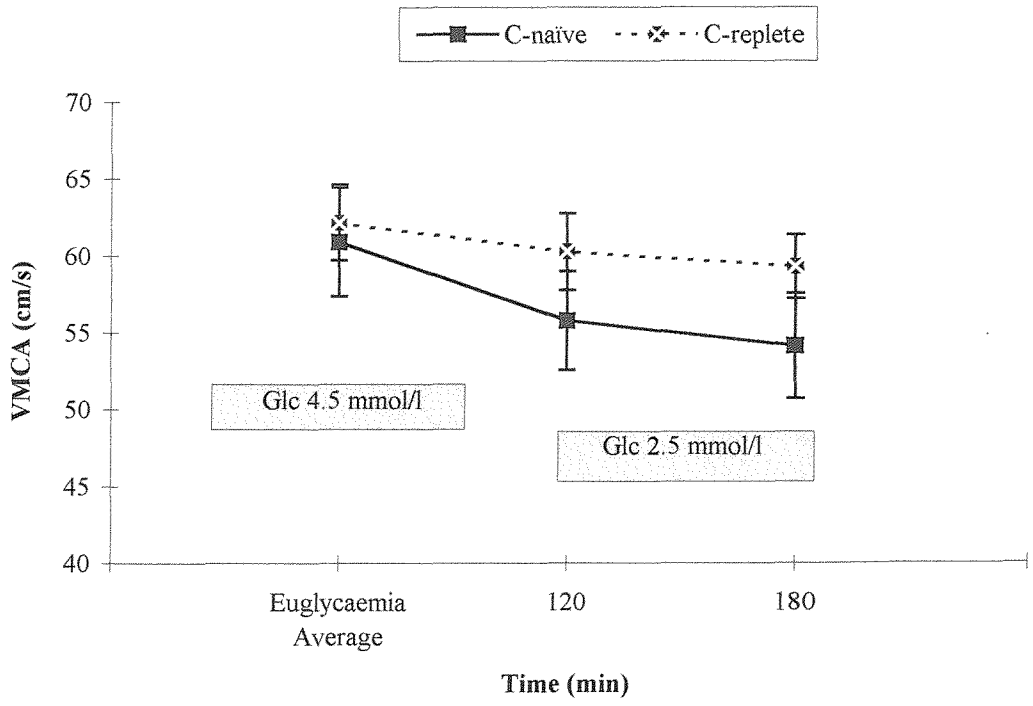
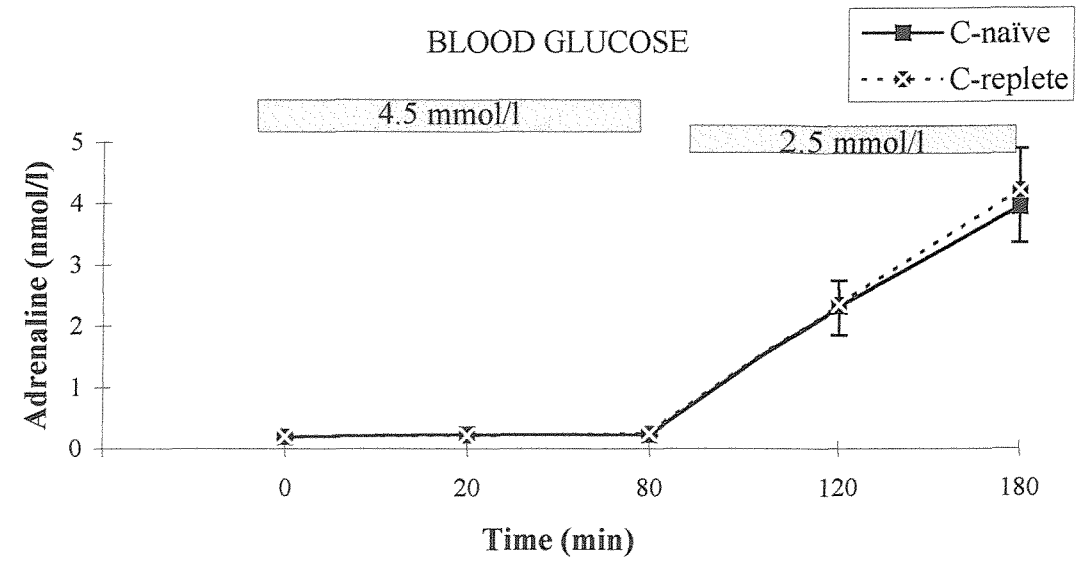


Figure VI.4.3 Catecholamine concentration during the two study conditions from euglycaemia to hypoglycaemia adrenaline (upper panel), noradrenaline (lower panel): (mean ± se).



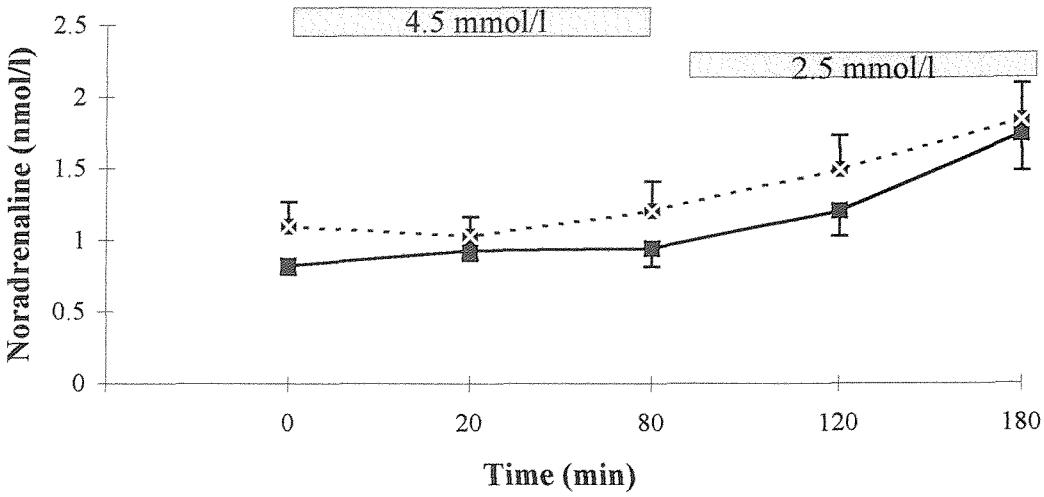
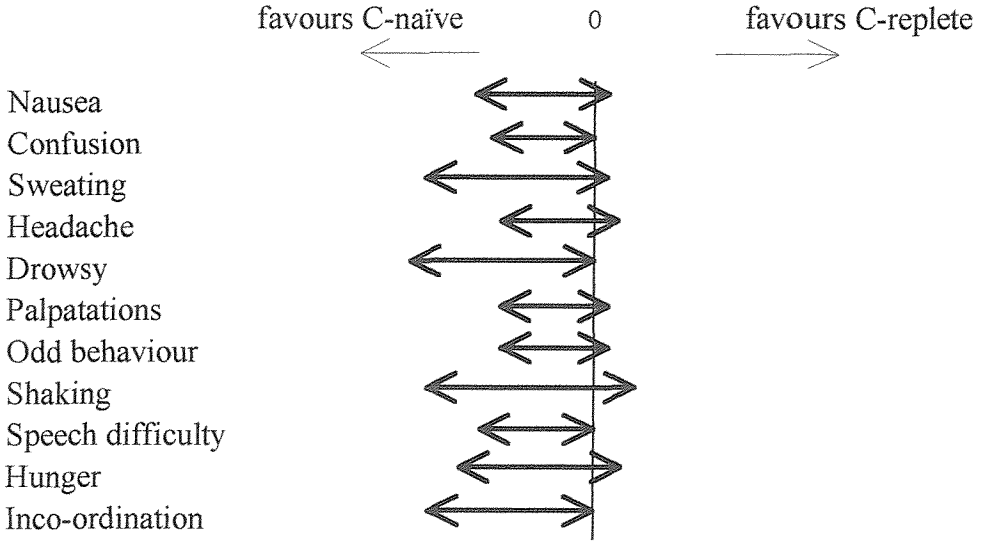
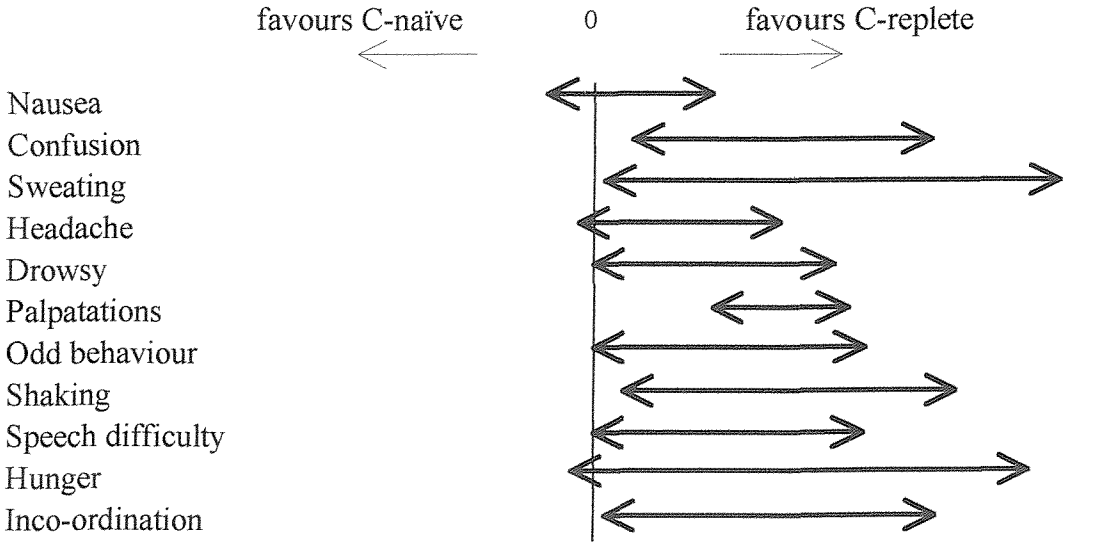


Figure VI.4.4 Difference between individual symptom scores experienced in the caffeine naive and caffeine replete conditions at start-time $^{+}$124 minutes (upper panel) and end of hypoglycaemia-time $^{+}$173 minutes (lower panel): (95% CI for mean difference).



Total symptom score C-naïve vs. C-replete at 123 min, p< 0.01



Total symptom score C-naïve vs. C-replete at 173 min, p< 0.05

Figure VI.4.5 Change in verbal processing tests during the two study conditions from euglcyaemia to hypoglycaemia: (mean difference ± se).

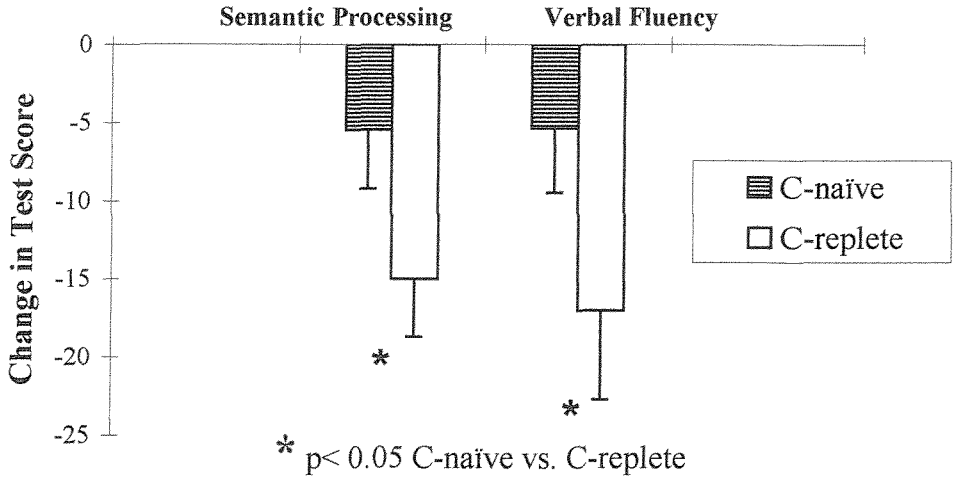


Table VI.4.1 Measurements of blood pressure (systolic and diastolic) and heart rate during the two study conditions from euglycaemia and hypoglycaemia (mean ± se; difference [95% CI]).

	C-replete			C-naïve		
	Eug-av.	Hypo-av.	Difference	Eug-av.	Hypo-av.	Difference
Systolic (mmHg)	112 ± 4	121 ± 4*	9 [1,18]	109 ± 3	123 ± 4*	13[7,19]
Diastolic (mmHg)	59 ± 2	57 ± 2	-2[-5,1]	61 ± 1.5	57 ± 1.4*	-4[-6,-1]
Heart rate (bpm)	64 ± 2	66 ± 2	2 [-2,7]	69 ± 1.9	69 ± 2.7	1 [-5,6]

* $p < 0.01$ Euglycaemia vs. Hypoglycaemia

Table VI.4.2 Measurements of the cognitive function tests (general, right hemisphere and visual perception threshold tests) during the two study conditions from euglycaemia and hypoglycaemia (mean ± se).

	C-replete		C-naïve	
	Euglycaemia	Hypoglycaemia	Euglycaemia	Hypoglycaemia
Trail-making B (s)	48 ± 7.2	62 ± 9.7	43 ± 5.7	56 ± 7.7
Digit symbol substitution (total score)	47 ± 1.5	44 ± 1.8	45 ± 2.2	41 ± 2.7
4 CRT (s)	0.53 ± 0.02	0.57 ± 0.03	0.52 ± 0.02	0.58 ± 0.03
Line matching (wrong score)	5 ± 1.2	5 ± 1.1	3 ± 0.8	4 ± 1.2
VCD (total score)	42 ± 1.3	40 ± 1.6	41 ± 1.5	40 ± 1.8
VMD (total score)	54 ± 0.9	54 ± 2.0	55 ± 1.1	55 ± 1.0

Discussion

After a period of abstinence, acute caffeine ingestion simultaneously decreases cerebral blood flow (Mathew R.et al 1985a) and increases brain glucose utilisation (Nehlig A. et al 1984). The clinical consequences of these effects of caffeine are a markedly augmented hormonal and symptomatic response to hypoglycaemia (Debrah K.et al 1996). In the current study, where acute withdrawal was avoided, ingestion of caffeine was associated with less suppression of middle cerebral artery velocity (a marker of cerebral blood flow) but virtually identical sympatho-adrenal and global cognitive responses to hypoglycaemia. In addition caffeine status also influenced the intensity of associated warning symptoms depending on the duration of the hypoglycaemic stimulus.

In humans, tolerance to the peripheral effects of caffeine is recognised (Robertson D.et al 1981) but controversial (James J. 1997b). Studies in young and middle-aged normotensive individuals suggest a minimum period of 12 hours abstinence is needed to avoid developing tolerance to the peripheral haemodynamic effects of acute caffeine ingestion (Shi J.et al 1993). In animals, tolerance to the effect of caffeine on cerebral energy metabolism does not appear to develop (Nehlig A. et al 1992). Here, sustained caffeine use was associated with a smaller change in middle cerebral artery velocity suggesting a degree of central tolerance to caffeine although the blood pressure and catecholamine responses were not influenced by caffeine status. In an earlier study, using orthostasis as a stimulus to sympathoadrenal activation, dissociation between central and peripheral tolerance to caffeine also developed (Debrah K.et al 1995), with tolerance developing in the latter only.

During euglycaemia, caffeine directly stimulates adrenomedullary catecholamine release with plasma adrenaline being more sensitive to caffeine than noradrenaline (Robertson D.et al 1978). The mechanism of action is unclear although in tetraplegic individuals, in whom sympathoadrenal responses are blunted, caffeine ingestion is not associated with a rise in adrenaline (Van Soeren M.et al 1996). Here there was a marked rise in plasma adrenaline levels during hypoglycaemia which was greater than that seen in healthy subjects who had not been challenged with caffeine at the onset of

hypoglycaemia (Bolli G.et al 1984; Debrah K.et al 1996). Tolerance to caffeine-augmented adrenaline rise during hypoglycaemia did not develop. The stimulus to a rise in plasma adrenaline levels in association with a low blood glucose level is mediated through the ventromedial hypothalamus with modulation by other higher centres (Borg W.et al 1995). Our data would suggest a similar degree of caffeine-associated neuroglycopenia in this region at the onset of hypoglycaemia, which is independent of caffeine exposure. This may be clinically relevant if caffeine proves to be a useful adjuvant treatment for diabetic patients who have abnormal counterregulatory responses to hypoglycaemia.

During the early phase of hypoglycaemia, warning symptoms were more intense in the caffeine-naïve state. However, with a more prolonged period of hypoglycaemia, symptom intensity was greater in the caffeine-replete state. The mechanisms involved are unknown but could reflect differences in baseline rates of cerebral glucose metabolism according to recent caffeine exposure. Although it is assumed that non-diabetic subjects always have symptoms when their blood glucose levels are low, during sustained hypoglycaemia warning symptoms and abnormalities in cognitive function wane with time despite sustained increments in counter-regulatory hormone levels (Frier B. et al 1999). Interestingly the increase in hypoglycaemic symptoms with sustained hypoglycaemia in the caffeine-replete state was associated with a less deleterious effect on cerebral blood flow (V_{MCA}). This supports the proposal that for patients at risk of prolonged low blood glucose levels, chronic caffeine use is better than abstention. An alternative explanation lies in the fact that brain lactate levels only rise in the caffeine-naïve state (Dager S. et al 1999) and that with sustained use this rise is lost. However lactate concentration increases within 20 minutes of caffeine consumption and does not correlate with anxiety.

In this study, tests of global cognitive performance deteriorated to the same extent in both the caffeine-replete and caffeine-naïve state. Previously Kerr et al reported that individual tests of right and left hemisphere function deteriorate to the same extent during hypoglycaemia in healthy, caffeine-naïve volunteers (Kerr D.et al 1993a). Here, chronic caffeine use was associated with greater deterioration in executive function as

illustrated by the verbal fluency tests. Although there is a large literature of formal studies examining the effect of caffeine on a variety of intellectual tasks, the results are often conflicting and inconclusive and may relate to increased arousal and suppression of boredom in repetitive tasks than a direct effect on intellectual performance (Nehlig A.et al 1992).

In summary, after a short period of abstinence, caffeine ingestion at the onset of hypoglycaemia is associated with markedly augmented hormonal and symptomatic responses. With regular caffeine use, the effects on middle cerebral artery blood velocity and early warning symptoms are attenuated but the hormonal responses are similar to the caffeine naïve state. Although, in general, deterioration in intellectual performance during hypoglycaemia is not influenced by caffeine status, specific tests of verbal processing are negatively affected by recent caffeine exposure. Whilst this finding could represent a type 2 error, the long-term consequences of this are unknown, warranting further investigation before caffeine can be suggested as an adjuvant treatment for patients who have difficulty in recognising the onset of hypoglycaemia.

Section VII CAFFEINE WITHDRAWAL

Study 5 Reversal of caffeine withdrawal by ingestion of a soft beverage

Caffeine, the most ubiquitous psychoactive substance in the world, continues to provoke controversy as to whether its use is associated with any adverse consequences for health (James J. 1997a). Whereas nicotine and alcohol are directly implicated in the deaths of more than 100,000 and 400,000 Americans each year (Pickworth W. 1995), the direct risk of caffeine ingestion is likely to be negligible (Goldstein A. et al 1990). However, potential problems associated with caffeine use maybe related to the issue of dependence and withdrawal.

Drug dependence is manifested by four criteria, namely withdrawal, tolerance, persistent desire and unsuccessful attempts to reduce consumption (American Psychiatric Association 1994). Unlike other major drugs of dependence, caffeine does not alter dopamine release or glucose utilisation within the nucleus accumbens (Nehlig A. 1998), nevertheless following regular caffeine ingestion acute cessation produces a specific symptom complex (Silverman K.et al 1992b), which is widely experienced (Strain E. et al 1994). Characteristic symptoms include headache, lethargy, fatigue and dysphoria (Griffiths R.et al 1990) and physiological changes e.g. a fall in blood pressure and rise in cerebral blood flow have also been reported in caffeine-withdrawn subjects (Couturier E.et al 1997). Such evidence has lead to the inclusion of caffeine withdrawal as a specific syndrome within the *Diagnostic and Statistical Manual of Mental Disorders* of the American Psychiatric Association, albeit as a proposed category only (American Psychiatric Association. 1994).

Previous work examining the syndrome of caffeine withdrawal have used doses in excess of 100 mg usually given in the form of coffee or caffeine capsules (Smith A.et al 1990). Depending on its duration of preparation, tea contains less caffeine than coffee (30mg compared to 85mg in a standard 150ml cup), as do cola soft drinks (33mg per can) (Barone J.et al 1996). Although tea is the most widely consumed drink in the world, the rise in consumption of cola beverages is phenomenal. This third vehicle of caffeine differs from coffee and tea in that approximately 95% of the caffeine is added during the manufacturing process (Institute 1983).

The aim of this study was to determine the effects of a small dose of caffeine given in the form of a market leading soft beverage on the physiological and psychological effects of the caffeine withdrawal syndrome.

Methods

Subjects

11 non-smoking nor drug taking, healthy women (aged 24-40 years), who were regular caffeine users (average daily consumption 143 to 773 mg). Subjects abstained from caffeine for 48 hours and fasting for 8 hours prior to testing.

Experimental procedure

On admission to the research unit, a retrograde cannula was inserted into the back of the non-dominant hand and placed in a 'hot-box'. After resting supine for 20 min, baseline measurements were taken of:

- Heart rate and blood pressure
- Brain blood flow using a transcranial Doppler technique to assess middle cerebral artery blood velocity (V_{MCA}).
- Mood using the UWIST mood score
- Cognitive function using 4-choice reaction time
- Plasma caffeine levels. Blood was taken from a vein draining the heated hand for subsequent measurement of caffeine.

Thereafter, subjects consumed, using a randomised and double-blind design, 660 mls (2 cans) of either regular (i.e. caffeinated) or caffeine-free DIET COKE (Coca-Cola Company, Atlanta, Georgia, USA) . On both occasions subjects also ate a candy bar (MARS Bar, Mars Company, Slough, UK) containing 70 g of sucrose to prevent hypoglycaemia. Thus, the total caffeine load on each occasion was 76 or 10 mg (Barone J.et al 1996) (assuming maximum caffeine content of MARS bar 10mg, as per communication with MARS company). All measurements were repeated in the same

position, at 30 min intervals for the next 2 hours with blood glucose levels measured every 15 min.

Statistical Analysis

Overall differences between serial measurements were examined by summary measures (Matthews J.et al 1993). Summary responses for each individual were calculated as area under the curve, or maximum response from baseline and contrasts in group means were compared by paired Student's t-test. Where data were not normally distributed comparisons were made after logarithmic transformation. Results are expressed as mean with 95% CI. Otherwise data are shown as mean ± se.

Results

Throughout both studies, there were no episodes of hypoglycaemia and blood glucose profiles were identical (peak value 8.5 ±0.6 mmol/l in both). Plasma caffeine levels were <0.1mg/L at the start of both studies and remained so following ingestion of caffeine-free DIET COKE. In contrast, plasma caffeine levels increased to average 2.0 mg/L after drinking regular DIET COKE.

At the start of both studies, Vmca was similar (72 ±2 and 70 ±2 cm/s). Within 30 min of drinking regular DIET COKE, this fell by 10% (95 % CI [6-14%], $p<0.005$) (Figure VII.5.1) associated with improvement in feelings of energy and hedonic mood (both $p<0.046$ and $p<0.037$ vs. caffeine-free DIET COKE) (Figure VII.5.2). Tense mood was unaffected.

At the start of both studies, total number of reactions (631 ±25 for DIET COKE and 621 ±23 for caffeine-free cola) and the number of correct reactions (620 ±18 and 618 ±19) performed over 5 minutes were similar. Both were unaffected by consumption of either cola drink (maximum total 654 ±22 and correct 648 ±21 for regular DIET COKE and 633 ±22 and 630 ±23 for caffeine-free cola, $p= 0.144$ and $p= 0.194$ respectively). On both occasions systolic blood pressure increased 30 minutes after ingesting the drinks (maximum increase $^{+}15 \pm 2$ and $^{+}12 \pm 2$ mmHg after regular and caffeine-free DIET COKE respectively, both $p<0.001$) and remained above baseline values for the duration of the studies (Figure VII.5.3). Diastolic pressure increased to a lesser extent ($^{+}9\pm1$ and $^{+}6\pm2$ mmHg, both $p<0.021$).

Figure VII.5.1 Middle cerebral artery blood velocity for all subjects following ingestion of 2 tins of regular or caffeine-free DIET COKE (mean ± se).

* = consumption of regular or caffeine-free DIET COKE and MARS Bar

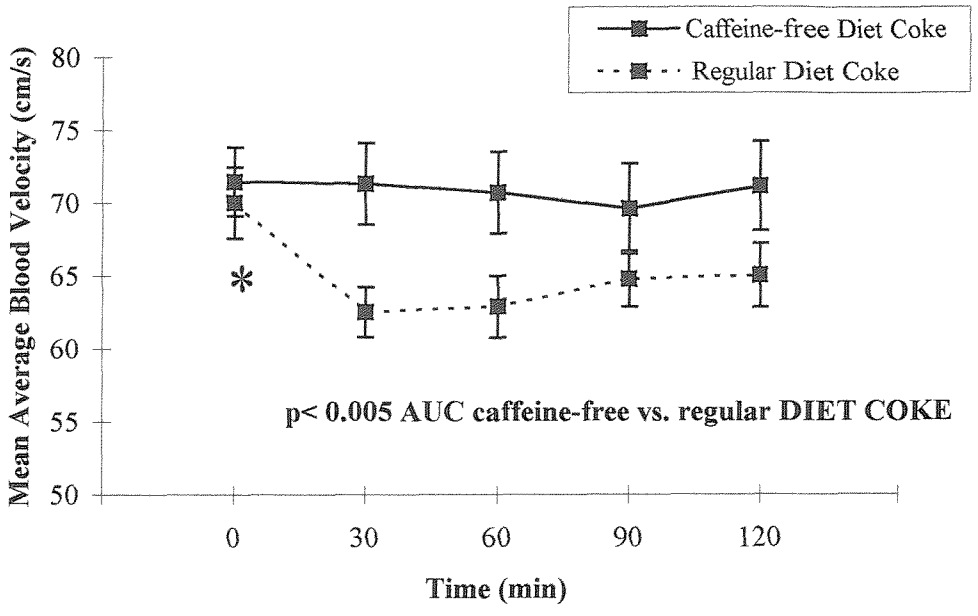
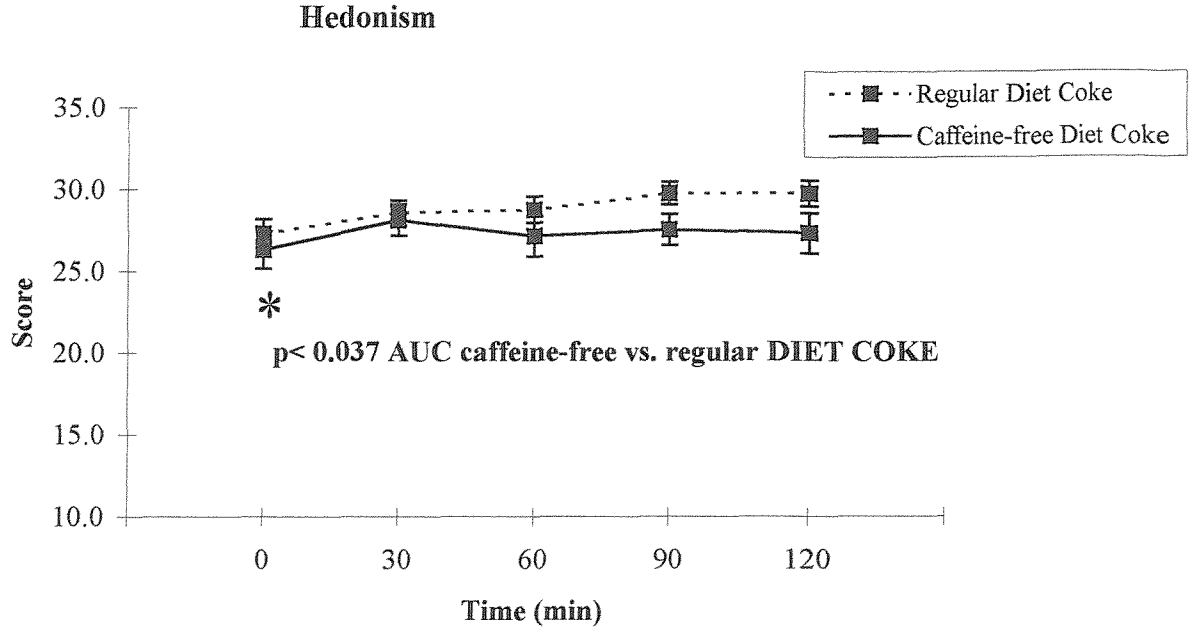


Figure VII.5.2 Mean (se) values for feelings of hedonism (upper panel) and energy (lower panel) for all subjects following ingestion of 2 tins of regular or caffeine-free DIET COKE .



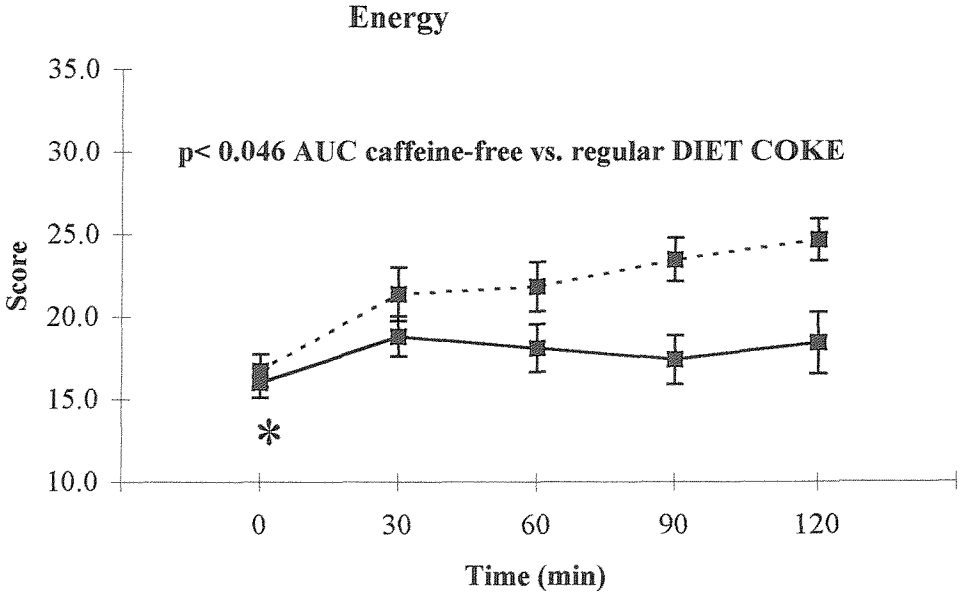
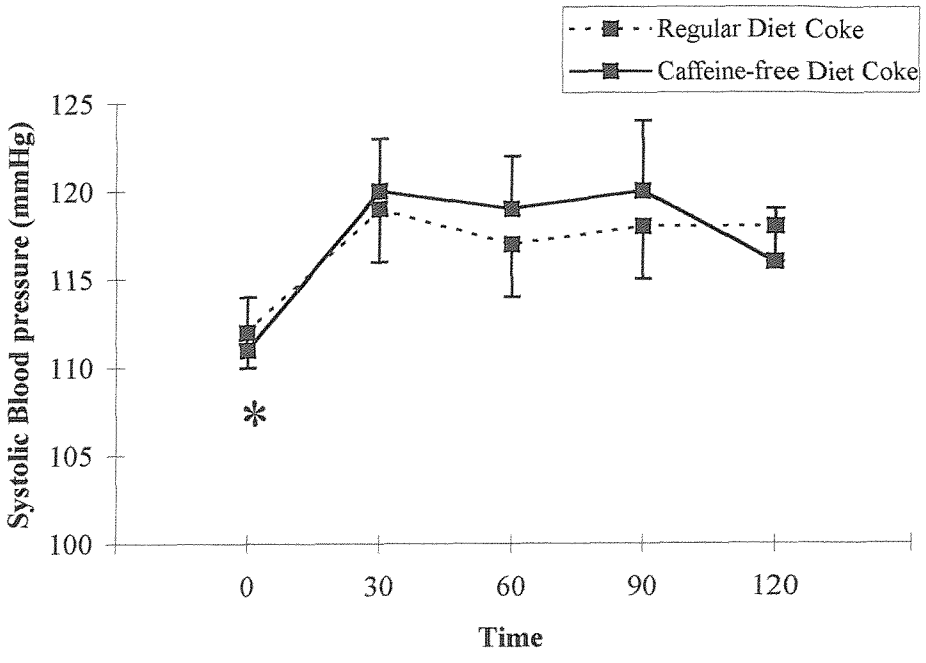
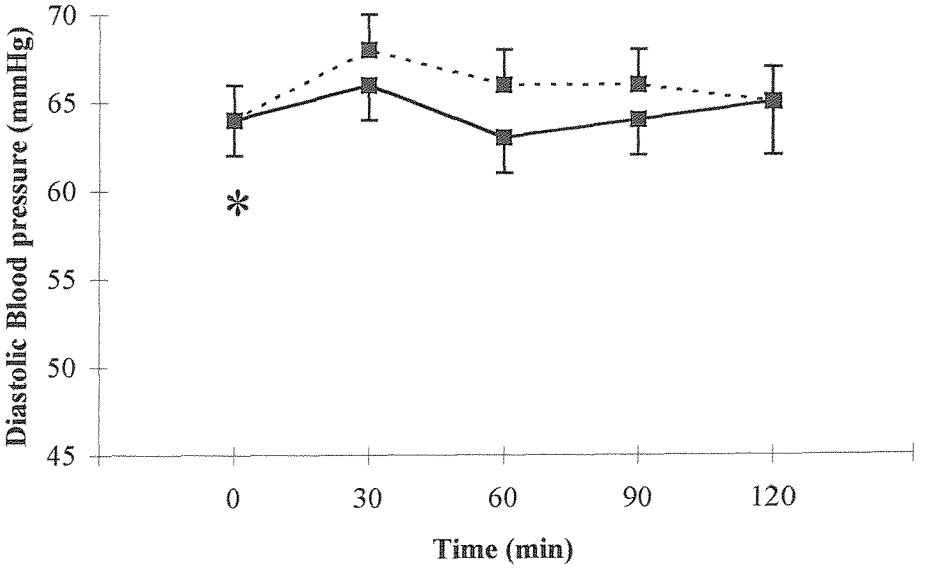


Figure VII.5.3 Mean (se) values for systolic (upper panel) and diastolic (lower panel) blood pressure following ingestion of 2 tins of regular or caffeine-free DIET COKE .





Discussion

In everyday life, the amount of caffeine consumed produces effects that are difficult to detect or so subtle as to go unnoticed. Caffeine is frequently added to non-alcoholic proprietary drinks as part of the manufacturing process although caffeine per se has no intrinsic nutritional value. The health consequences of this (if any) are unknown.

In the present study, subjects acutely withdrawn from caffeine ingestion of modest amounts of caffeine (76 mg) caused an almost immediate fall in middle cerebral artery blood velocity (an index of brain blood flow), together with marked improvement in subjects' mood without any change in cognitive function. Surprisingly, both systolic and diastolic blood pressure increased above baseline values after drinking both types of cola, irrespective of caffeine load.

Abstinence from caffeine leads to the symptoms of caffeine withdrawal. Consuming as little as 100mg each day is required before a person is at risk (Griffiths R.et al 1990). This state can be associated with marked functional impairment (Pickworth W. 1995) although this has not been consistently demonstrated (Lane J. 1997). Here we did not show any change in intellectual function as assessed by visual reaction time, with the reinstatement of caffeine consumption. This was unexpected as others have reported showing that as little as 32mg caffeine (less than in a single can DIET COKE) can increase 4-choice reaction time (Lieberman H.et al 1987) after 12 hours of caffeine abstinence. Durlach has also demonstrated significant speeding of the reaction time with 60mg of caffeine consumed as a cup of tea (Durlach P. 1998). Whilst it is possible that tea contains other psychoactive substances which may alter reaction time, other explanations for our results include differing plasma caffeine levels following administration (not measured in the 2 studies referenced). Alternatively differences could relate to fluctuation in blood glucose levels as hypoglycaemia negatively affects cognition. Interestingly 4-choice reaction time is particularly sensitive to mild hypoglycaemia (Mitrakou A.et al 1991; Maran A. et al 1995). Blood glucose levels were not reported in the earlier caffeine studies. Under normal circumstances, glucose is the sole metabolic fuel for normal brain function. In healthy people, prior ingestion of caffeine can be associated with the development of classical autonomic symptoms if

peripheral blood glucose levels fall into the low normal range as may occur after a large carbohydrate load. Here, subjects ate a candy bar in order to prevent neuroglycopenia. Subsequently, peripheral blood glucose levels remained well above the glycaemic thresholds release for activation of the sympathetic nervous system, release of adrenaline, alterations in cerebral blood flow and cognitive performance (Mitrakou A.et al 1991).

This study demonstrates a positive effect of a small amount of caffeine on its own withdrawal syndrome. Although in popular culture caffeine is thought to produce relief of fatigue and be mildly stimulating, it is possible that use continues in order to prevent the development of an unpleasant and perplexing withdrawal syndrome. Earlier work showed that when caffeine tolerant 92% of subjects would chose to drink caffeine compared to 52% of them when caffeine-naïve (Griffiths R.et al 1986a).

Caffeine has a vasoconstricting effect on the brain (Mathew R.et al 1985a). In caffeine naive individuals, ingestion of 250 mg reduces brain blood flow by approximately 20% associated with a rise in blood pressure and plasma adrenaline levels. In the present study, the pressor response to both types of DIET COKE was unexpected. Whilst it is well known that caffeine has a pressor response, the mechanism involved in the rise in blood pressure seen with caffeine-free cola is unknown but unlikely to relate to acute volume loading (DaCosta D. et al 1985). Unfortunately, we were unable to obtain information concerning other substances present in DIET COKE which might induce a pressor response.

Tea, coffee and in a lesser amount cola beverages all contain caffeine as it is present in the raw materials from which the beverage is made. Consumers can choose not to drink these beverages or select decaffeinated versions. For those who decide to continue with regular caffeine use, this maybe in order to prevent or ameliorate the syndrome of acute caffeine withdrawal.

Section VIII SUMMARY AND CONCLUSIONS

Hypoglycaemia is the commonest complication of insulin treatment. Patients with long-standing or well controlled diabetes are at risk of losing their awareness of hypoglycaemia in association with defective glucose counter-regulation. Whilst caffeine has been previously shown to increase the physiological and psychological responses to hypoglycaemia, to date the early work has not established the relevance of this in clinical practice.

The aims of this study were to extend the understanding of the interaction between caffeine and hypoglycaemic responses including the effects on the recovery period (study 2) and whether tolerance develops (studies 2-4). Furthermore, it was important to identify the effects of caffeine on hypoglycaemia recognition, in free-living patients (study 1). Whilst an extensive body of literature exists on the effects of caffeine on psychological function, blood pressure etc., previous studies have been criticised for not taking into account the effects of caffeine withdrawal prior to testing, thus a study looking at this phenomenon is included here (study 5). It is also considered in study 3. In combination these five studies describe the relationship between caffeine and hypoglycaemia further. The results are summarised below.

If caffeine is to be of benefit to hypoglycaemia awareness then the question of tolerance to the augmented responses seen in caffeine naïve subjects is an important one. Whether tolerance develops to the increased counter-regulatory hormones responses and symptoms and decreased CBF are considered in studies 1-4. The first of these was a clinical study of free-living diabetic patients and will be discussed separately, the other studies were laboratory based using healthy volunteers.

Study 2 required subjects to abstain from caffeine for 14 hours prior to the start of testing i.e. overnight abstention only. Compared to placebo, adrenaline and growth hormone production were significantly increased by the consumption of 200 mg caffeine prior to the induction of hypoglycaemia. Similarly the augmented symptom response with decreased CBF were demonstrated. Cognitive performance was equally compromised by hypoglycaemia, although caffeine influenced the recovery period negatively, following the restoration of euglycaemia. In studies 3 and 4 the effects of

tolerance were more directly studied. The two conditions studied were fully *caffeine-naïve* and fully *caffeine-replete*. With respect to the latter condition, subjects consumed 400 mg caffeine per day as 200 mg bd., for 7 days. When studied, subjects ingested a caffeine supplement prior to a second caffeine challenge several (approximately 3) hours later. Whilst some effects were diminished in the caffeine-replete state (e.g. V_{MCA}, blood pressure) significant differences from baseline were still observed, during the euglycaemic study (study 3) and hypoglycaemic study (study 4). Overall tolerance to the effects of caffeine was incomplete with respect to the specific parameters used namely V_{MCA}, blood pressure, mood (Study 3 only), cognition, catecholamine production (study 4 only), hypoglycaemia symptoms (study 4 only).

Study 2 also investigated the effects of caffeine into recovery from hypoglycaemia. In the placebo condition, V_{MCA} rose above baseline following the restoration of euglycaemia. Although V_{MCA} was rising at this time in the caffeine condition because of the initial fall, V_{MCA} failed to rise above baseline even after 70 minutes of euglycaemia. Hypoglycaemic symptoms were greater following caffeine ingestion prior to the induction of hypoglycaemia, this effect continued into recovery as well. Catecholamine production had returned to baseline levels at the beginning of the euglycaemic period, in contrast with GH production, which continued to rise with the restoration of euglycaemia. Thus caffeine consumption affects the recovery from hypoglycaemia with the negative consequences of prolonging symptoms and impairing improvements in cognition; although this latter finding was with the less sensitive test (visual movement detection) alone. This is of clinical importance in that caffeine potentially delays recovery from hypoglycaemia. Interestingly this study also showed that whilst catecholamine levels were higher in males than females during hypoglycaemia but with higher total symptom scores recorded reversely, cognition was not more adversely affected and there were gender specific V_{MCA} responses with V_{MCA} rising more significantly in females during recovery. In addition, Study 2 identified that caffeine whilst hypoglycaemia affects visual performance negatively with respect to P100 latencymeasurements, caffeine can have an additive effect on visually sensitive subjects.

Caffeine has variable effects on cognition as demonstrated by these studies. Whilst generally cognition was equally affected by hypoglycaemia in the caffeine-naïve and caffeine-replete conditions, a difference was seen with the verbal-logical tests. These tests, which relate directly to left (dominant) hemisphere function were more adversely affected in the caffeine-replete condition. As the hemispheres are equally affected by hypoglycaemia, this result was unexpected and may represent a type 2 error. Alternatively chronic caffeine ingestion may compromise higher cerebral function adversely.

Chronic caffeine consumption is associated with negative mood effects (study 3). Tense mood was significantly higher with regular caffeine consumption as opposed to the caffeine-naïve condition. This aspect of mood is positively affected by more caffeine consumption. This demonstrates that caffeine withdrawal should not be underestimated. Symptoms can develop over a short period of time. However, withdrawal from caffeine for 7 days was enough time for symptoms to regress. In study 3 caffeine withdrawal symptom scores were similar in both caffeine-naïve and caffeine-replete conditions. Caffeine withdrawal can be effectively treated with caffeine, the vehicle for which can be the less investigated cola soft-drinks (study 5). Improvements in cognitive performance however were not demonstrated here, only improvements in the positive aspects of mood (energy and hedonism).

The first study of this thesis is important because of its clinical relevance. The number of symptomatic mild hypoglycaemic events increased by 44%, which were associated with more intense warning symptoms. Thus patients were more aware of their blood glucose and able to take avoiding action by consuming carbohydrate in a relatively pure form. The number of severe hypoglycaemic episodes decreased in the caffeine consumption phase. However these were too infrequent for useful statistical comparison. Once again there was no evidence of tolerance developing across the 3 month phases of the cross-over trial. Neither diabetes control nor lipid profiles were compromised by caffeine consumption, although a pressor response was noted which was more marked in men.

It is relevant to contrast the different findings of study 1 and 4 here. In study 4 regular caffeine consumption was associated with more intense hypoglycaemic symptoms after approximately 60 minutes of hypoglycaemia; as opposed to the more intense symptoms of hypoglycaemia at the onset of hypoglycaemia in the caffeine-naïve state. This study was laboratory based using healthy volunteers and is in contrast to the increased symptoms reported by free-living subjects in study 1. Thus although regular caffeine consumption can affect the symptomatic repsonse to hypoglycaemia, this may be of less relevance clinically.

Caffeine has the potential to improve hypoglycaemic awareness. Increased responses to hypoglycaemia have been consistently demonstrated. Tolerance does not develop completely as demonstrated throughout the studies of this thesis. However, reservations would still apply. Tolerance does not develop to the pressor effects of caffeine either and whilst these may be minimal on normotensive populations, evidence exists to suggest that caffeine consumption is harmful to hypertensive people (borderline and established).

A second reservation before endorsing caffeine consumption for type 1 diabetic patients is the effects on cognition. Further work is needed to establish the effects during hypoglycaemia on cognitive function to fully elucidate the balance between increased neuroglycopenia (decreased CBF) and the psychostimulant action of caffeine. The finding of significantly increased deterioration in verbal-logical function, in study 4, requires that further work focus on the question of chronic caffeine consumption and cognitive function during hypoglycaemia. In addition caffeine affects recovery from hypoglycaemia as well. The clinical relevance needs to be fully determined once again. Furthermore the effects of caffeine on type 2 diabetes mellitus should be studied. Caffeine may be metabolised differently as well as having different affects on hypoglycaemic awareness.

When hypoglycaemia awareness becomes a major problem, as it does for a significant number of patients with diabetes, the aforementioned compromises associated with caffeine consumption could well exceed the problems described above, Thus when

quality of life is threatened by hypoglycaemia unawareness, caffeine could be incorporated into a programme to help increase hypoglycaemia awareness. The evidence presented here demonstrates that one of life's pleasures, enjoyed around the world, can be of benefit to patients with diabetes mellitus. It is not a perfect treatment to aid hypoglycaemic awareness and excessive consumption could have negative consequences as well as adversely affecting blood pressure control. However there is a group of patients with hypoglycaemia unawareness who could benefit from 1,3,7-trimethylxanthine.

REFERENCES

AMERICAN PSYCHIATRIC ASSOCIATION Diagnostic and statistical manual of mental disorders. Washington, DC. (1994)

AMIEL, S, D SIMONSON, W TAMBORLANE, R DEFRONZO AND R SHERWIN. Rate of glucose fall does not affect counterregulatory hormone responses to hypoglycemia in normal and diabetic humans. Diabetes (1987) 36: 518-522.

AMIEL, S. Cognitive function testing in studies of acute hypoglycaemia: rights and wrongs. Diabetologia (1998) 41: 713-719.

AMIEL, S, R SHERWIN, D SIMONSON AND W TAMBORLANE. Effect of intensive insulin therapy on glycemic thresholds for counterregulatory hormone release. Diabetes (1988) 37: 901- 907.

AMMON, H, P BIECK, D MANDALAZ AND E VERSPOHL. Adaptation of blood pressure to continuous heavy coffee drinking in young volunteers. Br J Clin Pharmacol (1983) 15: 701-706.

ANASTASI, A. Psychological Testing. New York, Macmillan (1990).

ANDERSON, K AND W REVELLE. Impulsivity, caffeine and proof-reading: a test of the Easterbrook hypothesis. J Exp Psychol (1982) 8: 614-624.

ARANDA, J, S MACLEOD, K RENTON AND N EADE. Hepatic microsomal drug oxidation and electron transport in newborn infants. J Pediatr (1974) 85: 534-542.

ARCIERO, P, A GARDNER, N BENOWITZ AND E POEHLMAN. Relationship of blood pressure, heart rate and behavioral mood state to norepinephrine kinetics in younger and older men following caffeine ingestion. Eur J Clin Nutrit (1998) 52: 805-812.

ARMITAGE, P AND G BERRY. Statistical method in medical research. Oxford, Blackwell Scientific Publications. (1987)

ARNAUD, M. The pharmacology of caffeine. Progress in Drug Res (1987) 31: 273-313.

ARNAUD, M AND C WELSCH. Theophylline and caffeine metabolism in man. Theophylline and other methylxanthines. N. Reitbrock, B. Woodcock and A. Staib. Zurich, Friedr. Vieweg and Sons (1982).

ARNOLD, M, T PETROS, B BECKWORTH, G COONS AND N NORMAN. The effects of caffeine, impulsivity and sex on memory for word lists. Physiol Behav (1987) 41: 25-30.

ARUNDA, J, J COLLINGE, R ZINMAN AND G WATTERS. Maturation of caffeine elimination in infancy. Arch Dis Childhood (1979) 54: 946-949.

AVOGARO, P, C CARPI, M PAIS AND G CAZOLATO. Plasma and urine cortisol behavior and fat mobilization in man after coffee ingestion. J Isr Med Sci (1973) 9: 114-119.

AZCONA, O, M BARBANOJ, J TORRENT AND F JANÉ. Evaluation of the central effects of alcohol and caffeine interaction. Br J Clin Pharmacol (1995) 40: 393-400.

BADDELEY, A. The cognitive psychology of everyday life. Brit J Psychol (1981) 72: 257-269.

BANTING, F, W CAMPBELL AND A FLETCHER. Further clinical experience with insulin (pancreatic extracts) on the treatment of diabetes mellitus. Brit Med J (1923) 1: 8-12.

BARONE, J AND H ROBERTS. Caffeine consumption. Food Chem Toxicol (1996) 34: 119-129.

BELLET, S, J KOSTIS, L ROMAN AND O DECASTRO. Effect of coffee ingestion on adrenocorticaal secretion in young men and dogs. Metabol (1969) 18: 1007-1012.

BENDER, A, R DONNERSTEIN, R SAMSON, D ZHU AND S GOLDBERG. Hemodynamic effects of acute caffeine ingestion in young adults. Am J Cardiol (1997) 79: 696-699.

BENTON, A AND K HAMSHER . Multilingual aphasia examination IV. Controlled word association. Benton Lab of Neuropsychology, University Hospitals, Iowa City (1978).

BENTON, A, H HANNEY AND N VARNEY. Judgement of line orientation. Neurology (1975) 25: 907-910.

BIAGGIONI, I, B OLAFSSON, R ROBERTSON, A HOLLISTER AND D ROBERTSON. Cardiovascular and respiratory effects of adenosine in conscious man. Evidence for chemoreceptor activation. Circ Res (1987) 61: 779-786.

BIGGERS, D, S MYERS, D NEAL, R STINSON, N COOPER, J JASPAN P WILLIAMS A CHERINGTON AND R FRIZZELL. Role of the brain in counterregulation of insulin-induced hypoglycaemia in dogs. Diabetes (1989) 37: 7-16.

BISHOP,C, S POWELL, D RUTT, N BROWSE. Transcranial Doppler measurement of middle cerebral artery blood flow velocity: a validation study. Stroke (1986) 17:913-915.

BLACKMAN, J, V TOWLE, G LEWIS, J SPIRE AND K POLONSKY. Hypoglycemic thresholds for cognitive dysfunction in humans. Diabetes (1990) 39: 828-835.

BLACKMAN, J, V TOWLE, J STURIS, G LEWIS, J SPIRE AND K POLONSKY. Hypoglycemic thresholds for cognitive dysfunction in insulin dependent diabetes. Diabetes (1992) 41: 392-399.

BOLLI, G, G CALABRESE AND P DE FEO. Lack of glucagon response in glucose counterregulation in type 1 (insulin-dependent) diabetics: absence of recovery after prolonged optimal insulin therapy. Diabetologia (1982) 22:100-105.

BOLLI, G, P DE FEO, G PERRIELLO, S DE COSMO, M VENTURA, P CAMPBELL, P BRUNETTI AND J GERICH. Role of hepatic autoregualtion in defense of hypoglycemia in humans. J Clin Invest (1985) 75: 1623-31.

BOLLI, G, P DE FEO, P COMPAGNUCCI, M CARTECHINI, G ANGELETTI,F SANTEUSANIO, P BRUNETTI AND J GERICH. Abnormal glucose counterregulation in insulin-dependent diabetes mellitus: interaction of anti-insulin antibodies and impaired glucagon and epinephrine secretion. Diabetes (1983) 32: 134-141.

BOLLI, G, I GOTTESMAN, P CRYER AND J GERICH. Glucose counterregulation during prolonged hypoglycemia in normal humans. Am J Physiol (1984) 247: E206-214.

BOLLI, G. Importance of catecholamines in defense against insulin hypoglycemia in humans. Adv Pharmacol (1998) 42: 627-630.

BONATI, M, R LATINI, F GALLETTI, J YOUNG, G TOGNONI AND S GARATTINI. Caffeine dispostion after oral doses. Clin Pharmacol Ther (1982) 32: 98-106.

BONATI, M, R LATINI, G TOGNONI, J YOUNG AND S GARATTINI. Interspecies comparison of in vivo caffeine pharmacokinetics in man, monkey, rabbit, rat and mouse. Drug Metab Rev (1984-85) 15: 1355-1383.

BORG, M, W BORG, W TAMBORLANE, M BRINES, G SHULMAN AND R SHERWIN. Chronic hypoglycemia and diabetes impair counterregulation induced by localized 2-deoxy-glucose perfusion of the ventromedial hypothalamus in rats. Diabetes (1999) 48: 584-587.

BORG, M, R SHERWIN, W BORG, W TAMBORLANE AND G SHULMAN. Local ventromedial hypothalamus glucose perfusion blocks counterregulation during systemic hypoglycaemia in awake rats. J Clin Invest (1997) 99: 361-365.

BORG, W, M DURING, R SHERWIN, M BORG, M BRINES AND G SHULMAN. Ventromedial hypothalamic lesions in rats suppress counterregulatory responses to hypoglycemia. J Clin Invest (1994) 93: 1677-1682.

BORG, W, R SHERWIN, M DURING, M BORG AND G SHULMAN. Local ventromedial hypothalamus glucopenia triggers counterregulatory hormone release. Diabetes (1995) 44: 180-184.

BOTT, S, U BOTT, M BERGER AND I MÜHLHAUSER. Intensified insulin therapy and the risk of severe hypoglycaemia. Diabetologia (1997) 40: 926-932.

BOTTINI, P, E BOSCHETTI, S PAMPANELLI, M CIOFETTA, P DEL SINDACO, L SCIONTI, P BRUNETTI AND G BOLLI. Contribution of autonomic neuropathy to reduced plasma epinephrine responses to hypoglycaemia in IDDM. Diabetes (1997) 46: 814-823.

BOYLE, P AND P CRYER. Growth hormone, cortisol or both are involved in defence against but are not critical to recovery from hypoglycemia. Am J Physiol (1991) 260: E395-402.

BOYLE, P, S KEMPERS, A O'CONNOR AND R NAGY. Brain glucose uptake and unawareness of hypoglycaemia in patients with insulin-dependent diabetes mellitus. New Eng J Med (1995) 333: 1726-1731.

BOYLE, P, N SCHWARTZ, S SHAH, W CLUTTER AND P CRYER. Plasma glucose concentrations at the onset of hypoglycemic symptoms in patients with poorly controlled diabetes and nondiabetics. New Eng J Med (1988) 318: 1487-1492.

BOYLE, P, R NAGY, A O'CONNOR, S KEMPERS AND R YEO. Adaptation in brain glucose uptake following recurrent hypoglycemia. Proc Natl Acad Sci (1994) 91: 9352-9356.

BRAATVEDT, G, M FLYNN, A STANNERS, M HALLIWELL AND R CORRALL. Splanchnic blood flow in man: evidence for mediation via a beta-adrenergic mechanism. Clin Sci (1993) 84:201-207.

BROWN, O, Y SOSA, M DARDENNE, J PLEAU AND R GOYA. Growth hormone-releasing activity of thymulin on pituitary somatotropes is age dependent. Neuroendocrinology (1999) 69: 20-27.

BRUCE, M, N SCOTT, M LADER AND V MARKS. The psychopharmacological and electrophysiological effects of single doses of caffeine in healthy human subjects. Br J Clin Pharmacol (1986) 22: 81-87.

BRUCE, M, N SCOTT, P SHINE AND M LADER. Anxiogenic effects of caffeine in patients with anxiety disorders. Arch Gen Psychi (1992) 49: 867-879.

BURKE, V, L BEILIN, R GERMAN, S GROSSKOPF, J RITCHIE, I PUDDEY AND P ROGERS. Association of lifestyle and personality characteristics with blood pressure and hypertension: a cross-sectional study in the elderly. J Clin Epidemiol (1992) 45: 1061-1070.

CASAS, M, S FERRE, A COBOS, J GRAU AND F JANE. Relationship between rotational behaviour induced by apomorphine and caffeine in rats with unilateral lesion of the nigrostriatal pathway. Neuropharmacol (1989) 28: 407-409.

CHU, C, D SINDELAR, D NEAL, E ALLEN AND P DONAHUE. Comparison of the direct and indirect effects of epinephrine on hepatic glucose production. Diabetes (1996) 45: 167A.

CHVASTA, T AND A COOK. Emptying and absorption of caffeine from the human stomach. Gastroenterology (1971) 61: 838-843.

CLARKE, W, L GONDER-FREDERICK, F RICHARDS AND P CRYER. Multifactorial origin of hypoglycemic symptom awareness in IDDM. Diabetes (1991) 40: 680-685.

CLARKE, W, D COX AND L GONDER-FREDERICK. Reduced awareness of hypoglycemia in adults with IDDM. A prospective study of hypoglycemic frequency and associated symptoms. Diabetes Care (1995) 18: 517- 522.

CLUBLEY, M, C BYE, T HENSON, A PECK AND C RIDDINGTON. Effects of caffeine and cyclizine alone and in combination on human performance, subjective effects and EEG activity. Br J Clin Pharmacol (1979) 7: U57-U63

COHEN, N, L ROSSETTI, P SHLIMOVICH, M HALBERSTAM, M HU AND H SHAMOON. Counterregulation of hypoglycemia: skeletal muscle glycogen metabolism during three hours of physiological hyperinsulinaemia in humans. Diabetes (1995) 44:423-430.

COLTON, T, R GOSSELIN AND R SMITH. The tolerance of coffee drinkers to caffeine. Clin Pharmacol Ther (1968) 9: 31-39.

COSTA, R AND I BIAGGIONI. Role of adenosine in the sympathetic activation produced by isometric exercise in humans. J Clin Invest (1994) 93: 1654-1660.

COUTURIER, E, D LAMAN, M VAN DUIJIN AND H VAN DUIJIN. Influence of caffeine and caffeine withdrawal on headache and cerebral blood flow velocities. Cephalagia (1997) 17: 188-190.

COX, D, L GONDER-FREDERICK, B ANTOUN, P CRYER AND W CLARKE. Perceived symptoms in the recognition of hypoglycemia. Diabetes Care (1993a) 16: 519-527.

COX, D, L GONDER-FREDERICK AND W CLARKE. Driving decrements in type 1 diabetes during moderate hypoglycemia. Diabetes (1993b) 42: 239-243.

COX, D, G IRVING, L GONDER-FREDERICK, G NOWACEK AND J BUTTERFIELD. Fear of hypoglycaemia: Quantification, validation and utilization. Diabetes Care (1987) 10: 617-621.

COX, D, B KOVATCHEV, D JULIAN, L GONDER-FREDERICK, W POLONSKY, D SCHLUNDT AND W CLARKE. Frequency of severe hypoglycaemia in IDDM can be predicited from self-monitoring blood glucose data. J Clin Endocrinol Metab (1994) 79: 1659-1662.

CRANSTON, I, J LOMAS, A MARAN, I MACDONALD AND S AMIEL. Restoration of hypoglycaemia unawareness in patients with long duration insulin-dependent diabetes mellitus. Lancet (1994) 344: 283-287.

CRYER, P. Glucose counterregulation in man. Diabetes (1981) 30: 261-264.

CRYER, P. Iatrogenic hypoglycemia as a cause of hypoglycemia-associated autonomic failure in IDDM: a vicious circle. Diabetes (1992) 41: 255-260.

CRYER, P. Hypoglycemia: the limiting factor in the management of IDDM. Diabetes (1994) 43: 1378-89.

CRYER, P. Glucagon and glucose counterregulation. Glucagon volume 3. P. Lefebvre. Berlin, Springer-Verlag (1996).

DACOSTA, D, C MCINTOSH, R BANNISTER, NJCHRISTENSEN AND C MATHIAS. Unmasking of the cardiovascular effects of carbohydrate in subjects with sympathetic denervation. J Hypertension (1985) 3: S447-S448.

DAGER, S, M LAYTON, W STRAUSS, T RICHARDS, A HEIDE, S FRIEDMAN, A ARTRU, C HAYES AND S POSSE. Human brain metabolic response to caffeine and the effects of tolerance. Am J Psychiatry (1999) 156: 229-237.

DAGOGO-JACK, S, S CRAFT AND P CRYER. Hypoglycemia-associated autonomic failure in insulin dependent diabetes mellitus. J Clin Invest (1993) 91: 819-828.

DAGOGO-JACK, S, C RATTARASARN AND P CRYER. Reversal of hypoglycemia unawareness but not defective glucose counterregulaiton, in IDDM. Diabetes (1994) 43: 1426-1434.

DALY, J . Mechanisms of action of caffeine. Caffeine, Coffee and Health. S.Garattini. New York, Raven Press (1993).

DANIELS, J, P MOLE, J SHAFFRATH AND C STEBBINS. Effects of caffeine on blood pressure, heart rate, and forearm blood flow during dynamic leg exercise. J Appl Physiol (1998) 85: 154-159.

DAVIS, P. Effect on electrencephalogram if changing the blood glucose level. Arch Neurol Psychiat (1943) 49: 186.

DAVIS, S, C SHEVERS, R MOSQUEDA-GARCIA AND F COSTA. Effects of differing antecedent hypoglycaemia on subsequent counterregulation in normal humans. Diabetes (1997) 46: 1328-1335.

DE FEO, P, G PERRIELLO, E TORLONE, M VENTURA, F SANTEUSANIO, P BRUNETTI, J GERICH AND G BOLLI. Demonstration of the role of growth hormone in glucose counterregulation. Am J Physiol (1989a) 256: E835-43.

DE FEO, P, G PERRIELLO, E TORLONE, M VENTURA, C FANELLI, F SANTEUSANIO, P BRUNETTI, J GERICH AND G BOLLI. Contribution of cortisol to counterregulation in humans. Am J Physiol (1989b) 257: E35-42.

DE FEO, P, G PERRIELLO, E TORLONE, C FANELLI, M VENTURA, F SANTEUSANIO, P BRUNETTI, J GERICH AND G BOLLI. Contribution of adrenergic mechanisms to glucose counterregulation in humans. Am J Physiol (1991a) 261: E35-42.

DE FEO, P, G PERRIELLO, E TORLONE, C FANELLI, M VENTURA, F SANTEUSANIO, P BRUNETTI, J GERICH AND G BOLLI. Evidence against important catecholamine compensation for absent glucagon counterregulation. Am J Physiol (1991b) 260: E203-212.

DE FRONZO, R, J TOBIN AND R ANDRES. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol (1979) 237: E214-E223.

DEL RIO, G, R MENOZZI, G ZIZZO, A AVOGARO, P MARRAMA AND A VELARDO. Increased cardiovascular response to caffeine in perimenopausal women before and during estrogen therapy. Eur J Endocrinol (1996) 135: 598-603.

DEAKINS, M. Effects of caffeine on human sugar-tolerance curves. Proc Soc Exp Biol Med (1939) 40: 588-589.

DEARY, I, D HEPBURN, K MACLEOD AND B FRIER. Partitioning the symptoms of hypoglycaemia using multi-sample confirmatory factor analysis. Diabetologia (1993a) 36: 771-777.

DEARY, I. Effects of diabetes on cognitive function. Hypoglycaemia and Diabetes: Clinical and Physiological Aspects. B. Frier and B. Fisher. London, Edward Arnold (1993b).

DEARY, I AND B FRIER. Severe hypoglycaemia and cognitive impairment. Brit Med J (1996) 313: 767-768.

DEBLER, E, I WAJDA, I MANIGAULT, A BURLINA AND A LAJTHA. Effects of caffeine on amino acid transport in the brain. Neurochem Int (1989) 14: 55-60.

DEBRAH, K, R HAIGH, R SHERWIN, J MURPHY AND D KERR. Effect of acute and chronic caffeine use on the cerebrovascular, cardiovascular and hormonal responses to orthostasis in healthy volunteers. Clin Sci (1995) 89: 475-480.

DEBRAH, K, R SHERWIN, J MURPHY AND D KERR. Effect of caffeine on the recognition of and physiological responses to hypoglycaemia in insulin-dependent diabetes. Lancet (1996) 347: 19-24.

DEBRY, G. Coffee and Health. Paris, John Libbey Eurotext (1994).

DENARO, C AND N BENOWITZ. Caffeine metabolism. Drug and Alcohol Abuse Reviews. R. Watson. Totowa, The Human Press Inc (1991).

DENARO, C, C BROWN, PJACOB III AND N BENOWITZ. Effects of caffeine with repeated dosing. Eur J Clin Pharmacol (1990) 40: 273-278.

DIABETES CONTROL AND COMPLICATIONS TRIAL RESEARCH GROUP. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-treated diabetes mellitus. New Eng J Med (1993) 329: 977-986.

DIABETES CONTROL AND COMPLICATIONS TRIAL RESEARCH GROUP. Adverse events and their association with treatment regimens in the Diabetes Conrol and Complications Trial. Diabetes Care (1995) 18: 1415-27.

DIABETES CONTROL AND COMPLICATIONS TRIAL RESEARCH GROUP. Effects of intensive diabetes therapy on neuropsychological function in adults in the Diabetes Control and Complications Trial. Ann Intern Med (1996a) 124: 379-388.

DIABETES CONTROL AND COMPLICATIONS TRIAL RESEARCH GROUP. Influence of intensive diabetes treatment on quality of life outcomes in the Diabetes Control and Complications Trial. Diabetes Care (1996b) 19: 195-203.

DIABETES CONTROL AND COMPLICATIONS TRIAL RESEARCH GROUP. Hypoglycaemia in the Diabetes Control and Complications Trial. Diabetes (1997) 46: 271-86.

DIBNER-DUNLAP, M, T KINUGAWA AND M THAMES. Activation of cardiac sympathetic afferents: effects of exogenous adenosine and adenosine analogues. Am J Physiol (1993) Heart Circ Physio 34: H395-H400.

DIMPFEL, W, F SCHOBER AND M SPULER. The influence of caffeine on human EEG under resting condition and during mental loads. Clin Investig (1994) 71: 197-207.

DONCHIN, E AND M COLES. Is the P300 component a manifestation of context updating? Behav Brain Sci (1988) 11: 357-374.

DRAELOS, M, A JACOBSON, K WEINGER, B WIDOM, C RYAN, D FINKELSTEIN AND D SIMONSON. Cognitive function in patients with insulin dependent diabetes mellitus during hyperglycemia and hypoglycemia. Am J Med (1995) 98: 135- 144.

DULLOO, A, J SEYDOUX AND L GIRARDIER. Paraxanthine (metabolite of caffeine) mimics caffeine's interaction with sympathetic control of thermogenesis. Am J Physiol (1994) 267: E801-804.

DURING, M, P LEONE, K DAVIS, D KERR AND R SHERWIN. Glucose modulates rat substantia nigra GABA release in vivo via ATP-sensitive potassium channels. J Clin Invest (1995) 95: 2403-2408.

DURLACH, P. The effects of a low dose of caffeine on cognitive performance. Psychopharmacol (1998) 140: 116-119.

EATON, W AND J MCLEOD. Consumption of coffee or tea and symptoms of anxiety. Am J Public Health (1984) 74: 66-68.

ECKERT, B, E RYDING AND C-D AGARDH. Sustained elevation of cerebral blood flow after hypoglycaemia in normal man. Diabetes Res Clin Pract (1998) 40: 91-100.

EGGERTSEN, R, A ANDREASSON, T HEDNER, B KARLBERG AND L HANSSON. Effect of coffee on ambulatory blood pressure in patients with treated hypertension. J Intern Med (1993) 233: 351-355.

EVANS, S AND R GRIFFITHS. Dose-related caffeine discrimination in normal volunteers: Individual differences in subjective effects and self-reported use. Behav Pharmacol (1991) 2: 345-356.

EVANS, S AND R GRIFFITHS. Caffeine withdrawal: A parametric analysis of caffeine dosing conditions. J Pharmacol Exp Ther (1999) 289: 285-294.

FANELLI, C, P DE FEO, F PORCELLATTI, G PERRIELLO, E TORLONE, F SANTEUSANIO, P BRUNETTI AND G BOLLI. Adrenergic mechanisms contribute to the late phase of hypoglycaemic glucose counterregulation in humans by stimulating lipolysis. J Clin Invest (1992) 89:2005-2013.

FANELLI, C, L EPIFANO, A RAMBOTTI, S PAMPANELLI, A DIVINCENZO, F MODARELLI, M LEPORE, B ANNIBALE, M CIOFETTA, P BOTTINI, F PORCELLATI, L SCIONTI, F SANTEUSANIO, P BRUNETTI AND G BOLLI. Meticulous prevention of hypoglycemia normalizes the glycemic thresholds and magnitude of most neuroendocrine responses to symptoms of and cognitive function during hypoglycemia in intensively treated patients with short-term IDDM. Diabetes (1993) 42: 1683-1689.

FANELLI, C, S PAMPANELLI, L EPIFANO, A RAMBOTTI, M CIOFETTA, F MODARELLI, A DI VINCENZO, B ANNIBALE, M LEPORE, C LALLI, P DEL SINDACO, P BRUNETTI AND GB BOLLI. Relative roles of insulin and hypoglycaemia on induction of neuroendocrine responses to, symptoms of, and deterioration of cognitive function in hypoglycaemia in male and female. Diabetologia (1994a) 37: 797-807.

FANELLI, C, S PAMPANELLI, L EPIFANO, A RAMBOTTI, A DIVINCENZO, F MODARELLI, M CIOFETTA, M LEPORE, B ANNIBALE, E TORLONE, G PERRIELLO, P DE FEO, F SANTEUSANIO, P BRUNETTI AND GB BOLLI. Long-term recovery from unawareness deficient counterregulation and lack of cognitive dysfunction during hypoglycaemia following institution of rational intensive therapy in IDDM. Diabetologia (1994b) 37: 1265-1276.

FANELLI, C, D PARAMORE, T HERSHEY, C TERKAMP, F OVALLE, S CRAFT AND P CRYER. Impact of nocturnal hypoglycemia on hypoglycemic cognitive dysfunction in type 1 diabetes. Diabetes (1998) 47: 1920-1927.

FASTBOM, J, A PAZOS AND J PALACIOS. The distribution of adenosineA1-reeptors and 5'-nucleotidase in the brain of some commonly used experimental animals. Neurosci (1987) 22: 813-826.

FELLOWS, I, I MACDONALD, H WHARRAD AND A BIRMINGHAM. Low plasma concentrations of adrenaline and physiological tremor in man. J Neuro Neurosurg Psych(1986) 49: 369-399.

FERRE, S, G VON EULER, B JOHANSSON, B FREDHOLM AND K FUXE. Stimulation of high affinity A2 receptors decrease the affinity of dopamine D2 receptors in rat striatal membranes. Proc Natl Acad Sci (1991) 88: 7238-7241.

FERRE, S, B FREDHOLM, M MORELLI, P POPLI AND K FUXE. Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. Trends Neurosci (1997) 20: 482-487.

FERRE, S, K FUXE, G VON EULER, B JOHANSSON AND B FREDHOLM. Adensine-dopamine interactions in the brain. Neurosci (1992) 51: 501-512.

FERRE, S, W O'CONNOR, P SVENNINGSSON, L BJORKLUND, J LINDBERG, B TINNER, I STROMBERG, M GOLDSTEIN, S OGREN, U UNGERSTEDT, B FREDHOLM, K FUXE. Dopamine D1 receptor-mediated facilitation of GABAergic neurotransmission in the rat strioentopenduncular pathway and its modulation by adenosine A1 receptor-mediated mechanisms. Eur J Neurosci (1996) 8: 1545-1553.

FERRE, S, P POPOLI, L GIMENEZ-LLORT, U FINNMAN, E MARTINEZ, A SCOTTI DE CAROLIS AND K FUXE. Postsynaptic antagonistic interaction between adensoine A1 and dopamine D1 receptors. Neuro Report(1994): 6: 73-76.

FILE, S, A BOND AND R LISTER. Interaction between effects of caffeine and lorazepam in performance tests and self-ratings. J Clin Psychopharmacol (1982) 2: 102-106.

FISHER, B, G GILLEN, H DARGIE, G INGLIS AND B FRIER. The effects of insulin-induced hypoglycaemia on cardiovascular function in normal man: studies using radionuclide ventriculography. Diabetologia (1987) 30: 841-845.

FLETCHER, A AND W CAMPBELL. The blood sugar following insulin administration and the symptom complex-hypoglycemia. J Met Res (1922) 2: 637-649.

FRANCE, C AND B DITTO. Cardiovascular response to occupational stress and caffeine in telemarketing employees. Psychosom Med (1989) 51: 145-151.

FREDHOLM, B. Adenosine actions and adenosine receptors after 1 week treatment with caffeine. Acta Physiol Scand (1982) 115: 283-286.

FREDHOLM, B. Astra Award lecture, adenosine receptors and the actions of caffeine. Pharmacol Toxicol (1995) 76: 93-101.

FREDHOLM, B, M ABBRACCHIO, G BURNSTOCK, J DALY, T HARDEN, K JACOBSON, P LEFF AND M WILLIAMS. Nomenclature and classification of purinoreceptors. Pharmacol Rev (1994) 46: 143-156.

FREDHOLM, B, K FUXE AND L AGNATI. Effect of some phosphodiesterase inhibitors on central dopamine mechanisms. Eur J Pharmacol (1976) 38: 31-38.

FREDHOLM, B. Are methylxanthines effects due to antagonism of endogenous adenosine? Trends Pharmacol Sci (1980) 1: 129-132.

FRENCH, E AND R KILPATRICK. The role of adrenaline in hypoglycaemic reactions in man. Clin Sci (1955) 14: 639-651.

FREWER, L AND M LADER. The effects of caffeine on two computerized tests of attention and vigilance. Hum Psychopharmacol (1991) 6: 119-128.

FRIER, B AND B FISHER. Impaired hypoglycaemia awareness. Hypoglycaemia in clinical diabetes. B. Frier and B. Fisher. Chichester, John Wiley & Sons (1999).

FRIZZEL, R, E JONES, SNDAVIS, D BIGGERS, S MYERS, C CONNOLLY, D NEAL, J JASPER AND A CHERRINGTON. Counterregulation during hypoglycemia is directed by widespread brain regions. Diabetes (1993) 42: 1253-1261.

GALE, E. Hypoglycaemia. Clinics Endocrinol Metabol (1980) 9: 461-475.

GARBER, A, P CRYER, J SANTIAGO, M HAMMOND, A PAGLIARA AND D KIPNIS. The role of adrenergic mechanisms in the substrate and hormonal response to insulin-induced hypoglycemia in man. J Clin Invest (1976) 58: 7-15.

GARRETT, B AND S HOLTZMAN. D1 and D2 dopamine receptor antagonsits block caffeine-induced stimulation of locomotor activity in rats. Pharmacol Biochem Behav (1994) 47: 89-94.

GERICH, J AND G BOLLI. Counterreulatory failure. Hypoglycaemia and Diabetes: Clinical and Physiological aspects. B. Frier and B. Fisher. London, Edward Arnold (1993).

GERICH, J, M LANGLOIS, C NOACCO, J KARAM AND P FORSHAM. Lack of glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic alpha cell defect. Sci (1973) 182: 171-173.

GIBBS, F, E GIBBS AND W LENNON. Cerebral blood flow in man as influenced by adrenaline, caffeine, amyl nitrite and histamine. Am Heart J (1935) 10: 916-924.

GILBERT, R AND J GOLDZIEHER. The mechanism and prevention of cardiovascular changes due to insulin. Ann Int Med (1946) 25: 928-940.

GILBERT, R. Caffeine consumption. The methlyxanthines beverages and foods: chemistry, consumption, and health effects. G. A. Spiller. New York, Alan R. Liss (1984).

GOLD, A, I DEARY, K MACLEOD AND B FRIER. The effect of IQ level on the degree of cognitive deterioration experienced during acute hypoglycemia in normal humans. Intelligence (1995a) 20: 267-290.

GOLD, A, B FRIER, K MACLEOD AND I DEARY. A structural equation model for predictors of severe hypoglycaemia in patients with insulin-dependent diabetes mellitus. Diabetic Med (1997) 14: 309-315.

GOLD, A, I DEARY AND B FRIER. Hypoglycemia-induced cognitive dysfunction in diabetes mellitus: effect of hypoglycemia unawareness. Physiol Behav (1995b) 58: 501-511.

GOLD, A, I. DEARY AND B FRIER. Recurrent severe hypoglycaemia and cognitive function in type 1 diabetes. Diabetic Med (1993) 10: 503-508.

GOLD, A, K MACLEOD AND B FRIER. Frequency of severe hypoglycaemia in patients with type 1 diabetes with impaired awareness of hypoglycaemia. Diabetes Care (1994) 17: 697-703.

GOLD, A AND S MARSHALL. Cortical blindness and cerebral infarction associated with chronic hypolgycaemia. Diabetes Care (1996) 19: 1001-1003.

GOLDFIEN, A, R MOORE, Z ZILELI, L HAVENS, L BOLING AND G THORN. Plasma epinephrine and norepinephrine levels during insulin-induced hypoglycemia in man. J Clin Endocrinol Metab (1961) 21: 296-303.

GOLDSTEIN, A AND M WALLACE. Caffeine dependence in schoolchildren? Exp Clin Psychopharmacol (1997) 5: 388-392.

GOLDSTEIN, A AND H KALANT. Drug policy. Sci (1990) 249: 1513-1521.

GOLDSTEIN, D, R MCCARTY, R POLINSKY AND I KOPIN. Relationship between plasma norepinephrine and sympathetic neural activity. Hypertension (1983) 5: 552-559.

GOLDSTEIN, I, D SHAPIRO, K HUI AND J YU. Blood pressure response to the "second cup of coffee". Psychosom Med (1990) 52: 337-345.

GONDER-FREDERICK, L, D COX, S BOBBITT AND J PENNEBAKER. Blood glucose symptom beliefs of diabetic patients: accuracy and implications. Health Psychol (1986) 5: 327-341.

GONDER-FREDERICK, L, D COX, N DRIESEN, C RYAN AND W CLARKE. Individual differences in neurobehavioral disruption during mild and moderate hypoglycemia in adults with IDDM. Diabetes (1994) 43: 1407-1412.

GOULD, L, K VENKATARAMAN, M GOSWAMI AND R GOMPRECHT. The cardiac effects of coffee. Angiology (1973) 24: 455-463.

GRADY, D. Don't get jittery over caffeine. Discover (1986) July: 73-79.

GRAHAM, D. Caffeine-its identity, dietary sources, intake and biological effects. Nutr Rev (1978) 36: 97-102.

GRANT, S AND D Redmond. Methylxanthine activation of noradrenergic unit activity and reversal by clonidine. Eur J Pharmacol (1982) 85: 105-109.

GREDEN, J. Anxiety of caffeinsim: A diagnostic dilemma. Am J Psychi (1974) 131: 1089-1092.

GREEN, P AND J SULS. The effects of caffeine on ambulatory blood pressure, heart rate and mood on coffee drinkers. J Behavioral Med (1996) 19: 111-128.

GREENLAND, S. A meta-analysis of coffee, myocardial infarction and coronary death. Epidemiol (1993) 4: 366-374.

GRIFFITHS, R, G BIGELOW, I LIEBSON, M O'KEEFFE, D O'LEARY AND N RUSS. Human coffee drinking: manipulation of concentration and caffeine dose. J Exper Analysis Behav (1986a) 45: 133-148.

GRIFFITHS, R, G BIGLOW AND I LIEBSON. Reinforcing effects of caffeine in coffee and capsules. J Exp Anal Behav (1989) 52: 127-140.

GRIFFITHS, R, S EVANS, S HEISHMAN, K PRESTON, C SANNERUD, B WOLF AND P WOODSON. Low dose caffeine physical dependence in humans. J Pharmacol Exper Therapeut (1990) 255: 1123-1132.

GRIFFITHS, R AND G MUMFORD. Caffiene- A drug of abuse? Psychopharmacology: the fourth generation in progress. F. Bloom and D. Kupfer. New York, Raven Press (1995).

GRIFFITHS, R AND P WOODSON. Reinforcing effects fo caffeine in humans. J Pharmacol Exp Ther (1988a) 246: 21-29.

GRIFFITHS, R AND P WOODSON. Caffeine physical dependence: a review of human and laboratory animal studies. Psychopharmacol (1988b) 94: 437-451.

GRIFFITHS, RR, GE BIGELOW AND IA LIEBSON. Human coffee drinking: reinforcing and physical dependence producing effects of caffeine. J Pharmacol Exp Ther (1986b) 239: 416-425.

GROME, J AND V STEFANOVICH. Differential effects of methylxanthines on cerebral blood flow and glucose utilization in the conscious rat. Naunyn Schiedeberg's Arch Pharmacol (1986) 333: 172-177.

GUPTA, U, G DUBEY AND B GUPTA. Effects of caffeine on perceptual judgement. Biolog Psychol Pharmacopsychol (1994) 30: 185-188.

GYNTELBERG, F, HO HEIN, P SUADICANI AND H STRENSEN. Coffee consuption and risk of ischaemic heart disease. J Intern Med (1995) 237: 55-61.

HADFIELD, M AND C MILO. Caffeine and regional brain monoamine utilisation in mice. Life Sci (1989) 45: 2637-2644.

HALE, K AND J HUGHES. Caffeine self-administration and subjective effects in adolescents. Exp Clin Psychopharmacol (1995) 3: 364-370.

HAMILTON-WESSLER, M, R BERGMAN, J HALTER, R WATANABE AND P CONOVAN. The role of gluco-sensors in the integrated sympathetic response induced by deep hypoglycemia in dogs. Diabetes (1994) 43: 1052-60.

HARGREAVES, R, A PLANAS, J CREMER AND V CUNNINGHAM. Studies on the relationship between cerebral glucose transport and phosphorylation using 2-deoxyglucose. J Cereb Blood Flow Metab (1986) 6: 708-716.

HARRAD, R, C COCKRAM, A PLUMB, S STONE, P FENWICK AND P SÖNKSEN. The effects of hypoglycaemia on visual function: a clinical and electrophysiological study. Clin Sci (1985) 69: 673-679.

HART, P, G FARRELL, W COOKSLEY AND L POWELL. Enhanced drug metabolism in cigarette smokers. Br Med J (1976) 2: 147-149.

HAVEL, P AND G TABORSKY. The contribution of the autonomic nervous system to increased glucagon secretion during hypoglycemic stress: update 1994. The Endocrine Pancreas, Insulin Action and Diabetes. L. Underwood. Bethseda, The Endocrine Soceity (1994).

HAVEL, P AND C VALVERDE. Autonomic mediation of glucagon secretion during insulin induced hypoglycemia in rhesus monkeys. Diabetes (1996) 45: 960-966.

HELLER, S AND P CRYER. Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after 1 episode of hypoglycemia in nondiabetic humans. Diabetes (1991) 40: 223-226.

HELLER, S, M HERBERT, I MACDONALD AND R TATTERSALL. Influence of sympathetic nervous system on hypoglycaemic warning symptoms. Lancet (1987) 2: 359-363.

HELLER, S AND I MACDONALD. The measurement of cognitive function during acute hypoglycaemia: experimental limitations and their effect on the study of hypoglycaemia unawareness. Diabetic Med (1996) 13: 607-615.

HEPBURN, D, I DEARY AND B FRIER. Classification of symptoms of hypoglycaemia in insulin treated diabetic patients using factor analysis: relationship to hypoglycaemia unawareness. Diabetic Med (1992) 9: 70-75.

HEPBURN, D, K MACLEOD, A PELL, I SCOURGAL AND B FRIER. Frequency and symptoms of hypoglycaemia experienced by patients with type 2 diabetes treated with insulin. Diabetic Med (1993) 10: 231-237.

HEPBURN, D, A PATRICK, D EADINGTON, D EWING AND B FRIER. Unawareness of hypoglycaemia in insulin treated diabetic patients: prevalence and relationship to autonomic neuropathy. Diabetic med 7:711-717.

HEPBURN, D, A PATRICK, H BRASH, I THOMSON AND B FRIER. Hypoglycaemia unawareness in type 1 diabetes: a lower plasma glucose is required to stimulate sympathoadrenal activation. Diabetic Med (1991) 8: 934-945.

HEPBURN, D, A PATRICK, D EADINGTON, D EWING AND B FRIER. Unawareness of hypoglycaemia in insulin treated diabetic patients: prevalance and relationship to autonomic neuropathy. Diabetic Med (1990) 7: 711-717.

HERRINGTON, J AND B HILLE Growth hormone-releasing hexapeptide elevates intracellular calcium in rat somatotrophes by two mechanisms. Endocrinol (1994) 135: 1100-1108.

HESELTINE, D, M EL-JABRI, F AHMED AND J KNOX. The effect of caffeine on postprandial blood pressure in the frail elderly. Postgrad Med J (1991) 67: 543-547.

HILSTED, J. Cardiovascular changes during hypoglycaemia. Clin Physiol (1993) 13: 1-10.

HOELDTKE, R, S CAVANAUGH, J HUGHES AND M POLANSKY. Treatment of orthostatic hypotension with dihydroergotamine and caffeine. Ann Int Med (1986) 105: 168-173.

HÖFER, I AND K BATTIG. Cardiovascular, behavioral and subjective effects of caffeine under field conditions. Pharmacol Biochem Behav(1994a) 48: 899-908.

HÖFER, I AND K BATTIG. Psychophysiological effects if seitching to caffeine tablets or decaffeinated coffee under field conditions. Pharmacopsychoecologia (1994b) 7: 169-177.

HOFFMAN, R, D SPEELMAN, D HINNEN, K CONLEY, R GUTHRIE AND R KNAPP. Changes in cortical functioning with acute hypoglycaemia and hyperglycaemia in type 1 diabetes. Diabetes Care (1989) 12: 193-197.

HOLMES, C. Metabolic control and auditory information processing at altered blood glucose levels in insulin dependent diabetes. Brain Cog (1987) 6: 161-174.

HOLMES, C, J HAYFORD, J GONZALEZ AND J WEYDERT. A survey of cognitive functioning at different glucose levels in diabetic persons. Diabetes Care (1983) 6: 180-185.

HOLMES, C, K KOEPKE AND R THOMPSON. Simple versus complex impairments at three blood glucose levels. Psychneuroendocrinol (1986) 11: 353-357.

HOLMES, C, K KOEPKE, R THOMPSON, P GYVES AND J WEYDERT. Verbal fluency and naming performance in type 1 diabetes at different blood glucose concentrations. Diabetes Care (1984) 7: 454-459.

HORINAKA, N, T-Y KUANG, H PAK, R WANG, J JEHLE, C KENNEDY AND L SOKOLOFF. Blockade of cerebral blood flow response to insulin-induced hypoglycemia by caffeine and glibenclamide in conscious rats. J Cerebral Blood Flow Metabol (1997) 17: 1309-1318.

HORNE, J AND O OSTBERG. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol (1976) 4: 97-110.

HORTON, R . Caffeine. The Observer (1998) June 15.

HUGHES, J. Clinical importance of caffeine withdrawal. New Eng J Med (1992) 327: 1160-1161.

HUGHES, J, G AMORI AND D HATSUKAMI. A survey of physician advice about caffeine. J Substance Abuse (1988) 1: 67-70.

HUGHES, J, S HIGGINS, W BICKEL, W HUNT, J FENWICK, S GULLIVER AND G MIREAULT. Caffeine self-adminsration, withdrawal and adverse effects among coffee drinkers. Arch Gen Psych (1991) 48: 611-617.

HUGHES, J, A OLIVETO, W BICKEL, S HIGGINS AND G BADGER. The ability of low doses of caffeine to serve as reinforcers in humans. Exp Clin Pharmacol (1995) 3: 358-363.

HUGHES, RN. Drugs which induce anxiety: caffeine. N. Z. J. Psychol (1996) 25: 36-42.

HVIDBERG, A, HOJBY, M RASMUSSEN, N CHRISTENSEN AND J HILSTED. Theophylline enhances glucose recovery after hypoglycemia in helathy men and in type 1 diabetic patients. Metabolism (1994) 43:776-781.

HVIDBERG, A, A ROSENFLACK, N CHRISTENSEN AND J HILSTED. Long-term administration of theophylline and glucose recovery after hypoglycaemia in patients with type 1 diabetes mellitus. Diabetic Med (1998) 15: 608-614.

INSTITUTE OF FOOD TECHNOLOGISTS' EXPERT PANEL ON FOOD SAFETY AND NUTRITION. Caffeine: A scientific status summary. Food Technol (1983) 37: 87-91.

IZZO, J, A GHOSAL, T KWONG, R FREEMAN AND J JAENIKE. Age and prior caffeine use alter the cardiovascular and adrenomedullary responses to oral caffeine. Am J Cardiol (1983) 52: 769-773.

JACOBSON, A. The psychological care of patients with insulin dependent diabetes mellitus. New Engl J Med (1996) 344: 1249-1253.

JAMES, J. Is habitual caffeine use a preventable cardiovascular risk factor? Lancet (1997a) 349: 279-281.

JAMES, J, I PAULL, E CAMERON-TRAUBB, J MINERS, A LELO AND D BIRKETT. Biochemical validation of self-reported caffeine consumption during caffeine fading. J Behav Med (1988) 11: 15-30.

JAMES, J. Chronic effects of habitual caffeine consumption on laboratory and ambulatory blood pressure levels. J Cardiovas Res (1994a) 1: 159- 164.

JAMES, J. Does caffeine enhance or merely restore degraded psychomotor performance? Neuropsychobiol (1994b) 30: 124-125.

JAMES, JE. Understanding caffeine: A biobehavioural analysis. Thousand Oaks, CA, Sage (1997b).

JASPER, H. The ten-twenty electrode system of the International Federation. Electroencephalography and Clinical Neurophysiology (1958) 10: 371-375.

JARVIS, M. Does caffeine intake enhance absolute levels of cognitive performance. Psychopharmacol (1993) 110: 45-52.

JEONG, D AND J DIMSDALE. The effects of caffeine on blood pressure in the work environment. Am J Hypertens (1990) 3: 749-753.

JETTON, T, Y LAING, C PETTEPHER, E ZIMMERMAN, F COX, K HORVATH, F MATSCHINSKY AND M MAGNUSON. Analysis of upstream glucokinase promotor activity in transgenic mice and identification of glucokinase in rare neuroendocrine cells in the brain and gut. Biol Chem (1997) 269: 3641-3654.

JOHANSSON, B, S AHLBERG, I VAN DER PLOEG, S BRENE, N LINDEFORS, H PERSSON AND B FREDHOLM. Effect of long term caffeine treatment on A1 and A2 adenosine receptor binding and on mRNA levels in rat brain. Naunyn Schmiedebergs Arch Pharmacol (1993) 347: 407-414.

JOHANSSON, B, V GEORGIEV, T KUOSMANEN AND B FREDHOLM. Long term treatment with some methylxanthines decreases the susceptibility to bicculline and pentylenetetrazol-induced seizures in mice. Relationship to c-fos expression and receptor binding. Eur J Neurosci (1996) 8: 2447-2458.

JONES, T, G MCCARTHY, W TAMBORLANE, S CAPRIO, R SHERWIN, E ROESSLER, D KRAEMER, K STARICKZYCH, T ALLISON AND S BOULWARE. Mild hypoglycemia and impairment of brain stem and cortical evoked potentials in healthy subjects. Diabetes (1990) 39: 1550-1555.

KAHN, K AND R MYERS. Insulin induced hypoglycaemia in the non human primate. I. Clinical consequences. Brain Hypoxia, ClinicS in Developmental Medicine. J. b. Brierley and B. S. Meldrum. London, Heineman: 185-193 (1971).

KAPLAN, G, D GREENBLATT, B EHRENBERG, J GODDARD, M COTREAU, S JEROLD, J HARMATZ AND R SHADER. Dose-dependent pharmacokinetics and psychomotor effects of caffeine in humans. J Clin Pharmacol (1997) 37: 693-703.

KAPLAN, G, D GREENBLATT, M KENT, M COTREAU, G ARCELIN AND R SHADER. Caffeine induced behavioral stimulation is dose dependent and associated with A1 adenosine occupancy. Neuropsychopharmacol (1992) 6: 145-153.

KATHOLI, R, G HAGEMAN, P WHITLOW AND W WOODS. Hemodynamic and afferent renal nerve responses to intrarenal adenosine in the dog. Hypertension (1983) 5: 1149-1154.

KAWACHI, I, G COLDWITZ AND C STONE. Does coffee drinking increase the risk of coronary heart disease? Results from a meta-analysisBr Heart J (1994) 72: 269-275.

KAWACHI, I, W WILLETT, G COLDITZ, M STAMPFER AND F SPEIZER. A prospective study of coffee drinking and suicide in women. Arch Int Med (1996) 156: 521-525.

KAWAMURA, N, H MAEDA, J NAKAMURA, K MORITA AND Y NAKAZAWA. Effects of caffeine on event-related potentials: comparison of oddball with single tone paradigms. Psychic Clin Neurosci (1996) 50: 217-221.

KENDALL, D, D ROONEY, Y SMETS, L BOLDING AND R ROBERTSON. Pancreas transplantation restores epinephrine response and symptom recognition during hypoglycemia in patients with long standing type 1 diabetes and autonomic neuropathy. Diabetes (1997) 46: 249-257.

KENEMANS, J AND M LORIST. Caffeine and selective visual procesing. Pharmacol Biochem Behav (1995) 52: 461-471.

KENEMANS, J AND M VERBATEN. Caffeine and visuo-spatial attention. Psychopharmacol (1998) 135: 353-360.

KERR, D, I MACDONALD, S HELLER AND R TATTERSALL. Alcohol causes hypoglycaemic unawareness in healthy volunteers and patients with type 1 (insulin dependent) diabetes. Diabetologia (1990) 33: 216-221.

KERR, D, M REZA, N SMITH AND B LEATHERDALE. Importance of insulin in subjective, cognitive and hormonal responses to hypoglycemia in patients with IDDM. Diabetes (1991) 40:1057-1062.

KERR, D, R SHERWIN, F PAVALKIS, P FAYAAD, L SIKORSKI, F RIFE, W TAMBORLANE AND M DURING. Effect of caffeine on the recognition of and responses to hypoglycemia in humans. Ann Intern Med (1993a) 119: 799-804.

KERR, D, J STANLEY, M BARRON, R THOMAS, B LEATHERDALE AND J PICKARD. Symmetry of cerebral blood flow and cognitive responses to hypolgycaemia in humans. Diabetologia (1993b) 36: 73-78.

KLEBANOFF, M, R LEVINE, R DERSSIMONIAN, J CLEMENS AND D WILKINS. Maternal serum paraxanthine, a caffeine metabolite and the risk of spontaneous abortion. N Engl J Med (1999) 341: 1639-1644.

KNUTTI, R, H ROTHWEILER AND C SCHLATTER. Effect of pregnancy on the pharmacokinetics of caffeine. Eur J Clin Pharmacol (1981) 21: 121-126.

KOVATCHEV, B, D COX, L GONDER-FREDERICK, D YOUNG-HYMAN, D SCHLUNDT AND W CLARKE. Assessment of risk for severe hypolgycemia among adults with IDDM. Diabetes Care (1998) 21: 1870-1875.

KUMAGAI, A, Y-S KANG, R BOADO AND W PARDRIDGE. Upregulation of blood brain barrier GLUT1 glucose transporter protein and mRNA in experimental chronic hypoglycemia. Diabetes (1995) 44: 1399-1404.

LAING, S, A SWERDLOW, S SLATER, J BOTHA, A BURDEN, N WAUGH, A SMITH, R HILL, P BINGLEY, C PATTERSON, Z QIAO, H KEEN. The British Diabetic Association Cohort Study, II: cause specific mortality in patients with insulin-treated diabetes mellitus. Diabetic Med (1999) 16: 466-471.

LALA, V, V VEDANARAYANA, S GANESH, C FRAY AND S IOSUB. Hypoglycemic hemiplegia in an adolescent with insulin-dependent diabetes mellitus; a case report and a review of the literature. J Emergency Med (1989) 7: 233-236.

LANCASTER, T, J MUIR AND C SILAGY. The effects of coffee on serum lipids and blood pressure in a UK population. J Royal Soc Med (1994) 87: 506-507.

LANDOLT, H-P AND E WORTH. Caffeine intake (200mg) in the morning affects human sleep and EEG power spectra at night. Brain Res (1995) 675:67-74.

LANE, J, A ADCOCK, R WILLIAMS AND C KUHN. Caffeine effects on the cardiovascular and neuroendocrine responses to acute psychosocial stress and their relationship to level of habitual caffeine consumption. Psychosomatic Med (1990) 52: 320-336.

LANE, J AND D MANUS. Persistent cardiovascular effects with repeated caffeine administration. Psychosomatic Med (1989) 51: 373-380.

LANE, J AND B PHILLIPS-BUTE. Caffeine deprivation affects vigilance performance and mood. Physiol Behav (1998) 65: 171-175.

LANE, J, J STEEGE, S RUPP AND C KUHN. Menstrual cycle effects on caffeine elimination in the human female. Eur J Clin Pharmacol (1992) 43: 543-546.

LANE, J AND R WILLIAMS. Cardiovascular effects of caffeine and stress in regular coffee drinkers. Psychphysiol (1987) 24: 157-164.

LANE, J. Effects of brief caffeinated-beverage deprivation on mood, symptoms, and psychomotor performance. Pharmacol Biochem Behav (1997) 58: 203-208.

LAU, C AND J FALK. Tolerance to oral and IP caffeine: locomotor activity and pharmacokinetics. Pharmacol Biochem Behav (1994) 48: 337-344.

LECAVALIER, L, G BOLLI, P CRYER AND J GERICH. Contributions of gluconeogenesis and glycogenolysis during glucose counterregulation in normal humans. Am J Physiol (1989) 256: E844-851.

LEDENT, C, J VAUGEOIS, S SCHIFFMANN, T PEDRAZZINI, M EL YACOUBI, J VANDERHAEGEN, J COSTENTIN, J HEATH, G VASSART AND M PARMENTIER. Aggressiveness hypoanalgesia and high blood pressure in mice lacking the adenosine A2a receptor. Nature (1997) 388: 674-678.

LEWIS, C, B CAAN, E FUNKHOUSER, J HILNER, C BRAGG, A DYER, J RACZYNSKI, P SAVAGE, M ARMSTRONG AND G FRIEDMAN. Inconsistent associations of caffeine-containing beverages with blood presure and with lipoproteins. Am J Epidemiol (1994) 138: 502-507.

LIEBERMAN, H, R WURTMAN, G EMDE, C ROBERTS AND I COVIELLA. The effects of low doses of caffeine on human performance and mood. Psychopharmacology (1987) 92: 308-312.

LIGUORI, A, J HUGHES AND J GRASS. Absorption and subjective effects of caffeine from coffee, cola and capsules. Pharmacol Biochem Behav (1997) 58: 721-726.

LINDGREN, M, B ECKERT, G STENBERG AND C AGARDH. Restitution of neurophysiological functions, performance and subjective symptoms after moderate insulin-induced hypoglycaemia in non-diabetic man. Diabetic Med (1996) 13: 218-225.

LIU, D, E MOBERG, M KOLLIND, P LINS, U ADAMSON AND I MACDONALD. Arterial, arterialized venous, venous and capillary blood glucose measurements during normal man in hypeinsulinaemic, euglycaemia and hypoglycaemia. Diabetologia (1992) 35: 287-290.

LORIST, M, J SNEL AND A KOK. Influence of caffeine on information processing stages in well rested and fatigued subjects. Psychpharm (1994a) 113: 411-421.

LORIST, M, J SNEL AND A KOK. Influence of caffeine on selective attention in well rested and fatigued subjects. Psychphysiol (1994b) 31: 525-534.

LORIST, M, J SNEL, G MULDER AND A KOK. Aging caffeine and information processing: an event-related potential analysis. Electroencephalogr Clin Neurophysiol (1996) 96: 453-467.

MACDONALD, T, K SHARPE, G FOWLER, D LYONS, S FREESTONE, H LOVELL, J WEBSTER AND J PETRIE. Caffeine restriction: Effect on mild hypertension. Br Med J (1991) 303: 1235-1238.

MACLEOD, K, D HEPBURN, I DEARY, G GOODWIN, N DOUGALL, K EBMEIER AND B FRIER. Regional cerebral blood flow in IDDM patients: effects of diabetes and of recurrent severe hypoglycaemia. Diabetologia (1994) 37: 257-263.

MAGGS, D, A SCOTT AND I MACDONALD. Thermoregulatory responses to hyperinsulinaemic hypoglycemia and euglycemia in humans. Am J Physiol (1994) 267: R1266-1272.

MARAN, A, J LOMAS, I MACDONALD AND S AMIEL. Lack of preservation of higher brain function during hypoglycaemia in patients with intensively treated IDDM. Diabetologia(1995) 38: 1412-1418.

MARANGOS, P, S PAUL, A PARMA, F GOODWIN, P SYNAPIN AND P SKOLNIK. Purinergic inhibition of diazepam binding to rat brain (in vitro). Life Sci (1979) 24: 851-857.

MARKS, V. The measurement of blood glucose and definition of hypolgycaemia. Metabol Res (1986) S1/6.

MARKS, V AND J KELLY. Absorption of caffeine from tea, coffee and coca-cola. Lancet (1973) i: 827.

MATHEW, R, D BARR AND M WEINMAN. Caffeine and cerebral blood flow. Brit J Psychiatry (1983) 143: 604-608.

MATHEW, R AND W WILSON. Caffeine consumption, withdrawal and cerebral blood flow. Headache (1985a) 25: 305-309.

MATHEW, R AND W WILSON. Caffeine induced changes in cerebral circulation. Stroke (1985b) 16: 814-817.

MATTHEWS, G, D JONES AND A CHAMBERLAIN. Redefining the measurements of mood: the UWIST Mood Adjective Checklist. Br J Psychol (1990) 81: 17-42.

MATTHEWS, J, D ALTMAN, M CAMPBELL AND P ROYSTAN. Analysis of serial measurements in medical research. Brit Med J (1990) 300: 230-5.

MATYKA, K, M EVANS, J LOMAS, I CRANSTON, I MACDONALD AND S AMIEL. Altered hierarachy of protective responses against severe hypoglycaemia in normal aging in healthy men. Diabetes Care (1997) : 135-141.

MCCALL, A. The impact of diabetes on the CNS. Diabetes (1992) 41: 557-570.

MCCRIMMON, R, I DEARY AND B FRIER. Auditory information processing during acute insulin-induced hypoglycaemia in non-diabetic human subjects. Neuropsychologia (1997) 35: 1547-1553.

MCCRIMMON, R, B FRIER AND I DEARY. Appraisal of mood and personality during hypoglycaemia in human subjects. Physiol Behav (1999a) 67: 27-33.

MCCRIMMON, R, F EWING, B FRIER AND I DEARY. Anger state during acute insulin-induced hypoglycaemia. Physiol Behav (1999b) 67: 35-39.

MCCRIMMON, R, A GOLD, I DEARY, C KELNAR AND B FRIER. Symptoms of hypoglycaemia in children with IDDM. Diabetic Med (1995) 18: 858-861.

MCCRIMMON, R, I DEARY, B HUNTLY, K MACLEOD AND B FRIER. Visual information processing during controlled hypoglycaemia in humans. Brain (1996) 119: 1277-1287.

MCPHERSON, P, Y KIM, H VALDIVIA, C KNUDSON, H TAKEKURA, C FRANZINI-ARMSTRONG, R CORONADO AND K CAMPBELL. The brain ryanodine receptor: A caffeine-sensitive calcium release channel. Neuron (1991) 7: 17-25.

MEINEKE, I, E STUWE, E HENNE, G RUSTEBERG, E BRENDEL, D MEY. Routine measurement of plasma catecholamines in clinical pharmacology by high-performance liquid chromatography with electochemical detection. J Chromatogr (1989) 493:287-303.

MILLER, L AND S MILLER. Caffeine enhances initial but not extended learning of a proprioceptive-based discrimination task in nonsmoking moderate users. Perceptual and Motor Skills(1996) 82: 891-898.

MITRAKOU, A, C RYAN, T VENEMAN, M MOKAN, T JENSSEN, I KISS, J DURRANT, P CRYER AND J GERICH. Hierachy of glycemic thresholds for counterregulatory hormone secretion, symptoms and cerebral dysfunction. Am J Physiol (1991) 260: E67-74.

MORGAN, M AND R VESTRAL. Methylxanthine effects on caudate dopamine release as measured by in vivo electrochemistry. Life Sci (1989) 45: 2025-2039.

MUHLHAUSER, I, L HEINEMANN, E FRITSCHE, K VON LENNEP AND M BERGER. Hypoglycemic symptoms and frequency of severe hypoglycemia in patients treated with human and animal insulin preparations. Diabetes Care (1991) 14: 745-749.

MUKHOPADHYAY, S AND MK PODDAR. Is GABA involved in the development of caffeine tolerance. Neurochemical Research (1998) 23: 63-68.

MUMFORD, G, S EVANS, B KAMINISKI, K PRESTON, C SANNERUD, K SILVERAMN AND R GRIFFITHS. Discriminative stimulus and subjective effects of theobromine and caffeine in humans. Psychopharmacol (1994) 115: 1-8.

MUMFORD, G AND S HOLTZMAN. Qualitative differences in the discriminative stimulus effects of low and high doses of caffeine in the rat. J Pharmacol Exp Ther (1991) 258: 857-865.

MUNTE, T, D BERGER, C TERKAMP, C SCHOFL, C JOHANNES AND G BRABANT. Cognitive functioning in experimental hypoglycaemia assessed with event-related potentials. Neuroreport (1995) 6: 1509-1512.

MYERS, M AND L HARRIS. High dose caffeine and venticular arrhythmias. Can J Cardiol (1990) 6: 95-98.

MYERS, M AND R REEVES. Effect of caffeine on daytime ambulatroy blood pressure. Am J Hypertens (1991) 4: 427-431.

NAATANEN, R AND T PICTON. N2 and automatic versus controlled processes. Electrocephal Clin Neurophysiol (1986) Suppl 38: 169-186.

NEHLIG, A. Are we dependent upon coffee and caffeine? A review on human and animal data. Neurosci Biobehav Rev (1998) 23: 563-576.

NEHLIG, A, J DAVAL, S BOYET AND P VERT. Comparative effects of acute and chronic administration of caffeine on local cerebral glucose utilisation in the conscious rat. Eur J Pharmacol (1986) 129: 93-103.

NEHLIG, A, J DAVAL AND G DEBRY. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain (1992) 17: 139-170.

NEHLIG, A, G LUCIGNANI, M KADEKARO, LJ PORRINO AND L SOKOLOFF. Effects of acute administration of caffeine on local cerebral glucose utilization in the rat. Eur J Pharmacol (1984) 101: 91-100.

NELSON, H AND J WILLISON. National Adult Reading Test Manual. Windsor, NFER-Nelson (1991).

NEWMAN, F, M STEIN, J TRETTAU, R COPPOLA AND T UHDE. Quantitative electroencephalographic effects of caffeine in panic disorder. Psychiatr Res (1992) 45: 105-113.

OKADA, M, K KIRYU, Y KAWATA, K MIZUNO, K WADA, H TASAKI AND S KANEKO. Determination of the effects of caffeine and carbamazepine on striatal dopamine release by in vivo microdialysis. Eur J Pharmacol (1997) 321: 181-188.

ORCHARD, T, R MASER, D BECKER, J DORMAN AND A DRASH. Human insulin use and hypoglycaemia: insights from Pittsburgh Epidemiology of Diabetes Complications Study. Diabetes Med (1991) 8: 469-474.

OBORNE, D AND Y ROGERS. Interactions of alcohol and caffeine on human reaction time. Aviat Space Environ Med (1983) 54: 528-534.

OVALLE, F, C FANELLI, D PARAMORE, T HERSHEY, S CRAFT AND P CRYER. Brief twice weekly episodes of hypoglycaemia reduce detection of clinical hypoglycaemia in type 1 diabetes mellitus. Diabetes (1998) 47: 1472-1479.

OWEN, O, A MORGAN, H KEMP, J SULLIVAN, M HERRERA AND G CAHILL. Brain metabloism during fasting. J Clin Invest (1967) 46: 1589-1595.

PALMER, J, D HENRY, J BENSON, D JOHNSON AND J ENSINCK. Glucagon response to hypoglycemia in sympathectomized man. J Clin Invest (1976) 57: 522-525.

PAMPANELLI, S, L FANELLI, C LALLI, M CIOFETTA, P SINDACO, M LEPORE, F MODARELLI, A RAMBOOTTI, L EPIFANO, A VINCENZO, L BARTOCCI, B ANNIBALE, P BRUNETTI AND G BOLLI. Long term intensive insulin therapy in IDDM: effects on HbA1c, risk for severe and mild hypoglycaemia, status for counterregulation and awareness of hypoglycaemia. Diabetologia (1996) 39:677-686.

PANG, C. Vasopressin and angiotensin in the control of arterial pressure in anaesthetised surgically stressed rats. Can J Physiol Pharmacol (1983) 61: 1494-1500.

PARAMORE, D, C FANELLI, S SHAH AND P CRYER. Hypoglycemia per se stimulates sympathetic neural as well as adrenomedullary activity, but, unlike the adrenomedullary response, the forearm sympathetic neural response is not reduced after recent hypoglycemia. Diabetes (1999) 48: 1429-1436.

PARSONS, W AND NEIMS A. Effect of smoking on caffeine clearance. Clin Pharmacol Ther (1978) 24:40-45.
PASSMORE, A, G KONDOWE AND G JOHNSTON. Renal and cardiovascular effects of caffeine: a dose response study. Clin Sci (1987) 72: 749-756.

PATRICK, A, D HEPBURN, K CRAIG, I THOMPSON, C SWAINSON AND B FRIER. The effects of acute insulin-induced hypoglycaemia on renal function in normal human subjects. Diabetic Med (1989) 6: 703-708.

PATRICK, G, REEVES, P AND F STRUVE. Does caffeine cessation increase firing rates of diffuse paroxysmal slowing dysrhythmia? A serendiptious observation. Clin Electroencephal (1996) 27: 78-83.

PATWARDHAN, R, P DESMOND, R JOHNSON AND S SCHENKER. Impaired elimination of caffeine by oral conraceptive steroids. J Lab Clin Med (1980) 95: 603-608.

PAXINOS, G AND W WATSON. The Rat Brain in Stereotaxic Coordinates. London, Academic Press (1986).

PENNEBAKER, J, D COX, L GONDER-FREDERICK, M WUNSCH, W EVANS AND S POHL. Physical symptoms related to blood glucose in insulin-dependent diabetics. Psychosomatic Med (1981) 43: 489-500.

PETERS, A, F ROHLOFF AND W KERNER. Preserved counterregulatory hormone release and symptoms after short term hypoglycemic episodes in normal man. J Clin Endo Met (1995) 80: 2894-2898.

PFEIFER, R AND R NOTARI. Prediciting caffeine plasma concentrations resulting from consumption of food or beverages: a simple method and its origin. Drug Intell Clin Pharmacy (1988) 22: 953-959.

PHILLIPS, WA. On the distinction between sensory storage and short term visual memory. Percept Psychophysics (1974) 16: 283-90.

PHILLIPS-BUTE, B AND J LANE. Caffeine withdrawal symptoms following brief caffeine deprivation. Physiol Behav (1998) 63: 35-39.

PHILLIS, J, Z ZHIANG, B CHELAK AND P WU. The effect of morphine on purine and aectylcholine deaminase, dipyridamole and aminophylline on acetylcholine release of adenosine analogs. Pharmacol Biochem Behav (1980) 13: 421-427.

PICKWORTH, W. Caffeine dependence. Lancet (1995) 345: 1066.

PIHA, S. Effect of acute dose of caffeine on cardiovascular autonomic responses in healthy subjects. Clin Physiol (1994) 14: 411-417.

PINACOMB, G, W LOVALLO, R PASSEY, T WHITSETT, S SILVERSTEIN AND M WILSON. Effect of caffeine on vascular resistance, cardiac output and myocardial contractility in young men. Am J Cardiol (1985) 56: 119-122.

PINCOMB, G, W LOVALLO, R PASSEY AND M WILSON. Effect of behavior state on caffeine's ability to alter blood pressure. Am J Cardiol (1988) 61: 798-802.

PINCOMB, G, M WILSON, B SUNG, R PASSEY AND W LOVALLO. Effects of caffeine on pressor regulation during rest and exercise in men at risk of hypertension. Am Heart J (1991) 122: 1107-1115.

PINCOMB, G, W LOVALLO, B MCKEY, B SUNG, R PASSEY, S EVERSON AND M WILSON. Acute blood pressure elevations with caffeine in men with borderline systemic hypertension. Am J Cardiol (1996) 77: 270-274.

PIZZIOL, A, V TIKHONOFF, CD PALEARI, E RUSSO, A MAZZA, G GINOCCHIO, C ONESTO, L PAVAN, E CASIGLIA AND AC PESSINA. Effects of caffeine on glucose tolerance: a placebo- controlled study. Eur J Clin Nutr (1998) 52: 846- 849.

POWELL, K AND S HOLTZMAN. D1 and D2 dopamine receptor involovement in the discriminative-stimulus effects of caffeine in rats. Behav Pharmacol (1996) 7: 90-91.

POWERS, W, I HIRSCH AND P CRYER. Effect of stepped hypoglycemia on regional cerebral blood flow response to physiological brain activation. Am J Physiol (1996) 270: H554-H558.

PRAMMING, S, B THORSTEINSSON, I BENDSTON AND C BINDER. Symptomatic hypoglycaemia in 411 type 1 diabetic patients. Diabetic Med (1991) 8: 217-222.

PRAMMING, S, B THORSTEINSSON, I BENDTSON AND C BINDER. The relationship between symptomatic and biochemical hypoglycaemia in insulin-dependent diabetic patients. J Int Med (1990) 228: 641-646.

PRAMMING, S, B THORSTEINSSON, I BENDTSON, B RONN AND C BINDER. Nocturnal hypoglycaemia in patients receiving conventional treatment with insulin. Brit Med J (1985) 291: 376-379.

PRAMMING, S, B THORSTEINSSON, A THEILGAARD, E PINNER, B STIGSBY AND C BINDER. Cognitive function during hypoglycaemia in type 1 diabetes mellitus. Brit Med J (1986) 292: 647-650.

QUINLAN, P, J LANE AND L ASPINALL. Effects of hot tea, coffee and water ingestion on physiological responses and mood: the role of caffeine, water and beverage type. Psychopharmacol (1997) 134: 164-173.

QUINLAN, P, J LANE, K MOORE, J ASPEN, J RYCROFT AND D O'BRIEN. The acute physiological and mood effects of tea and coffee: the role of caffeine level. Pharmacol Biochem Behav (2000) 66: 19-28.

RALL, T. Drugs used in the treatment of asthma: The methylxanthines, cromolyn sodium, and other agents. Goodman and Gilman's the Pharmacological Basis of Therapeutics. A. Gilman, T. Rall, A. Nies and P. Taylor. New York, Pergamon (1990).

REEVES, R, F STRUVE, G PATRICK AND J BULLEN. Topographic quantitative EEG measures of alpha and theca power changes during caffeine withdrawal: preliminary findings ffrom normal subjects. Clin Electroencephalogr (1995) 26:154-162.

REEVES, R, F STRUVE AND G PATRICK. The effects of caffeine withdrawal on cognitive P300 auditiory and visual evoked potentials. Clin Electroencephal (1999) 30: 24-27.

REGINA, E, G SMITH, C KEIPER AND R KELVEY. Effects of caffeine on alertness in simulated automobile driving. J Appl Psychol (1974) 59: 483-489.

REICHARD, P, B BERGLUND, A BRITZ, I CARS, B NILSSON AND U ROSENQVIST. Intensified conventional insulin treatment retards the microvascular complications of insulin-dependent diabets mellitus (IDDM): the Stockholm Diabetes Intervention Study (SDIS) after 5 years. J Intern Med (1991) 230: 101-108.

REITAN, R AND L DAVISON. Clinical neuropsychology: current status and applications. New York, Hemisphere (1974).

REVELLE, W, M HUMPHREYS, L SIMON AND K GILLILAND. The interactive effect of personality, time of day and caffeine: a test of the arousal model. J Exp Psychol Gen (1980) 109: 1-31.

RICHARDSON, N, P ROGERS, N ELLIMAN AND R O'DELL. Mood and performance effects of caffeine in reation to acute and chronic caffeine deprivation. Pharmacol Biochem Behav (1995) 52: 313-320.

RICHARDSON, P, H KASE AND P JENNER. Adenosine A2a receptor anatgonists as new agents for the treatment of Parkinson's disease. Trends Pharmacol Sci (1997) 18: 338-344.

RIHS, M, C MULLER AND P BAUMANN. Caffeine consumption in hospitalized psychiatric patients. Eur Arch Psychiatry Clin Neurosci (1996) 246: 83-92.

RIZZO, A, L STAMPS AND L FEHR. Effects of caffeine withdrawal on motor performance and heart rate changes. Int J Psychophysiol (1988) 6: 9-14.

ROBELIN, M AND P ROGERS. Mood and psychomotor peformance effects the first but not of subsequent cup-of-coffee equivalant doses of caffeine consumed after overnight abstinence. Behav Pharmacol (1998) 9: 611-618.

ROBERTSON, D, C JÜRGEN, J FRÖLICH, R CARR, J WATSON, J HOLLIFIELD, D SHAND AND J OATES. Effects of caffeine on plasma renin activity, catecholamines and blood pressure. New Eng J Med (1978) 298: 181-186.

ROBERTSON, D, AS HOLLISTER, D KINCAID, R WORKMAN, M GOLDBERG, C TUNG AND B SMITH. Caffeine and hypertension. Am J Med (1984) 77: 54-60.

ROBERTSON, D, D WADE, R WORKMAN, R WOOSLEY AND J OATES. Tolerance to the humeral and hemodynamic effects of caffeine in man. J Clin Invest (1981) 67: 1111-1117.

RODRIGUEZ, G, F NOBILI, M CELESTINO, S FRANCIONE, G GULLI, K HASSAN, S MARENCO, G ROSADINI AND R CORDERA. Regional cerebral blood flow and cerebrovascular reactivity in IDDM. Diabetes Care (1993) 16: 462-468.

ROGERS, P, N RICHARDSON AND N ELLIMAN. Overnight caffeine abstinence and negative reinforcement of preference for caffeine-containing drinks. Psychopharmacol (1995) 120: 457-462.

ROGERS, P AND C DERNONCOURT. Regular caffeine consumption: a balance of adverse and benficial effects for mood and psychomotor performance. Pharmacol Biochem Behav (1998) 59: 1039-1045.

RONGEN, G, J FLORAS, W LENDERS, T THIEN AND P SMITS. Cardiovascular pharmacology of purines. Clin Sci (1997) 92: 13-24.

ROSEN, B, M MIODOVNIK, G HOLCBERG, J KHOURY AND T SIDDIQI. Hypoglycaemia: the price of intensive insulin therapy for pregnant women with insulin dependent diabetes mellitus. Obstet Gynaecol (1995) 85: 417-422.

RUGG, M AND M COLES. Electrophysiology of mind: event-related brain potentials and cognition. Oxford, Oxford university Press (1995).

RUIJTER, J, M LORIST, J SNEL AND M DE RUITER. The influence of caffeine on sustained attention: an ERP study. Pharmacol Biochem Behav (2000) 66 : 29-37.

RUSH, C, S HIGGINS, W BICKEL AND J HUGHES. Acute behavioral effects of lorazepam and caffeine, alone and in combination, in humans. Behav Pharmacol (1994a) 5: 245-254.

RUSH, C, S HIGGENS, J HUGHES AND W BICKEL. Acute behavioral effects of trizolam and caffeine, alone and in combination, in humans. Ex Clin Pharmacol (1994b) 2: 211-222.

SAMOLS, E AND J STAGNER. Intra-islet cell-cell interactions and insulin secretion. Diabetes Rev (1996) 4: 207-223.

SCHENK, S, A VALADEZ, B HORGER, S SNOW AND P WELLMAN. Interactions between caffeine and cocaine in tests of self-administration. Behav Pharmacol (1994) 5: 153-158.

SCHOLZ, H. Inotropic drugs and their mechanisms of action. J Am Coll Cardiol (1984) 4: 389-397.

SCHWARZ, N, W CLUTTER, S SHAH AND P CRYER. Glycaemic thresholds for activation of glucose counterregulatory systems are higher than the threshold for symptoms. J Clin Invest (1987) 79: 777-781.

SCOTT, N, J CHAKRABORTY AND V MARKS. Caffeine consumption in the United Kingdom: a retrospective survey. Food Sci Nutrition (1989) 42F: 183-191.

SETCHELL, K. Rapid high performance liquid chromatography assay for salivary and serum caffeine following an oral load. J Chromat (1987) 385: 267-274.

SHI, J, N BENOWITZ, C DENARO AND L SHEINER. Pharmacokinetic-pharmacodynamic modeling of caffeine: Tolerance to pressor effects. Clin Pharmacol Ther (1993) 53: 6-14.

SILVERMAN, K, S EVANS, E STRAIN AND R GRIFFITHS. Withdrawal syndrome after the double blind cessation of caffeine consumption. New Eng J Med (1992a) 327: 1119-1114.

SILVERMAN, K AND R GRIFFITHS. Low-dose caffeine discrimination and self-reported mood effects in normal volunteers. J Exp Anal Behav (1992b) 57: 91-107.

SILVERMAN, K, G MUMFORD AND R GRIFFITHS. Enhancing caffeine reinforcemnat by behavioral requirement following drug ingestion. Psychopharmacology (1994) 114: 424-432.

SIPERSTEIN, M, D FOSTER, H KNOWLES, R LEVINE, L MADISON AND J ROTH. Control of blood glucose and diabetic vascular disease. New Eng J Med (1977) 296: 1060-1063.

SMELLIE, F, C DAVIS, J DALY AND J WELLS. Alkylxanthines: Inhibition of adenosine-elicited accumulation of cyclic AMP in brain slices and of brain phosphodiesterase activity. Life Sci (1979) 24: 2475-2482.

SMID, HO, B TRUMPER, G POTTAG, K WAGNER, R LOBMANN, H SCHEICH, H LEHNERT AND H-J HEINZE. Differentiation of hypoglycaemia induced cognitive impairments: An electrophysiological approach. Brain (1997) 120: 1041-1056.

SMITH, A, A MABEN AND P BROCKMAN. Effects of evening meals and caffeine on cognitive perforamnce , mood and cardiovascular functioning. Appetite (1994) 22: 57-65.

SMITH, A, J RUSTED, P EATON WILLLIAMS, M SAVORY AND P LEATHWOOD. Effects of caffeine given before and after lunch on sustained attention. Neuropsychobiol (1990) 23: 160-163.

SMITH, A, M THOMAS, K PERRY AND H WHITNEY. Caffeine and the common cold. J Psychopharmacol (1997) 11: 319-324.

SMITS, P, T THIEN AND A VAN'T LAAR. Circulatory effects of coffee in relation to the pharmacokinetics of caffeine. Am J Physiol (1985) 56: 958-963.

SNIDER, S AND B WALDBECK. Increased synthesis of adrenomedullary catecholamines by caffeine and theophyline. Naunyn Schmiedebergs Arch Pharmacol (1974) 281:257-260.

SORTEBERG, W Cerebral artery blood velocity and cerebral blood flow. Transcranial Doppler. R. Aaslid and D. W. Newall. New York, Raven Press (1992).

SPINDEL, E, R WURTMAN, A MCCALL, D CARR, L CONLAY, L GRIFFITH AND M ARNOLD. Neuroendocrine effects of caffeine in normal subjects. Clin Pharmacol Ther (1984) 36: 402-407.

STAGNER, J AND E SAMOLS. Intra-islet regulation. Am J Med (1988) 85:31-35.

STEBBINS, C AND J SYMONS. Role of angiotensin II in hemodynamic responses to dynamic exercise in miniswine. J Appl Physiol (1995) 78: 185-190.

STEVENS, A, W MCKANE, P BELL, P BELL, D KING AND J HAYES. Psychomotor performance and counterregulatory responses during mild hypoglycemia in healthy volunteers. Diabetes Care (1989) 12: 12-17.

STRAIN, E, G MUMFORD, K SILVERMAN AND R GRIFFITHS. Caffeine dependence syndrome: evidence from case histories and experimental evaluations. JAMA (1994) 272: 1043-1048.

SUENAGA, N, T INAMITSU, M HATAKENAKA, F YANAGA AND F SHIRAISHI. Effect of caffeine on the mental calculation. Fukuoka Acta Med (1997) 88: 27-29.

SUNG, B, W LOVALLO, G PINACOMB AND M WILSON. Effects of caffeine on blood pressure response during exercise in normotensive healthy young men. Am J Cardiol (1990) 65: 909-913.

SUNG, B, T WHITSETT, W LOVALLO, M AL'ABSI, G PINACOMB AND M WILSON. Prolonged increase in blood pressure by a single oral dose of caffeine in mildly hypertensive men. Am J Hypertension (1994) 7: 755-758.

SUPERKO, H, J MYLL, P WILLIAMS, W BORTZ AND P WOOD. Effects of cessation of caffeinated-coffee consumption on ambulatory and resting blood pressure in man. Am J Cardiol (1994) 73: 780-784.

SURWIT, R, A SCOVERN AND M FEINGLOS. The role of behaviour in diabetes care. Diabetes Care (1982) 5: 337-342.

SUTHERLAND, D, D MCPHERSON, K RENTON, C SPENCER AND T MONTAGUE. The effect of caffeine on cardiac rate rhythm and ventricular replarization. Analysis of 18 normal subjects and 18 patients with primary ventricular dysrhythmia Chest (1985) 87: 319-324.

SVENNINGSSON, P, G NOMIKOS, E ONGINI AND B FREDHOLM. Antagonism of Adenosine A2a receptors underlies the behavioural activating effect of caffeine and is associated with reduced expression of messenger RNA for NGFI-A aand NGFI-B in caudate-putamen and nucleus accumbens. Neurosci (1997) 79: 753-764.

TALLROTH, G, E RYDING AND C AGARDH. Regional cerebral blood flow in normal man during insulin-induced hypoglycemia and in the recovery period following glucose infusion. Metabol (1992) 41: 717-721.

TAMBURRANO, G, A LALA, N LOCURATOLO, F LEONETTI, P SBRACCIA, A GIACCARI, S BUSCO AND S PORCU. Electroencephalography and visually evoked potentials during moderate hypoglycemia. J Clin Endo Metabol (1988); 66: 1301-1306.

TATTERSALL, R AND G GILL. Unexplained deaths of type 1 diabetic patients. Diabetic Medicine (1991) 8: 49-58.

THELLE, DS, E ARNESEN AND OH FORDE. The Tromsø heart study: Does coffee raise serum cholesterol? New Eng J Med (1983) 308: 1454- 1457.

THOMAS, M, R SHERWIN, J MURPHY AND D KERR. Importance of cerebral blood flow to the recognition of and physiological responses to hypoglycemia. Diabetes (1997) 46: 829-833.

THORSTEINSSON, B, S PRAMMING, T LAURITZEN AND C BINDER. Frequency of daytime biochemical hypolgycaemia in insulin-treated diabetic patients: relationship to daily median blood glucose concentration. Diab Med (1986) 3: 147-151.

TOWLER, D, C HAVLIN, S CRAFT AND P CRYER. Mechanism of awareness of hypoglycemia: perception of neurogenic (predominantly cholinergic) rather than neuroglycopenic symptoms. Diabetes (1993) 42: 1791-1798.

TRIBL, G, K HOWORKA, G HEGER, P ANDERER, H THOMA AND J ZEITHOFER. EEG topography during insulin-induced hypoglycemia in patients with insulin-dependent diabetes mellitus. Eur Neurol (1996) 36: 303-309.

URGERT, R, N ESSED, G VAN DER WEG, T SCHUIL AND M KATAN. Separate effects of the coffee diterpenes cafestol and kahweol on serum lipids and liver aminotransferases. Am J Clin Nutr (1997) 65: 519- 24.

VAN SOEREN, M, T MOHR, M KJAER AND TE GRAHAM. Acute effects of caffeine ingestion at rest in humans with impaired epinephrine responses. J Appl Physiol (1996) 80: 999-1005.

VAN SOEREN, M, P SATHASIVAM, L SPRIET AND T E GRAHAM. Caffeine metabolism and epinehrine responses during exercise in users and nonusers. J Appl Physiol (1993) 75: 805-812.

VENEMAN, T, A MITRAKOU, M MOKAN, P CRYER AND J GERICH. Induction of hypoglycemia unawareness by asymptomatic nocturnal hypoglycemia. Diabetes (1993) 42: 1233-1237.

WACHMAN, A, R HATTNER, B GEORGE AND D BERNSTEIN. Effects of decaffeinated and nondecaffeinated coffee on blood glucose and plasma radioimmunoreactive insulin responses to rapid intravenous infusion of glucose in normal men. Metabol (1970) 19: 539-546.

WALTHER, F, R ERICKSON AND M SIMS. Cardiovascular effects of caffeine therapy in preterm infants. Am J Dis Child (1990) 144: 1164-1166.

WARBURTON, D. Effects of caffeine on cognition and mood without caffeine abstinence. Psychopharmacol (1995) 119: 66-70.

WECHSLER, D AND C STONE. Wechsler Memory Scale- revised manual. New York, Psychology Corporation (1981).

WENNMALM, M, B FREDHOLM AND P HEDQVIST. Adenosine as a modulator of sympathetic nerve-stimulation-induced release of noradrenaline from the isolated rabbit heart. Acta Physiol Scand(1988); 132: 487-494.

WHITSETT, T, C MANION AND H CHRISTENSEN. Cardiovascular effects of coffee and caffeine. Am J Cardiol (1984) 53: 918-922.

WIDOM, B AND D SIMONSON. Intermittant hypoglycemia impairs glucose counterregulation. Diabetes (1992) 41: 1597-1602.

WIETHOP, B AND P CRYER. Glycaemic actions of alanine and terbutaline in IDDM. Diabetes Care (1993) 16: 1124-30.

WILKINSON, R AND D HOUGHTON. Portable four choice reaction time test with magnetic tape memory. Behav Res Meth Instrumentation (1975) 7: 441-446.

WIRSÉN, A, G TALLROTH, M LINDGREN AND C AGARDH. Neuropsychological performance differs between type 1 diaebtic and normal man during insulin-induced hypoglycaemia. Diabetic Med (1992) 9: 156-165.

ZEIGLER, D. A HÜBINGER AND H MÜHLEN. Effects of previous glycaemic control opn the onset and magnitude of cognitive dysfunction suring hypoglycaemia in type 1 (insulin-dependent) diabetic patients. Diabetologia (1992) 35: 828-834.

ZINI, S, E TREMBLAY, M ROISIN AND Y BEN-ARI. Regional distribution of sulfonylurea receptors in the brain of rodent and primate. Brain Res (1991) 542: 151-154.

ZWYGHUIZEN-DOORENBOS, A, T ROEHRS, T LIPSCHUTZ, V TIMMS AND T ROTH. Effects of caffeine on alertness. Psychopharmacology (1990) 100: 36-39.

ZYSSET, T AND H WIETHOLTZ. Pharmacokinetics of caffeine in patients with decompensated type I and type II diabetes mellitus. Eur J Clin Pharmacol (1991) 41: 449-452.

APPENDIX

PUBLICATIONS

The work described in this thesis has previously been published in the following:

PAPERS

Influence of caffeine on the frequency and perception of hypoglycemia in free-living patients with type 1 diabetes mellitus.
J. Watson, E. Jenkins, P. Hamilton, M. Lunt. D. Kerr.
(*Diabetes Care* 2000, 23:455-459)

Reversal of the symptoms of caffeine withdrawal by a soft beverage.
J. Watson, M. Lunt, S. Morris, M. Weiss, D. Hussey, D. Kerr
(Pharmacology, Biochemistry and Behavior 2000, 66: 19-28)

Effects of caffeine on frequency, physiological responses and perception hypoglycemia.
J. Watson and D. Kerr.
(*Diabetes Therapeutics and Treatment* 1999, 1:193-200)

ABSTRACTS

Influence of caffeine on neurophysiological function during hypoglycaemia.
J. Watson, G. Owen, G. Barrett, J. Ingleby, M. Weiss, I. Deary, D. Cavan, D. Kerr
***(Diabetologia* 1999, 42:S1:A219)**

Ingestion of modest amounts of caffeine delays the expected recovery from hypoglycemia.
J. Watson, M. Weiss, J. Ingleby, M. Lunt, I. Deary, D. Kerr.
(Diabetes 1999, 48:S1:P123)

Daily consumption of caffeine and its effects on the perception of and catecholamine responses to hypoglycaemia.
J. Watson, L. Scott, J. Ingleby, M. Weiss, D. Cavan, D. Kerr.
(Diabetic Medicine 1999, 16:S1:P144)

Does the brain become tolerant to sustained caffeine use? implications for treatment of hypoglycaemia unawareness.
J. Watson, M. J. Weiss, M. J. Lunt, D. A. Cavan, D. Kerr.
(Diabetologia 1998, 41:S1:A241)

Does tolerance develop to the effects of caffeine on the perception and physiological responses to hypoglycemia with sustained use?
J. Watson, L. Scott, J. Ingleby, D. Kerr.
(Diabetes 1998, 47:S1:109A)

Effect of acute caffeine withdrawal on the perception and frequency of hypoglycemia in free-living patients with IDDM.
E. Jenkins, P. Hamilton, J. Watson, M. Lunt, D. Kerr.
(Diabetes 1998, 47:S1:109A)

The effects of a caffeinated cola drink on cerebral blood flow.
J. Watson M. Lunt, M. Weiss, D. Kerr.
(European Journal of Ultrasound 1998 **7:S2:S5)**

The effects of caffeine ingestion on visual sensation during hypoglycaemia.
G. Owen, G. Barrett. J. Watson, D. Kerr.
(British Psychological Society **1998)**

Effects of caffeine-containing soda on brain blood flow and function in healthy women.
J. Watson, M. Lunt, M. Weiss, D. Kerr.
(Diabetes 1997, 46:S1:295A)

DATA (MEAN ±SE) FOR GRAPHIC REPRESENTATIONS IN THESIS

Values for **Figure V.1.2**

Total No. Hypos	symptomatic	biochemical
Placebo	362	296
Caffeine	534	228

Values for **Figure V.1.3**

Total symptom score		score
Placebo	mean	26.66
	se	2.57
Caffeine	mean	29.09
	se	2.78

Values for **Figure V.1.4**

VMCA (cm/s)			
Males	baseline	mean	52.09
		se	2.28
	caffeine	mean	47.80
		se	2.29
	placebo	mean	50.38
		se	2.14
Females	baseline	mean	58.91
		se	3.03
	caffeine	mean	54.49
		se	2.65
	placebo	mean	60.67
		se	3.89

Values for **Figure VI.2.2**

Change in VMCA (all subj; cm/s)		Time (min) 20	80	120	180	210	270
E+C	mean	0.00	2.25	-0.31	-2.63	1.13	0.00
	se	0.00	1.42	0.90	1.58	1.44	1.32
H+P	mean	0.00	0.56	-1.56	-2.63	0.31	-0.63
	se	0.00	1.27	0.76	1.29	1.66	1.31
H+C	mean	0.00	1.56	-7.94	-9.00	-2.88	-1.13
	se	0.00	1.36	1.94	2.37	1.91	1.88

Change in VMCA (males; cm/s)		Time (min) 20	80	120	180	210	270
E+C	mean	0.00	1.15	-1.98	-2.97	-0.08	-0.80
	se	0.00	1.24	0.84	1.05	1.10	0.97
H+P	mean	0.00	0.22	0.97	-1.38	2.75	3.88
	se	0.00	0.96	1.38	1.18	1.51	1.90
H+C	mean	0.00	0.94	-9.28	-7.63	-2.53	-0.69
	se	0.00	0.99	1.30	1.60	1.58	1.51

Change inVMCA (females; cm/s)		Time (min) 20	80	120	180	210	270
E+C	mean	0.00	-0.11	-3.88	-3.36	-1.46	-1.71
	se	0.00	2.13	1.16	1.45	1.66	1.45
H+P	mean	0.00	-0.13	3.50	-0.13	5.19	8.38
	se	0.00	1.45	2.28	1.85	2.16	2.66
H+C	mean	0.00	0.31	-10.63	-6.25	-2.19	-0.25
	se	0.00	1.41	1.60	2.02	2.53	2.37

Values for **Figure VI.2.3**

		Time (min)					
Systolic BP (mmHg)		20	80	120	180	210	270
E+C	mean	104.2	104.7	107.0	109.3	109.0	108.4
	se	1.6	1.5	2.3	2.3	1.9	2.1
H+P	mean	102.2	102.9	104.0	108.1	101.8	97.6
	se	2.1	1.6	1.6	2.3	3.1	2.4
H+C	mean	105.0	106.3	109.6	109.2	101.8	101.9
	se	1.9	1.1	2.0	2.7	2.2	2.0

		Time (min)					
Diastolic BP (mmHg)		20	80	120	180	210	270
E+C	mean	56.6	57.4	59.0	57.5	58.7	58.5
	se	1.8	1.3	1.6	1.8	1.6	1.4
H+P	mean	56.3	54.3	53.8	47.2	51.7	53.6
	se	1.6	1.7	1.6	1.6	1.7	1.7
H+C	mean	58.0	57.7	58.2	49.9	55.2	57.3
	se	1.5	1.5	1.7	1.5	2.5	2.2

		Time (min)					
HR (bts/min)		20	80	120	180	210	270
E+C	mean	58.7	58.7	56.4	59.2	60.1	58.4
	se	1.9	1.6	1.4	1.7	1.7	1.2
H+P	mean	59.0	59.2	63.4	62.4	54.4	52.5
	se	2.1	2.0	2.3	2.2	2.0	2.2
H+C	mean	55.5	58.1	61.2	58.1	52.1	51.6
	se	1.9	2.0	2.4	2.1	2.0	2.2

Values for **Figure VI.2.4**

		Time (min)						
Adrenaline (pmol/l)		0	20	80	120	180	210	270
E+C	mean	198.8	165.3	182.5	214.8	232.7	175.0	235.084
	se	7.8	6.2	7.3	6.9	7.5	5.7	9.32586
H+P	mean	233.2	205.7	231.6	510.9	2185.9	508.8	312.198
	se	7.8	6.2	9.3	37.6	80.0	18.8	8.87268
H+C	mean	189.6	289.6	178.7	848.9	2817.4	676.4	507.594
	se	5.7	21.1	8.3	60.7	107.1	39.4	30.4523

		Time (min)						
Nordrenaline (pmol/l)		0	20	80	120	180	210	270
E+C	mean	1.03	1.17	1.19	1.18	1.15	1.16	1.00
	se	0.02	0.03	0.03	0.04	0.03	0.04	0.03
H+P	mean	1.07	1.00	1.16	1.13	1.38	1.18	0.96
	se	0.03	0.03	0.04	0.03	0.03	0.03	0.02
H+C	mean	1.14	1.35	1.35	1.53	1.53	1.68	1.48
	se	0.03	0.05	0.04	0.03	0.02	0.08	0.05

		Time (min)						
Growth Hor (pmol/l)		0	20	80	120	180	210	270
E+C	mean	2.46	2.65	1.29	1.11	2.80	4.20	2.40
	se	0.69	1.21	0.33	0.21	1.10	1.60	0.90
H+P	mean	2.19	2.91	1.23	1.55	14.24	12.45	6.08
	se	0.51	0.88	0.18	0.24	1.85	1.80	1.07
H+C	mean	1.28	1.46	0.89	2.73	16.88	19.39	9.19
	se	0.27	0.40	0.09	0.90	1.82	2.00	1.94

Values for **Figure**
VI.2.5

		Time (min)					
Neuroglycopenic (sym score)		20	80	120	180	210	270
E+C	mean	5.93	6.46	5.80	5.27	5.36	5.54
	se	0.37	0.45	0.24	0.12	0.17	0.22
H+P	mean	6.13	6.67	8.00	8.63	6.88	6.71
	se	0.29	0.41	1.21	0.81	0.55	0.33
H+C	mean	5.63	6.38	7.69	9.79	7.50	6.64
	se	0.24	0.22	0.71	0.92	0.89	0.39

		Time (min)					
Autonomic (sym score)		20	80	120	180	210	270
E+C	mean	5.20	5.23	6.07	6.40	6.43	6.00
	se	0.40	0.28	0.32	0.62	0.25	0.39
H+P	mean	5.00	5.13	7.19	9.75	6.50	6.29
	se	0.34	0.30	0.95	0.83	0.39	0.33
H+C	mean	4.94	5.15	9.31	11.79	8.19	6.86
	se	0.23	0.17	1.31	1.05	0.78	0.34

		Time (min)					
Other (sym score)		20	80	120	180	210	270
E+C	mean	2.40	2.38	2.13	2.13	2.14	2.07
	se	0.19	0.31	0.13	0.13	0.09	0.07
H+P	mean	2.06	2.06	2.25	2.31	2.18	2.14
	se	0.06	0.06	0.14	0.11	0.10	0.09
H+C	mean	2.50	2.23	2.52	2.52	2.25	2.28
	se	0.30	0.15	0.22	0.21	0.14	0.15

Values for **Figure VI.2.6**

		Time (min)		
Change in VCD (score)		20	120	210
E+C	mean	0.00	2.43	3.07
	se	0.00	1.14	1.12
H+P	mean	0.00	-1.44	-0.88
	se	0.00	1.25	0.90
H+C	mean	0.00	-0.38	-1.06
	se	0.00	1.22	1.18

		Time (min)		
Change in VMD (score)		20	120	210
E+C	mean	0.00	3.27	4.07
	se	0.00	1.41	1.25
H+P	mean	0.00	-0.19	-0.94
	se	0.00	1.15	1.60
H+C	mean	0.00	-1.19	-2.75
	se	0.00	1.32	1.47

Values for **Figure VI.2.9**

		Blood Glucose (mmol/l)			
Latency (ms)		base	4.5	2.5/4.5	4.5
gp1 Eug	mean	97.6	97.7	96.3	97.6
	se	0.6	0.5	1.1	0.8
gp1 H+C	mean	97.6	99	99.1	99.1
	se	0.8	0.7	0.6	0.6
gp2 Eug	mean	99.5	99.5	97.4	97.6
	se	0.5	0.6	1	0.8
gp2 H+C	mean	98.9	99.1	107.9	101.1
	se	0.8	0.7	0.5	0.8

		Blood Glucose (mmol/l)			
Latency (ms)		base	4.5	2.5/4.5	4.5
gp1 H+P	mean	98.1	97.4	98.0	98.2
	se	0.6	0.7	1.0	0.7
gp1 H+C	mean	97.6	99.0	99.1	99.1
	se	0.8	0.7	0.6	0.6
gp2 H+P	mean	95.2	96.1	100.6	97.8
	se	0.6	0.6	0.6	0.6
gp2 H+C	mean	98.9	99.1	107.9	101.1
	se	0.8	0.7	0.5	0.8

Values for **Figure VI.2.10**

Change in VMD (score)		Blood glc (mmol /l) 4.5	2.5/4.5	4.5
gp1 H+P	mean	0.0	2.0	1.8
	se	0.4	0.4	0.4
gp1 H+C	mean	0.0	0.9	-0.1
	se	0.3	0.3	0.3
gp2 H+P	mean	0.0	-2.0	-3.8
	se	1.0	1.5	0.7
gp2 H+C	mean	0.0	-2.1	-6.1
	se	0.8	1.2	1.7

Values for **Figure VI.3.2**

		Time (min)				
VMCA (cm/s)		0	30	60	90	120
C-naïve	mean	0	-8.0	-7.0	-4.9	-5.5
	se	0	1.0	1.2	1.2	1.1
C-replete	mean	0	-4.9	-2.5	-2.1	0.4
	se	0	1.0	0.9	0.9	1.7

Values for **Figure VI.3.3a**

		Time (min)				
Systolic BP (mmHg)		0	30	60	90	120
C-naïve	mean	0	8.7	6.6	9.1	9.1
	se	0	2.3	2.3	3.0	2.2
C-replete	mean	0	4.5	1.5	2.6	4.6
	se	0	1.9	1.9	1.3	1.4

Values for **Figure VI.3.3b**

		Time (min)				
Diastolic BP (mmHg)		0	30	60	90	120
C-naïve	mean	0	9.5	14.5	21.2	27.5
	se	0	1.6	1.5	1.9	1.4
C-replete	mean	0	1.1	0.8	0.3	2.0
	se	0	1.0	1.6	0.8	1.1

Values for **Figure VI.3.4**

Mood		**Hedonic mood**		**Tense mood**		**Energetic mood**	
(score)		Baseline	Maximum	Baseline	Minimum	Baseline	Maximum
C-naïve	mean	28.0	30.8	**16.3**	12.0	**21.4**	24.6
	se	1.3	0.4	**1.6**	0.7	**1.6**	1.2
C-replete	mean	28.9	31.1	**11.6**	9.7	**19.8**	25.2
	se	1.0	0.4	**0.6**	1.0	**1.4**	1.3

Values for **Figure VI.3.5**

4CRTT			
(s)		Baseline	Minimum
C-naïve	mean	0.51	0.49
	se	0.01	0.01
C-replete	mean	0.51	0.49
	se	0.01	0.01

Values for **Figure VI.4.2**

VMCA (*cm/s*)		Time (min) baseline	120	180
Caffeine free	mean	60.9	55.8	54.1
	se	3.5	3.2	3.4
Caffeine	mean	62.2	60.3	59.3
	se	2.4	2.5	2.1

Values for **Figure VI.4.3**

Adrenaline (*nmol/l*)		Time (min) 0	20	80	120	180
Caffeine free	mean	195	235	228	2326	3949
	se	36	15	20	470	582
Caffeine	mean	198	221	242	2353	4222
	se	32	22	46	395	679

Noradren (*nmol/l*)		Time (min) 0	20	80	120	180
Caffeine free	mean	0.82	0.93	0.94	1.21	1.74
	se	0.06	0.07	0.13	0.18	0.26
Caffeine	mean	1.10	1.03	1.21	1.49	1.84
	se	0.17	0.14	0.21	0.24	0.25

Values for **Figure VI.4.5**

Adrenaline (nmol/l)		Change in test score **VF**	**SP**
Caffeine free	mean	-5.5	-5.36
	se	3.8	4.09
Caffeine	mean	-15.1	-17.0
	se	3.7	5.7

Values for **Figure VII.5.1**

VMCA (cm/s)		Time (min) 0	30	60	90	120
Caffeine free	mean	71.5	71.3	70.7	69.6	71.1
	se	2.3	2.8	2.8	3.1	3.1
Caffeine	mean	70.0	62.5	62.9	64.8	65.0
	se	2.4	1.7	2.1	1.9	2.2

Values for **Figure VII.5.2**

Hedonic Mood (UWIST score)		Time (min) 0	30	60	90	120
Caffeine free	mean	26.3	28.1	27.1	27.5	27.3
	se	1.2	1.0	1.2	1.0	1.2
Caffeine	mean	27.3	28.5	28.7	29.7	29.7
	se	0.9	0.8	0.8	0.7	0.8

Energetic Mood (UWIST score)		Time (min) 0	30	60	90	120
Caffeine free	mean	16.0	18.8	18.1	17.4	18.4
	se	0.9	1.2	1.5	1.5	1.9
Caffeine	mean	16.7	21.4	21.8	23.5	24.6
	se	1.0	1.6	1.5	1.3	1.3