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**THE EFFECT OF POSTPRANDIAL
GLUCOSE METABOLISM ON COGNITION
AND MOOD ACROSS THE LIFESPAN**

By

Hayley Anne Young BA (Hons)

Submitted to Swansea University
in fulfilment of the requirements for the
Degree of Doctor of Philosophy

2013

**Department of Psychology
Swansea University**



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ABSTRACT

The aim of this thesis was to examine the effects of postprandial glycaemia on cognition and mood. Three studies provided evidence that low GL meals/drinks result in cognitive superiority. Study 1 found that Children's (n = 75) memory and mood were improved 180 minutes after eating a breakfast (cereal, yogurt, orange flavour drink, 337kcal) sweetened with 40g isomaltulose (low GL) rather than 40g glucose (high GL).

Study 2 examined the interaction between gluco-regulatory status of older adults (n=153) and the GL of breakfast. Older adults with better, but not poorer glucose tolerance, had better memory and mood if they ate breakfast (toast, yogurt, orange flavour drink, 275kcal) sweetened with 40g isomaltulose (low GL), rather than 40g glucose (high GL) or 40g sucrose (medium GL). Conversely, older adults with poorer glucose tolerance had better memory and mood 30 minutes after glucose but not a sucrose or isomaltulose based meal. Individual differences in gluco-regulatory control also interacted with age to predict cognitive performance, cognitive decline and mood. Adults aged 61 or above, with poorer glucose tolerance, had poorer memory than those 61 or over with better glucose tolerance, or those 60 and younger with poorer glucose tolerance. In addition, in older adults with poor glucose tolerance, developing subsequent low blood glucose levels was associated with better cognitive performance, mood and less cognitive decline.

Study 3 investigated the interaction between caffeine (80mg) and the GL of its vehicle. After drinking caffeine young adults (n= 345) had poorer glucose tolerance. Caffeine, regardless of vehicle, improved young adult's memory, reaction times and vigilance. Young adults remembered more words, after 150 minutes, if they drank milk (250ml, 155kcal, low GL), rather than glucose (250ml drink with 30g glucose, 155kcal, high GL), and had better working memory, after 90 and 150 minutes, if they drank water (250ml), or milk, rather than glucose. After 30 minutes, caffeine increased subjective energy levels, however, when caffeine was taken with water energy levels were reduced after 90 and 150 minutes. In contrast, when caffeine was consumed with milk greater energy was reported after 90 and 150 minutes. Caffeine did not affect energy levels when it was drunk with glucose.

These results were discussed in relation to an emerging understanding of the pathologies that underlie disturbed glucose homeostasis and how these relate to the brain and cognitive performance. A theoretical framework was put forward which aims to direct future research.

DECLARATION

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

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ABBREVIATIONS

GI	Glycaemic Index
GL	Glycaemic Load
CHO	Carbohydrate
PRO	Protein
KCAL	Kilocalorie
BBB	Blood Brain Barrier
ANOVA	Analysis of Variance
ANCOVA	Analysis of Co-variance
MCI	Mild Cognitive Impairment
SIP	Speed of Information Processing
RT	Reaction Time
ST	Short Term
LT	Long Term
AUC	Area under the Curve
T2DM	Type 2 Diabetes Mellitus
IGT	Impaired Glucose Tolerance
NGT	Normal Glucose Tolerance
GT	Glucose Tolerance
WHO	World Health Organisation
OGTT	Oral Glucose Tolerance Test
GTT	Glucose Tolerance Test
PH	Postprandial Hypoglycaemia
MMSE	Mini Mental State Examination
PCA	Principal Component analysis
ED	Energy Drink
RCT	Randomised Controlled Trial
VAS	Visual Analogue Scale
NART	National Adult Reading Test
GHQ	General Health Questionnaire
SNS	Sympathetic Nervous System
LBG	Lowest Blood Glucose
SGLT	Sodium Dependent Glucose Transporters
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
ENOS	Endothelial Nitric Oxide Synthase
NNOS	Neuronal Nitric Oxide Synthase
INOS	Inducible Nitric Oxide Synthase
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
FAD	Flavin Adenine Dinucleotide
FMN	Flavin Mononucleotide
BH4	Tetrahydrobiopterin
BH2	Dihydrobiopterin
cGMP	Cyclic Guanosine Monophosphate
cGC	Guanylyl Cyclase
ATP	Adenosine Triphosphate
AMPK	Adenosine 3',5' Monophosphate activated Protein Kinase
ROS	Reactive Oxygen Species

CHAPTER 1

The influence of postprandial glycaemia on cognition and mood: a review of the literature.

1.1 General Introduction.

This first section provides a conceptual overview of the relevant scientific background of the studies contained in this thesis. First, a description of the concept of glycaemic index (GI) and glycaemic load (GL) is provided, and their potential importance to the metabolism and transport of glucose in the brain highlighted. The literature examining the psychological effect of GI/GL in different populations is then systematically considered. The dietary and physiological factors that influence postprandial glycaemia are discussed, including the consumption of caffeine, individual differences in glucose tolerance, and reactive hypoglycaemia. Finally, the evidence supporting these factors in relation to mood and cognition is reviewed.

1.2 Glycaemic index and Glycaemic load.

The concept of the GI was developed by Jenkins et al (1981) to obtain a numeric classification of carbohydrates based on their rate of digestion and absorption. GI defines carbohydrate (CHO) foods according to their postprandial glycaemic impact (Jenkins et al. 1981). The GI is calculated by measuring the glucose incremental area under the curve (AUC) following ingestion of a test food providing 50 g of CHO, compared to the area under the curve (AUC) following 50g CHO intake from the reference food (usually glucose or white bread) (Jenkins et al., 1981; Brouns et al. 2005). Capillary blood samples are taken in the fasted state and at 15 - 30 minutes intervals for 2 hours after ingestion, with all tests being conducted on the same individual after an overnight fast (Hätönen et al. 2006;

Brouns et al. 2005). The reference value is set to be 100 and GI is expressed by dividing the AUC of the test food by the AUC of the reference food and multiplied by 100. The average GI value is calculated from data collected in at least ten human subjects (Brouns et al. 2005). A food is said to have a high GI if it has a value of more than 70, a moderate GI if it has a value of between 56 and 69, and a low GI if lower than 55 (Foster-Powell, 2002).

The GI of a food depends on the rate at which the CHO is digested in the gastrointestinal tract, and the rate of absorption into the bloodstream (Jenkins et al. 1981; Englyst et al., 1999). The postprandial insulin response is related to the postprandial glycaemic response (Wolever and Bolognesi, 1996b), such that quickly digested and absorbed CHOs will produce a rapid increase in plasma glucose, which stimulates a rapid pancreatic insulin secretion. Insulin then promotes glucose uptake to counteract the rise in plasma glucose concentrations. An extreme insulin response will lead to reactive hypoglycaemia; that is a blood glucose nadir that is undesirably low. In contrast, carbohydrates that have a low GI are absorbed at a slower rate than carbohydrates that have a high GI, resulting in reduced postprandial glycaemia and insulinaemia (Figure 1).

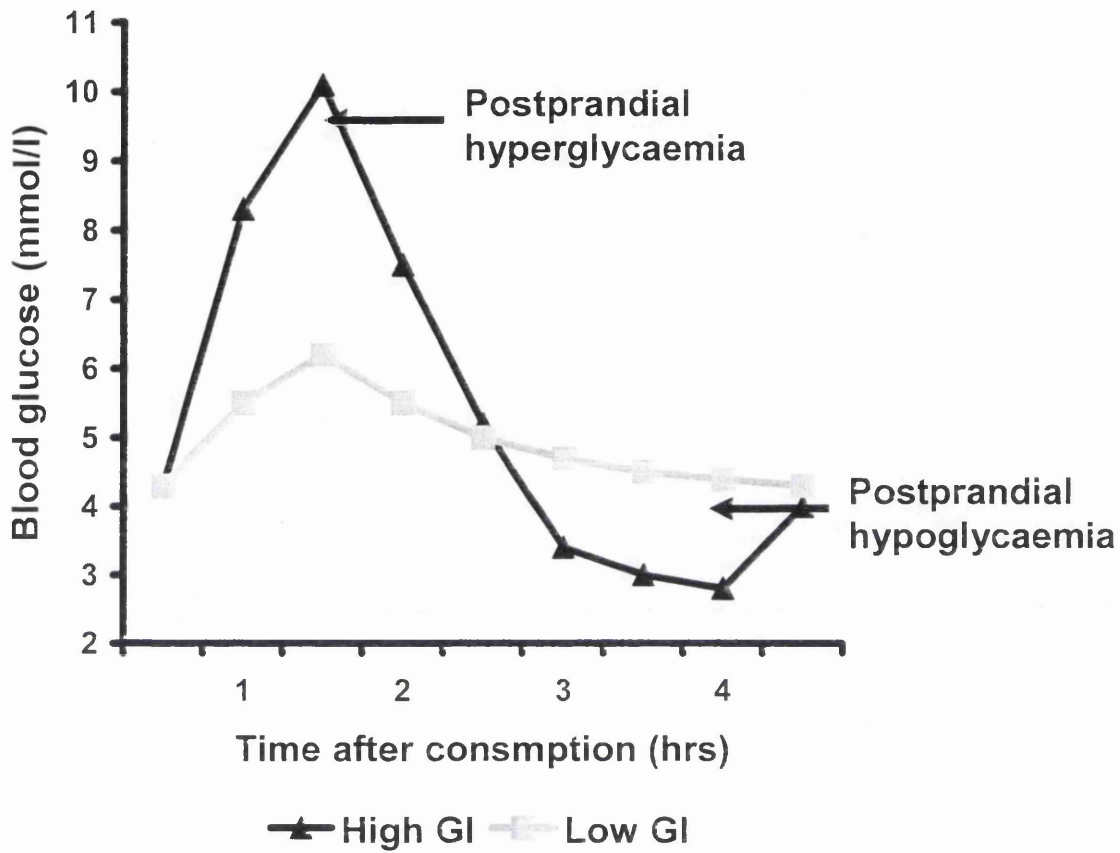


Figure 1. Blood glucose response (mmol/l) to a high compared to a low GI food.

Some, but not all, studies support an association between GI and a range of health benefits, such as decreased incidence of diabetes (Brand-Miller et al. 2003) and heart disease (Barclay et al. 2008). However, GI has been questioned on both conceptual and methodological grounds (Brouns et al. 2005). For example, low GI foods may be energy dense and contain high proportions of fats that, by their nature, delay gastric emptying. Conversely, some fruits that have high amounts of the low GI sugar fructose also contain a number of other potentially beneficial nutrients. Thus health related claims based solely on GI may not be justified. In addition, GI measures carbohydrate quality but not quantity and allows comparison between similar amounts of carbohydrate, usually 50g. The methodological requirement for the GI determination applies solely to situations where food servings contain only the amount of carbohydrate used. However, CHO foods are seldom eaten alone, but rather as a part of a mixed meal. In common day-to-day situations, the amount of CHO content ingested in foods and meals varies greatly. Therefore, the concept of glycaemic load (GL) was introduced by Salmeron et al. (1997). The GL is estimated by multiplying the GI of the food with the amount of CHO (in grams) present in a specified serving size and dividing by 100. GL is a much better predictor of glycaemic response within the context of everyday eating patterns (Wolever and Bolognesi, 1996; Brand-Miller et al. 2003). A low GL food is considered to have a GL of less than 10, a medium GL food to have a GL of between 10 and 20, whilst a high GL food is considered to have a GL of 20 or more (Salmeron et al., 1997).

1.3 The relevance of peripheral glucose metabolism to brain functioning.

When it is considered that glucose is the major fuel for the brain (Magistretti et al, 2000) the importance of blood glucose homeostasis to cognitive functioning is clear. It was reported that during cognitive demand there was a measurable drop in peripheral blood glucose concentrations (Scholey et al. 2001). Furthermore, the greater the decline in blood glucose the better participants performed (Perlmutter et al. 2009). It is possible that a higher rate of decline in peripheral blood glucose reflects greater glucose utilisation by the brain, facilitating cognition. Given these associations any factor that disrupts peripheral glucose homeostasis may compromise the brains functioning, and the ingestion of a meal that has a high glycaemic GL may be one such factor. The following section provides a brief overview of glucose transport in the brain including evidence that changes in peripheral glucose levels can affect these processes.

Glucose is transported across the blood brain barrier (BBB) by the glucose transporter GLUT 1, that is present on both the luminal and abluminal membranes of the BBB endothelial cells. Contrary to the glucose transporters of muscle cells, GLUT 1 is not dependent on insulin for glucose transport. Rather a concentration gradient between the blood and brain compartment is necessary to facilitate glucose transport. GLUT1 can function at less than its maximum functional capacity under normal conditions; therefore it is viewed as less of a rate limiting factor for brain function. However, during neuronal activation it might be necessary for the transport parameters to be adapted to the increased glucose demand (Leybaert, 2005; Leybaert et al. 2007), to prevent it from becoming rate-limiting. Neuronal activation, and the resulting glucose consumption, leads to a drop in

brain interstitial glucose concentrations (McNay and Gold, 2001), such that transport of glucose across the BBB needs to be increased. It has been observed that BBB glucose transport changes under certain conditions. For example, sustained or repeated decreases or increases in plasma glucose concentration, that occur in poorly controlled diabetes, are known to up regulate or down regulate BBB glucose transport respectively. Such adaptation presumably helps keep the supply of glucose to the brain constant. (Christensen et al, 1981; Gjedde and Crone, 1981; Hasselbalch et al, 2001b, 1995; McCall et al, 1982; Mooradian and Morin, 1991; Pardridge et al, 1990; Pelligrino et al, 1990).

Within the brain, glucose is rapidly distributed over the intercellular and intracellular space and taken up by the different brain cells. Specifically, neurons express GLUT3, a high 'affinity' and high capacity glucose transporter compared to GLUT1 (Simpson et al. 2007). There are also reports that neurons in particular areas such as the hippocampus express the insulin dependent GLUT4, and also GLUT8. Astrocytes, whose end-feet surround 99% of the BBB endothelia, express several isoforms of the GLUT family of transporters including, GLUT1, GLUT2, and to a small extent GLUT4 (Qutub and Hunt, 2005). There is evidence that changes in blood glucose metabolism can alter the expression of the majority of these glucose transporters (McCall, 2005; Shah et al, 2012). For example, the neuronal glucose transporter, GLUT 3, is upregulated following low blood glucose levels (Kumagai, 1995; Koranyi et al.1991; Boado and Pardridge,1993; Uehara et al 1997) and these adaptations lead to increased brain glucose content (Lej and Gruetter, 2006).

Brain adaptations to acute and chronic changes in peripheral glucose metabolism are necessarily studied using animal models, such that their relation to cognition has been rarely considered. However, alterations in brain functioning, affected by changes in peripheral glucose metabolism, are likely to have cognitive consequences. Whether changes in brain functioning occurs in response to acute changes in blood glucose during the postprandial period is unknown, and is an interesting question. Nonetheless, the biological evidence supports the need for further studies exploring the relationship between peripheral glucose metabolism and cognitive functioning. The next section considers the evidence that altering the GL of a meal can have cognitive consequences.

1.4 The effect of GL on cognition

A high GL provides a substantial but rather short-lived rise in blood glucose levels which subsequently fall rapidly (Figure 1). It may be predicted that the rapid swings in blood glucose, following a high GL, could disrupt cognition, particularly during the late postprandial period when plasma glucose falls to very low levels. On the other hand, a low GL, which provides a steady and consistent supply of glucose to the brain, may provide a sustained benefit to cognition that extends into the late postprandial period. The following section gives a critical overview of the literature concerning GL and cognition.

1.4.1 Methodology

Online electronic databases; Medline, Google 'scholar', Cochrane Library, Embase, Web of Science and psychINFO (up until 07/02/13) were searched for trials investigating the effects of GL/GI on cognition and mood. The following search terms were used to search for relevant publications: 'glycaemic index' or

'glycaemic load' or 'breakfast' or 'snack' or 'meal' or 'drink' and 'cognition' or 'memory' or 'cognitive performance' or "mood". To minimise publication bias ClinicalTrials.gov was also searched and secondary references were checked. Figure 2 shows the selection process.

Inclusion/exclusion criteria

- Studies were included that investigated the effects of GI/GL on cognition and mood.
- Studies investigating the cognitive effects of isomaltulose, compared to another sugar, were also included.
- Studies were excluded if their attempt at manipulating GL was unsuccessful, or if not enough dietary information were provided for an estimate of GL to be made.
- Studies were also excluded if they were not appropriately randomised and counterbalanced.
- Also excluded were studies examining the cognitive effects of macronutrients in which GL was not the focus.
- Studies on the effects of glucose vs placebo were excluded.
- All populations were included ranging from school aged children to older adults with the exception of very young infants.
- Only studies conducted on samples from industrialised countries were included.
- Survey type studies were excluded.

Data were extracted using defined measures, including sample size, mean age of participants, dietary manipulation given, cognitive and subjective measures examined, time scale of measurement, study design and statistical method (Table 1). For standardisation and more direct comparison between trials, when possible GL of each meal/snack were calculated from the information provided and GI provided by Foster-Powell et al. (2002).

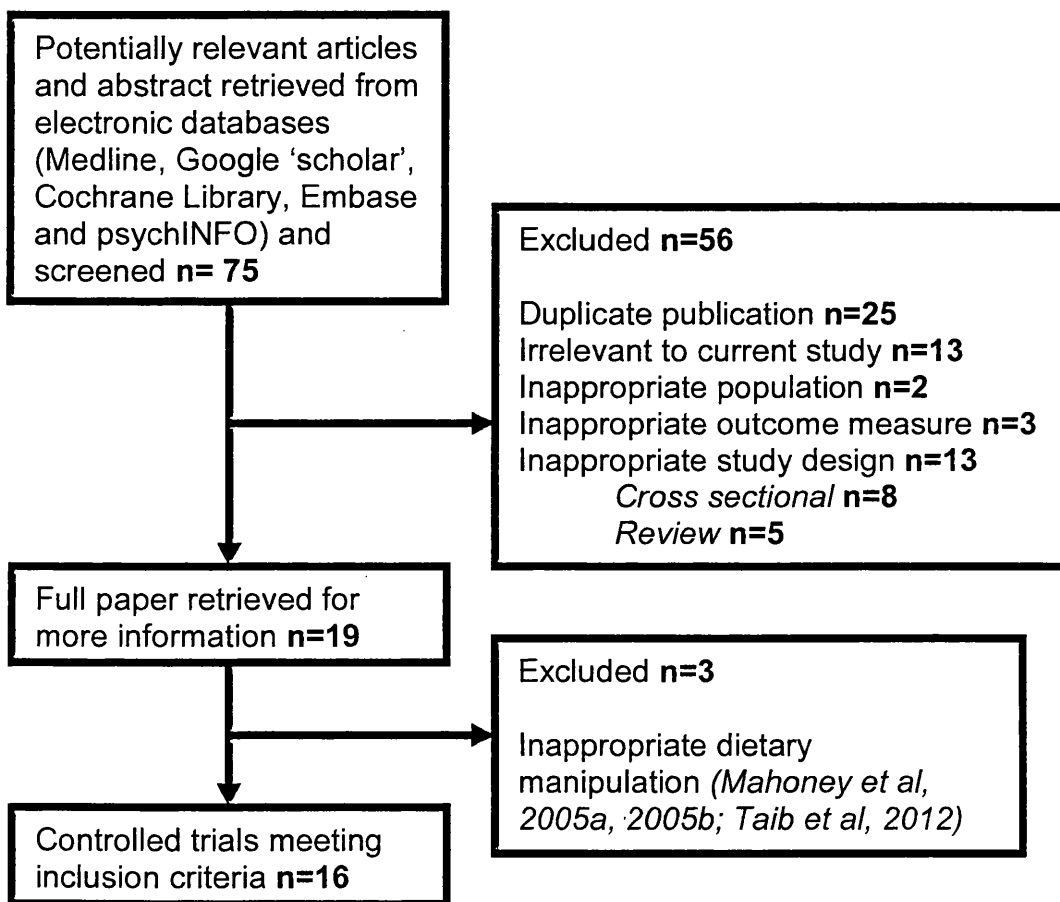


Figure 2. Flow diagram of the screening process. n - number.

1.4.2 Results

A total of seventy five articles were found on an initial search of the electronic databases, sixteen of which were included in the final review (Table 1). Two studies involving the effect GL on children's cognition were excluded (Mahoney et al, 2005a, 2005b; Taib et al, 2012) because they failed to successfully manipulate the GL of their treatments. Three studies examined effects of GL on cognition in primary school children (age 6-9 years) and five studied effect in older children and adolescents (age 11- 15.6 years). All studies included both males and females. The effects in younger children and older children are considered separately given that after the age of about 11 hormonal changes begin to occur that may have an impact on behaviour, and may also have an effect on blood glucose levels (Hindmarsh et al, 1988) and hence the glycaemic response to meals. Three further studies examined effects in young adults (age 20-23). Of these one study used only females (Benton et al. 2003), whereas the other two used both genders. One also examined the effect of individual differences in fasting glucose as a potential modifier (Nabb and Benton 2006). Six studies examined the effect of modulating GL in older adults; all included both males and females and of these five also considered individual differences in glucose regulation.

Author (date)	Design	N	Time scale	Measures	Meals	GL	GT	Findings	Comments
Ingwersen et al. (2007)	WS	26M 38F Mean 9 years.	10, 70, 130 min.	Episodic memory: (Immediate and Delayed). Selective attention.	1) 35 g All Bran breakfast cereal with Semi-skimmed milk. 2) 35 g Coco Pops breakfast Cereal with semi skimmed milk.	7 23	Not measured.	Positive effect of lower GL on memory based on combined % accuracy scores from delayed word list recognition, delayed picture recognition, immediate memory and delayed memory. Low GL reduced the decline in accuracy of attention later in the morning.	Effect of time, low GL better during late PPP. Macronutrients not matched. Lower GL better.
Wesnes et al. (2003)	WS	10 F 9 M Mean 6 years.	0, 60, 120, 180, 240 min.	Episodic memory (Immediate Delayed). Selective Attention.	1) 30 g breakfast cereal with milk containing 29 g total CHO including 16 g as complex CHO (Cheerios). 2) 45 g breakfast cereal with milk containing 38 g total CHO including 25 g as complex CHO (Shreddies). 3) 38g orange-flavoured glucose drink. 4) Fasting condition	15 15 38	Not measured.	Breakfast cereal conditions Reduced declines in attention over the morning and prevented the decline in immediate word recall.	Effect of time, low GL better during late PPP. Macronutrients not matched. Lower GL better.

Author (date)	Design	N	Time scale	Measures	Meals	GL	GT	Findings	Comments
Benton et al. (2007)	WS	10 F 9 M Mean 6 years.	110-180 min.	Episodic memory: (Immediate and Delayed). Reaction times. Sustained Attention. Reaction to Frustration. Classroom behaviour (observational).	1) 25 g Cornflakes, 115 ml semi-skimmed milk, two spoons sugar, one waffle, one tablespoon maple syrup. 2) 60 g scrambled egg, one slice bread, 8 g low-fat spread, 10 g jam. 3) 125 g low-energy yoghurt, 30 g ham, 40 g cheese, 30 g bread, 8 g spread.	18 12 3	Not measured.	Performance on day 1 of the trials of the difficult video game was poorer in those who had consumed highest GL meal but not the other breakfasts. When eating the lowest GL meal significantly more time was spent working on the classroom task at hand compared with the other breakfasts. Lower GL related to better immediate memory and fewer lapses of attention (12sec delay, second half).	Effects only measured mid-late PPP, not clear if there were any earlier effects. Macronutrients not matched. Lower GL better.
Micha et al. (2011)	GI - WS GL - BS	37M 37F Mean 12.6 years.	103-136 min.	Semantic memory. Episodic memory: (Immediate and Delayed). Working memory. Stroop task. SIP. POMS. Hunger and thirst. VAS.	1) High GL Low GI: 66g Alpen muesli, 200ml milk, 245ml apple juice, 7g sugar. 2) High GL High GI: 55g cornflakes, 300ml milk, 200ml apple juice, 7g sugar. 3) Low GL Low GI: 40g Alpen muesli, 250ml milk, 5g sugar. 4) Low GL high GI: 30g cornflakes, 300ml milk, 5g sugar.	41 55 21 28	Not measured.	Low-GI meals associated with feeling less nervous, happier, more alert and less thirsty compared to high GI meals. High-GI meals associated with feeling more confident, less sluggish, less hungry and less thirsty compared the low-GI meals. Semantic memory better after low GI. Stroop and SIP and working memory better after high GI.	Not clear when (time frame) these effects occurred. Macronutrients not matched. High GL better so long as they are also low GI.

Author (date)	Design	N	Time scale	Measures	Meals	GL	GT	Findings	Comments
Smith and Foster (2008)	BS	19M 19F Mean 15.6 years.	20, 60, 100 min.	Episodic memory: (Immediate and Delayed). Bond-Lader VAS.	1) 30 g All-Bran, 125ml milk. 2) 30 g Cornflakes, 125ml milk.	6 16	Not measured.	High GI associated with better delayed memory at 100min.	Effects only considered during early-mid PPP, not clear what effects may have occurred during late PPP. Macronutrients not matched. High GL better.
Brindal et al, (2012a)	WS	13F 26M Mean 11.6 years.	60, 120, 180 min.	Appetite. SIP. Immediate episodic and working memory. Perceptual speed. Attention switching.	1) 70g white bread, 10g margarine, 5g vegemite or jam, 200ml fruit drink. 2) 100g low fat yogurt, 20g full fat cheese, 35g white bread, 5g vegemite or jam, 100ml fruit drink. 3) 100ml full fat milk, 100g low fat yogurt, 20g full fat cheese, 35g white bread, 5g vegemite or jam.	33 24 18	Not measured.	No effect of meal.	Effects only considered during mid-late PPP, not clear what effects may have occurred earlier. Macronutrients not matched. No effect of GL.

Author (date)	Design	N	Time scale	Measures	Meals	GL	GT	Findings	Comments
Cooper et al (2011)	WS	41 (M and F unknown n) Mean 13 years.	30 and 120 min.	Stroop task. Working memory. Selective attention.	1) Cornflakes, milk, white bread and margarine. 2) Muesli, milk and apple. 3) No breakfast	54 36	Not measured.	Stroop – low GL associated with faster responses late morning compared to no breakfast. More correct responses after low rather than high GL or no breakfast. Low rather than high GL associated with faster and more accurate working memory and selective attention (incongruent stimuli).	Effect of time, low GL better during late PPP. Macronutrients not matched. Lower GL better.
Brindal et al. (2012b)	WS	21F, 19M Mean 11.6 years.	60, 120 and 180min.	Speed of Processing. Episodic memory. Attention. Perceptual speed. Appetite.	1) 65g Glucose. 2) 400g Full milk. 3) 200g/32g Half milk/glucose.	65 5 35	Not measured.	Both milk drink improved episodic memory but only in girls.	Effects only measured mid- late PPP, not clear if there were any earlier effects. Macronutrients not matched. Lower GL better.
Benton et al (2003)	BS	71 F Mean 21 years	30-220 min	Episodic memory Immediate: 30, 90, 150, and 210 min. Delayed: 40, 100, 160, and 220 min.	1) High-SAG (low GI) biscuit, 50 g: 34 g CHO (8 g SAG + 20 g RAG). 2) Low-SAG (high GI) cereal bar, 50 g: 31 g CHO (0.05 g SAG + 21 g RAG).	14 20	Not measured	Episodic memory: Based on sum of immediate and delayed recall: better performance after the high SAG (low GI) breakfast at 150 and 210 min.	Effect of time, low GL better during late PPP. Macronutrients not matched. Lower GL better.

Author (date)	Design	N	Time scale	Measures	Meals	GL	GT	Findings	Comments
Nabb and Benton (2006)	BS	168 F Mean 20.41 years.	Battery (30 and 90 min) Mood (20, 50, 80, 110 min)	Episodic memory: (Immediate and Delayed). Vigilance. Simple and choice RT. POMS.	1) LCLF 15.10g CHO 1.46g fibre. 2) LCMF 14.45g CHO 6.09g fibre. 3) MCLF 30.44g CHO 1.56g fibre. 4) MCMF 29.79 CHO 6.19g fibre. 5) MCHF 30.25 CHO 13.05g fibre. 6) HCLF 49.84 CHO 1.44g fibre. 7) HCMF 50.85 CHO 6.13g fibre. 8) HCHF 49.65 CHO 12.93g fibre.	47 71	Fasting blood glucose below and above 6 mmol/l.	Better GT = Better mood. More CHO = more forgetting in those with poorer GT. MED rather than LOW fibre = more word recalled in those with poorer GT in those with better GT MED and HIGH CHO with LOW fibre = faster decision times.	Effects only considered during early-mid PPP, not clear what effects may have occurred during late PPP. Macronutrients not matched. Interaction with fasting glucose. Lower GL better for those with higher fasting glucose (Memory) but higher GL better for those with lower fasting glucose (Decision times).
Dye et al (2010)	WS	24M Mean 23 years.	35 and 115 min	Episodic memory: (Immediate and Delayed). Working memory SIP	1) ISO (50 g) milk based drink. 2) SUC (50 g) milk based drink. 3) Water.	16 32 0	Not measured.	No effect of drink.	Effects only considered during early-mid PPP, not clear what effects may have occurred during late PPP. Macronutrients were matched. No effect of GL.

Author (date)	Design	N	Time scale	Measures	Meals	GL	GT	Findings	Comments
Kashimur et al (2002)	BS	11M 3F Mean 40.1 years.	90 and 150 min	Uchida-Kraepelin psychodiagnostic test.	1) ISO (40g) in water. 2) SUC (40g) in water.	12 26	Not measured.	No significant difference in performance between the ISO and SUC however, after ISO decline over time was less.	Effects only measured mid-late PPP, not clear if there were any earlier effects. Macronutrients were matched. No effect of GL.
Kaplan (2000)	WS	10M 10F Mean 72.5 years.	15,60, 105 min.	Episodic memory: (Immediate and Delayed). Trails part B. Attention (video clip).	1) 50g glucose drink. 2) 50g potato. 3) 50g barley. 4) Water.	50 37 12 0	B cell function. HOMA. gAUC.	BMI and gAUC related to immediate paragraph recall. B cell function, HOMA, BMI and gAUC related to delayed paragraph recall. B cell function, BMI and gAUC related to word list recall. B cell function, HOMA, and gAUC related to trails. Those with poorest B cell function benefit most from 50g carbohydrate regardless of its source.	Effects only considered during early-mid PPP, not clear what effects may have occurred during late PPP. Macronutrients not matched. No effect of GL.
Papanikolaou et al. (2006)	WS	10M 11F Mean 65 years.	15, 60, 100, 140 min.	Episodic memory: (Immediate and Delayed). Digit span. Trail making A and B. Elevator task (Attention).	1) 50 g pasta. 2) 50g white bread. 3) Water.	17 37 0	T2DM diagnosis.	Higher gAUC = poorer memory. No differences between meals @15min but after bread performance poorer compared to pasta (working memory, executive function and auditory selective attention) later in morning.	Effect of time, lower GL better during mid-late PPP. Macronutrients not matched. Lower GL better.

Author (date)	Design	N	Time scale	Measures	Meals	GL	GT	Findings	Comments
Lampport et al. (2012)	WS Glu tol - BS	12M 12 F (T2DM) Mean 61 years. 4M 6F (control) Mean 56 years.	30 and 120 min.	Episodic memory: (Immediate and Delayed). Visual Spatial Learning Test. Corsi Block task. Tapping Test. Grooved Peg Board. Tower of Hanoi.	1) 438g Lucozade Energy Original. 2) 68g bread, 101g yogurt, 19g margarine. 3) Water.	71 3 12 4	T2DM diagnosis.	Type 2 poorer immediate and delayed verbal and spatial memory and slower pegboard than control after water. LGL meal better than water for pegboard (irrespective of glu tol).	Effects only considered during early-mid PPP, not clear what effects may have occurred during late PPP. Not matched on macronutrients No effects of GL. Effects measured mid-late PPP, not clear if there were any earlier effects. Macronutrients matched. Lower GL better.
Nilsson et al. (2012)	WS	12M 28F Mean 62 years.	75 to 225 min.	Working memory. Selective attention.	1) 124 g White bread (WB). 2) 179 g White bread with guar gum (GWB).	-	AUC after WB - median split.	GWB associated with better selective attention in the later postprandial period. Better GT = better cognition. Subjects with better GT had better attention after WB.	Effects measured mid-late PPP, not clear if there were any earlier effects. Macronutrients matched. Lower GL better.
Nilsson et al. (2009)	WS	12 M 28 F Mean 62 years.	35, 90, 120 and 150 min.	Working memory. Selective attention.	1) 50g glucose as bolus. 2) 50g glucose sipped every 30 min (total of 6 sips 8.3g each time).	50 -	Median split (5.4mmol/l) of blood glucose values 3h after 50g bolus	Better GT = better cognition. Working memory - order effect found so only day 1 analysed. Performance increased most during day in those that drank bolus. Sipping associated with better selective attention.	Effect of time, lower GL better (attention) during late PPP. Macronutrients matched. Higher GL better (memory) but lower GL better (attention).

Table 1. Studies examining the effect of modulating the GL of meals/drinks on cognition in children, adolescents, young adults and older adults. GI-glycaemic index, GL-glycaemic load, WS-within subjects, BS-between subjects, AUC-area under the curve, T2DM-type 2 diabetes, WB-white bread, CHO-carbohydrate, PPP-postprandial period, SIP-speed of information processing, POMS-profile of mood states, VAS-visual analogue scale, ISO-isomaltulose, SUC-sucrose, GT - glucose tolerance, LCLF - Low carbohydrate, low fibre, LCMF - Low carbohydrate, medium fibre, MCLF - Medium carbohydrate, low fibre, MCMF Medium carbohydrate, medium fibre, MCHF - medium carbohydrate, high fibre, HCLF - high carbohydrate, low fibre, HCMF - high carbohydrate, high fibre, HCHF - high carbohydrate, high fibre.

1.4.2.1 The effect of GL on cognition and mood in younger children.

The GL of the meal/drink varied considerably between studies ranging from 3 (Benton et al. 2007) to 38 (Wesnes et al. 2003), however, the difference in GL between the lower and the higher GL within each study were similar between studies with the smallest difference being 15 (Benton et al. 2007) and largest being 23 (Wesnes et al. 2003). All studies manipulated breakfast after children had abstained from food overnight. Ingwersen et al. (2007) manipulated GL by providing two breakfast cereals that differed in GL (All Bran and Coco pops), however, in addition to differing in GL these cereals also offered different amounts of available carbohydrates making interpretation difficult. Similarly, Wesnes et al. (2003) provided two breakfast cereals (Cheerios and Shreddies), however; they did not differ significantly in GL. They also provided a highly glycaemic glucose drink (38g) that was compared with each breakfast cereal, again the meals differed in their degree of available carbohydrate. The cereal products were ingested with milk, which is insulinotropic, and would probably result in lower GIs than calculated from the individual carbohydrate components, as well as shortened blood glucose response profiles. Benton et al. (2007) provided meals that differed in their macronutrient content as a means of manipulating the GL. Although Benton et al. (2007) entered the various macronutrients into a regression model to account for their influence on cognition and mood, no study successfully matched meals on their macronutrient content.

All studies reported a beneficial effect of a lower rather than a higher GL. Although it has been reported that the 'glucose facilitation effect' has the strongest effect on memory (Hoyland et al. 2008), the positive effect of a lower GL meal does not

appear to be limited to this domain, at least in young children. Benefits were observed on episodic memory (Ingwersen et al. 2007; Wesnes et al. 2003; Benton et al. 2007), selective (Ingwersen et al. 2007; Wesnes et al. 2003) and sustained attention (Benton et al. 2007), reaction to frustration and time spent concentrating in class (Benton et al. 2007). Lowering the GL of breakfast does not seem to influence young children's simple reaction times (Benton et al, 2003). The beneficial effect of a lower GL occurred during the late rather than the early postprandial period; young children's performance declined over the morning after they had eaten a higher GL breakfast (Ingwersen et al. 2007; Wesnes et al. 2003), and this decline was prevented by the consumption of a lower GL meal. The GL of a meal had similar effects in both boys and girls (Ingwersen et al. 2007 Benton et al. 2007).

An important consideration is that in Benton et al (2007) study, no effects were observed when the data were considered using a series of ANOVAs; effects only occurred when multiple regression were used which may have been caused by differences in the amount of breakfast children ate (they were not forced to consume everything). Memory and attention were the domains most studied and hence effects of GL on other cognitive domains such as executive function remain unstudied. In addition, no study measured the blood glucose response to the meals, presumably due to the children young age. It should be noted that although GI/GL may be calculated or estimated from international tables of GI values (which are based on adult responses), it is unknown whether children and adults differ in their glycaemic responses since GI studies have not been conducted in children (Hoyland et al. 2009).

All studies employed a within subjects (WS) design, however, only Benton et al. (2007) considered order of meal presentation as part of their analysis. Indeed when order was considered there was an effect of order where children's reaction to frustration only improved after the lower GL breakfast on day 1. Presumably this effect occurred because by day 2 the children had learned that the task was impossible.

1.4.2.2 The effect of GL on cognition and mood in older children and adolescents.

Again the GL of the meal/drink varied considerably between studies. GLs ranged from 5 to 65. Interestingly both the highest and the lowest GL were found in the same study; Brindal et al. (2012b). The difference in GL between the lower and the higher GL within each study also varied from 6 (Brindal et al. 2012a) to 60 (Brindal et al. 2012b). A range of methods were used to manipulate GL. Three studies used various combinations of different breakfast cereals (Smith and Foster 2008; Cooper et al. 2011; Micha et al. 2011). Both studies by Brindal et al. varied the amount of dairy in each meal/drink to manipulate GL (Table 1). All studies examined breakfast after an over night fast. Again no study successfully matched meals based on their macronutrient composition.

In contrast to younger children the effect of modulating GL on older children's cognition is less clear. Cooper et al. (2011) reported that adolescents' working memory and attention were better later in the morning after the lower rather than a higher GL breakfast. Similarly, Brindal et al (2012b) found that episodic memory was improved after the low and medium GL drink compared to the high GL drink

but this effect was limited to girls. In contrast, two studies (Micha et al. 2001; Smith and Foster, 2008) reported that delayed memory and mood were improved after a high rather than a low GL meal and a further study reported no effect of either (Bridal et al. 2012a). In regards to Brindal et al. (2012a) their manipulation of dairy as well as GL may have caused a significant confound. The mechanisms involved in the effect of GL on cognition have not been determined but increasing reports suggest that insulin itself can modulate memory (Banks et al, 2012). If the effect of GL on memory is mediated, even in part, by an effect of insulin then the insulinotropic nature of dairy may disguise any benefits associated with a lower rather than a higher GL. Although in older children and adolescents more domains were used and speed of information processing (SIP), reaction times (RT) and appetite were measured there were no effects of GL reported (Bindal et al. 2012a; 2012b; Micha et al. 2011). Domains that appear most susceptible to modulation of GL are memory and attention.

Again effects were strongest later in the morning (Cooper et al. 2011; Brindal et al. 2012b) and all but one study considered these longer term effects. Smith and Foster (2008) only considered effects up until 100 minutes post consumption and this may explain their finding that the higher GL meal was cognitively superior. Glycaemic measurements were performed up to 90 min, and it was clear that no significant differences in glycaemia were observed during this time. Beneficial effects of a low GL meal tend to occur two to three hours after eating (Ingwersen et al. 2007; Cooper et al. 2011), such that their time frame may have been too short to capture these effects.

Three out of the five studies employed a cross over design (Bindal et al. 2012a; 2012b; Cooper et al. 2011), however, no study included order as a factor in their analysis.

1.4.2.3 The effect of GL on cognition and mood in young adults.

Studies have also been performed in young healthy adults (Table 1). Again studies have provided a range of different meals and used a variety of methods to modulate GL. Benton et al. (2003) investigated the impact of two cereal based breakfasts in young females. The breakfast meals contained similar carbohydrate contents and GL characteristics (42 and 66, respectively; Table 1). Nabb and Benton (2006) provided meals that differed in their fibre and carbohydrate content (Cornflakes vs All Bran). Dye et al (2010) was the only study to successfully match the macronutrient content of drinks while varying GL. They used a novel low glycaemic sugar: isomaltulose (GL 32) and compared its cognitive effects to that of sucrose which has a higher GL (GL 65). However, again the sugars were consumed in a milk based drink, potentially confounding the affect of GL.

Benton et al. (2003) reported that the lower GL breakfast significantly improved memory function, particularly in the late postprandial phase (150 and 210 min after breakfast). However, there were no concomitant differences in blood glucose suggesting that cognitive outcomes could not be attributed to late glycaemia per se. On the other hand despite controlling for macronutrient composition, Dye et al (2010) reported no beneficial effects of the low rather than the high GL drink. Interestingly capillary and interstitial blood glucose after both isomaltulose and sucrose had returned to or were below fasting level, respectively, after

approximately 95 min, which may be attributed to an enhanced insulin response after a milk-based vehicle. If this were the case then the glycaemic differences between the two sugars may have been ameliorated to the extent they were no longer cognitively significant. In addition, Dye et al (2010) only studied effects up until 115 minutes post consumption, which may not have been long enough for the superior effects of isomaltulose to emerge, given that the Benton et al. (2003) effects became apparent after 150 minutes.

Nabb and Benton (2006b) found that consuming more carbohydrates resulted in more forgetting, but only in those with higher fasting blood glucose levels, suggesting that individual differences in physiology may be important moderators. However, higher amounts of carbohydrates improved reaction time after 90 min in the poor regulators suggesting beneficial effects of a lower GL may be domain specific. The lowest levels of fibre (1.5 g) were associated with poorer memory in participants with poorer glucose tolerance. However, blood glucose responses were not affected by dietary fibre content, indicating that the expected variation in GL may not have been achieved. Of the studies in young adults two employed a between subjects (Benton et al. 2003; 2006b) and one a within subjects design (Dye et al. 2010). Dye et al. (2010) considered order as part of their analysis but found no interaction with GL.

1.4.2.4 The effect of GL on cognition and mood in older adults.

Six studies were identified that considered the effects of meals differing in GL on older adults' cognition. A range of methods were used to vary the GL. Five of these studies matched meals based on their carbohydrate content. Kaplan et al.

(2000) studied three test meals that provided 50 g of available carbohydrates from pearl barley (GL = 14), potatoes (GL = 28 - 59), glucose (GL = 50), rather than a placebo drink with no carbohydrates. Similarly, in a group of older adults with type 2 diabetes mellitus (T2DM) Papanikolaou et al. (2006) provided breakfasts consisting of pasta (GL = 15 - 25), toasted white bread (37 - 56) or water.

Kashimur et al (2002) provided either 40g isomaltulose (GL = 12.8) or 40g sucrose (GL = 28) taken in water, however statistical effects were considered thus Kashimur et al's findings should be interpreted with caution. Nilsson et al. (2009) provided a 50g glucose solution that was either drunk as a bolus or sipped across the morning to mimic the glycaemic effect of a lower GL and Nilsson et al. (2012) gave 124g of white bread with or without the addition of guar gum, which slows absorption. In the final study GL was manipulated by comparing 438g of glucose (Lucozade Energy Original GL - 71.3) with a standard breakfast (68g bread, 101g yogurt, 19g margarine, GL - 12.4) (Lampert et al. 2012). All studied the effects of breakfast after participants had fasted overnight.

Importantly, the glucoregulatory status of the participants was considered in five out of six studies; however, this was defined in various ways such that it is difficult to compare studies. Both Lampert et al (2012) and Papanikolaou et al. (2006) studied older adults with T2DM and it should be noted that, although it would be unethical to study unmediated diabetics, in both studies participants were taking some form of oral hypoglycaemic. Lampert et al. (2012) found no effects of high or low GL meal in those with T2DM or healthy controls. However, it is possible that the high GL glucose drink (Lucozade Energy Original) contained 48mg of caffeine which may have masked any negative effects of a higher GL.

Papanikolaou et al. (2006) did not employ a control group of healthy volunteers therefore it is not known whether the beneficial effects of a lower, rather than a higher, GL meal observed in their sample of T2DM, would have occurred similarly in a healthy sample. Kaplan et al (2000) measured B cell function, insulin sensitivity (estimated by The Homeostasis Model Assessment (HOMA)) and glucose area under the curve (AUC) after the 50g glucose drink to determine which indices best predicted cognition; older adults with the poorest B cell function appeared to benefit most from consuming 50g of carbohydrates regardless of their composition. Nilsson et al. (2009) used blood glucose concentration 3 hours after drinking 50g glucose as a bolus, and Nilsson et al. (2012) considered AUC after participants ate white bread. However, both studies by Nilsson et al (2009; 2012) failed to directly compare the effects of GL in those with better or poorer GT, thus it is unclear how/if GT status interacts with the effect of GL in these studies. Given the variation in definitions used to define glucose tolerance it is hard to draw any conclusion about how the GL of a meal interacts with individual glucoregulatory states.

Effects have been considered across a wide number of domains including immediate and delayed episodic memory, working memory, selective and sustained attention and executive function (Table 2). However, surprisingly subjective measures, for example mood, remain unstudied in this population. In addition, no study considered the effects of immediate episodic memory during the late postprandial period (PPP) (121 minutes onwards).

	Early PPP (0- 60min)	Mid PPP (61-120min)	Late PPP (121min +)	Total
Working memory/ executive function.	0/11	1/4	1/3	2/18
Verbal ST memory.	2/6	0/4		2/10
Spatial ST memory.	0/2	0/2		0/4
Verbal delayed memory.	0/3	1/5	2/3	3/11
Spatial delayed memory.	0/1	0/1		0/2
Attention, concentration, vigilance.	0/3	1/7	3/5	4/15
Psychomotor skill.	0/1	0/1		0/2
Total *	2/27	3/24	6/11	11/62

Table 2. The ratio of significant to non-significant findings for studies of early mid or late PPP for each cognitive domain (significant/non-significant). Only significant differences between higher and lower GL meals are reported. * Does not add up to sum of above as some tests are classified in more than one cognitive domain. PPP – post prandial period.

The majority of studies have considered effects of GL during the early to mid PPP and Table 2 displays the ratio of significant to non-significant findings for studies of early mid or late PPP. A total of twenty seven measures of cognition were taken within the 60 minutes post consumption and of these only two significant effects of GL were observed (Papanikolaou et al. 2006). Similarly, twenty four measures were taken between 60 and 120 minutes after eating and again only three significant effects were observed (Papanikolaou et al. 2006; Kaplan et al. 2000). Given the large number of measures during these time periods it is possible that the small number of significant observations may have occurred by chance. During the late postprandial period (120 minutes onwards) only eleven measures were taken, and of these six found beneficial effects of a lower rather than a higher GL.

(Papanikolaou et al. 2006; Nilsson et al. 2009; 2012). This suggests that the beneficial of a lower GL may be more apparent during this time.

In conclusion, although beneficial effects of a lower GL have been noted in older adults (Nilsson et al 2009, 2012; Papanikolaou et al, 2006; Kashimur et al 2002) effects are far from consistent (Lamport et al, 2012; Kaplan et al, 2000). The vast majority of cognitive measures have been taken during the early or mid PPP (up to 120 minutes) however, any beneficial effect of a lower GL meal appear stronger during the late PPP (Table 2). Although studies have considered the moderating influence of glucose tolerance, the variation in definitions used to define glucose tolerance makes it hard to draw any conclusions.

1.4.3 Discussion and conclusions.

Of the sixteen studies presently considered two do not allow conclusions to be drawn about how differences in GL influence cognition and mood (Kashimur et al. 2002; Micha et al. 2011). Of the remaining fourteen, two found no effect of GL (Kaplan et al 2000; Dye et al. 2010 Brindal et al, 2012) but methodological weaknesses may account for these findings; the manipulation of dairy presents a possible confound in two of these studies (Kaplan et al 2000; Dye et al. 2010). In addition, Dye et al (2010) ended the testing procedure slightly short of the time frame at which the cognitive effects of GL are likely to emerge. One study reported benefits of high GL vs low GL breakfasts (Smith and Foster, 2008) with the effect in question occurring after 100min; during the mid rather than the late postprandial period, again this effect may reflect the shorter time frame.

A total of ten studies provided evidence that a lower GL meal was cognitively superior to a higher GL meal (Ingwersen et al. 2007; Wesnes et al. 2003; Benton et al. 2007; Cooper et al 2011; Brindal et al. 2012b; Benton et al. 2003; Nabb and Benton 2006; Papanikolaou et al. 2006; Nilsson et al. 2009; Nilsson et al. 2012) but the quality of the evidence in these studies varied, and in many cases the GL of the test breakfasts are estimates from international tables, when no actual glycaemic profiles were provided. Most studies report effects on cognitive outcomes in the late postprandial phase. Although benefits in cognitive function in the late postprandial phase coincided with higher late glycaemia (Nilsson et al. 2009), such benefits are also reported with low GL breakfasts in the absence of differences in blood glucose levels (Benton et al. 2003), suggesting that the benefits of a lower GL may not be directly attributed to differences in the level of blood glucose as such.

Although only three studies (Ingwersen et al. 2007; Wesnes et al. 2003; Benton et al. 2007) met the inclusion criteria for this review effects in younger children are the most consistent and this may reflect a very high rate of brain tissue glucose utilisation (Chugani 1998). However, effects in older children are less consistent with only two (Cooper et al 2011; Brindal et al. 2012b) out of four supporting the benefits of a lower GL. It may be that older children are less vulnerable to changes in postprandial glycaemia or that poor experimental designs could not detect differences. The evidence in young adults is similarly unclear; while one study reported that a low GL is cognitively better (Benton et al. 2003) another reported no effect of either a high or a low GL (Dye et al. 2010). The third study in this population suggested that while a lower GL may improve some tasks for some

people, a higher GL may improve other tasks for others (Benton et al. 2006) making interpretation complex.

Only three out of five studies in older adults reported a benefit of a lower GL (Papanikolaou et al. 2006; Nilsson et al. 2009; 2012). However, the available data examining the effects of differences in GL on cognition on older adults highlights an important issue. If lower GL meals do confer greater cognitive benefits than higher GL meals it is important to identify potential physiological differences that may moderate this relationship. There is evidence that individuals with poorer glucose tolerance have poorer cognition, in particular memory, and that these effects are exacerbated with age (see section 1.5). Five out of the six studies conducted in older adults considered the impact of glucose tolerance and there is some evidence for an interaction with the effects of a meal (Kaplan et al. 2000) and possibly GL (Nilsson et al, 2009; 2012). However, given the heterogeneity in the way GT was defined no conclusions can be made. For example, different pathologies underlie impaired glucose tolerance and impaired fasting glucose (Abdul-Ghani et al, 2006) and these pathologies may result in different cognitive problems. It is critical that authors are clear about which aspect of glucose tolerance they wish to tap if research in this area is to move forward. Furthermore, although in both studies by Nilsson et al. (2009; 2012) an interaction between glucose tolerance and GL was implied, comparisons between the effects of high and low GL in those with and without poor glucose tolerance were not conducted and as such no conclusions can be drawn.

The conclusions from studies available to date are tempered by a range of methodological limitations, e.g. poor choice of meals ingested; the use of milk

products; little consideration given to the matching of meals based on their macronutrient content; a limited range cognitive tests administered; lack of adequate information on, or physiological confirmation of, the course of postprandial glycaemia; insufficient duration of the time after the meal; inappropriate statistical methods; too few participants. Taken together, the studies generally favour LGI meals for improved memory and/or attention, particularly in children and older adults who may be more cognitively vulnerable; the effects have been noted most often during the late postprandial phase. Besides the glycaemic effects no consideration has been given to underlying mechanisms. Similarly to previous reviews (Gilsenan et al. 2009) it is concluded that more studies are needed to better elucidate the cognitive effects of manipulating GL.

1.5 Physiological factors effecting GI and GL.

1.5.1 Glucose tolerance and postprandial hypoglycaemia.

Physiological factors can influence the glycaemic response to a food or meal. An individual difference in the ability to regulate blood glucose levels is one such factor. The normal control of postprandial glucose requires the suppression of hepatic glucose output, an increase in insulin secretion that is proportional to the glycaemic response of the meal and adequate insulin sensitivity in the muscle and liver for glucose uptake. Impaired glucose tolerance (IGT) is a pre-diabetic state of hyperglycaemia in which either insulin secretion is impaired, or there is reduced insulin sensitivity, most importantly in skeletal muscle (Ryden and Mellbin, 2012).

According to the world health organisation (WHO) a person's glucose tolerance should be measured using a two hour 75g oral glucose tolerance test (OGTT). After fasting over-night, 1.75 grams of glucose per kilogram of body weight are

consumed, to a maximum of 75 g. Every 30 minutes for 120 minutes either capillary or venous blood glucose is monitored. The category in which an individual is placed depends on their blood glucose levels after 2 hours, (Table 3). Impaired glucose tolerance (IGT) is considered a metabolic state that lies between normal glucose homeostasis and diabetic hyperglycaemia. Although chronic hyperglycaemia is associated with an array of negative symptoms, acute hyperglycaemia is not easily recognisable and minor symptoms may include increased thirst and weakness or feeling tired (O'Connor et al. 2006).

	Venous	Capillary
Diabetes	> 10.0	> 11.0
IGT	6.7–9.9	7.8–11.0
Normal	< 6.7	<7.8

Table 3. Whole blood (mmol/l) values, 2-h post- glucose load, for diagnosis of diabetes mellitus or impaired glucose tolerance (WHO, 1999).

The most likely cause of impaired glucose tolerance is insulin resistance; that is a reduced cellular response to insulin despite its presence. The usual response to insulin resistance is compensatory hyperinsulinaemia (Polonsky et al. 2000), therefore hyperglycaemia and glucose intolerance do not occur until insulin secretion becomes impaired (Seino et al. 2011). However, if too much insulin is produced blood glucose levels may fall too low. Postprandial hypoglycaemia (PH) is a term describing recurrent episodes of symptomatic hypoglycaemia occurring within 4 hours after a high carbohydrate meal (or oral glucose load) in people who

do not have diabetes. It is thought to represent a consequence of excessive insulin release triggered by a carbohydrate meal (Brun et al, 2000). Individuals with PH often suffer from an associated adrenergic hormone response which is responsible for the major symptoms of hypoglycaemia.

The boundaries between hypoglycaemia and normoglycaemia have been the matter of debate and there are large individual differences in the threshold at which symptoms occur (Blackman et al, 1990; Gonder-Frederick et al, 1994). Harris (1924) first described PH and reported that symptoms of hypoglycaemia occurred at blood glucose values below 3.9 mmol/l, however, the blood glucose value defining hypoglycaemia has fluctuated since this time. It has been argued that neuroglycopenia and its associated cognitive dysfunction precede adrenal discharge and may occur at thresholds as high as 4mmol/l (De Feo et al, 1988). In both IGT and PH a low GL diet is indicated to help maintain glycaemic control (Brun et al, 2000; Wolever et al, 2002; Gellar and Nansel, 2009). The following section reviews the evidence that an impaired ability to regulate postprandial glucose levels, the result of IGT, PH or both, may result in cognitive deficits.

1.5.2 The effect of individual differences in glucoregulation on cognition.

1.5.2.1 Methods

Literature searches were conducted for RCT using PubMed, Psych Info, and Google Scholar with keywords that included 'glucose tolerance', glucose regulation' or 'glucoregulation' and 'cognitive performance' or 'memory' or 'mood'. Only English-language articles or abstracts were retrieved. The search primarily sought peer-reviewed publications, but some published dissertations were also

evaluated. A total of 24 studies met the inclusion criteria. Of these studies fifteen related cognition to glucose tolerance in the absence of any further dietary manipulation (Table 4) and nine examined cognition either during the OGTT or following a subsequent glucose drink (Table 5). Six were longitudinal and the rest were cross sectional. Given that evidence suggests glucose consumption may affect the relationship between glucose tolerance and cognition (Awad et al, 2002; Messier et al, 1999; Riby et al, 2008) studies which administer glucose during testing are distinguished from those that do not and are discussed separately.

Author (date)	N	GT measurement	Definition	Cognitive tests	Findings	Design
Vanhanen et al. (1998)	Recruited from study of risk factors for CVD, n = 586, 369 female, 217 male. Mean age 73 yrs.	75 g OGTT	WHO criteria for NG and IGT	Buschke Selective Reminding Test (BSR), Visual Retention Test, Trail Making Test, Verbal Fluency Test for letters, and category, MMSE. Performed at follow up	IGT participants had significantly lower BSR-L scores. IGT men were significantly lower in MMSE and there was a trend of poorer cognition for IGT women.	Longitudinal - 3 ½ yr follow up OGTT at baseline and follow-up, cog tests at follow up.
Scott et al. (1998)	Recruited from the Rancho Bernardo study for risk factors for CVD. 643 men and 875 women, mean age 73.9 yrs.	75 g OGTT	WHO criteria for DM, IGT, and NG	3 years after glucose assessment. BSR, Visual Reproduction Test, MMSE, Blessed Information Memory Concentration Test, Trails B from the Halstead-Reitan Neuropsychological Battery	ns	Longitudinal - OGTT at baseline cog tests 3 yrs later.
Helkala et al. (2001)	Recruited from the Kuopio Ischemic Heart Disease Risk Factor Study, n = 528 men. There was a 54-yr-old age group and a 60-yr-old age group.	75 g OGTT	WHO criteria for DM, and IGT.	Buschke Selective Reminding Test, Trail Making Test A, Trail Making Test B. Assessed at 4 years follow up	Only participants with AGT (IGT and DM combined) and apoE E2 allele had poorer executive control compared with NGT participants.	Longitudinal 4 yr follow up.
Kanava et al. (2004)	Recruited from the Rancho Bernardo study for risk factors for CVD, n = 999 men and women, mean age 71 yrs.	75 g OGTT.	WHO criteria for NGT, IGT, and DM.	MMSE, Verbal Fluency, Trail Making Test B. Cognitive tests performed at baseline and 4-year follow up.	No differences at baseline or after 4 years. Major decline (25 th percentile) in verbal fluency in females with IGT after 4 years.	Follow up 4 yr - OGTT only at baseline
Kumari and Marmot (2005)	Participants from the Whitehall II cohort, n = 5647 males and females, mean age 56 yrs.	75 g OGTT.	WHO criteria for NGT, IGT, and DM (types 1 and 2).	Verbal Memory Test, The Alice Heim 4 (reasoning), the Mill Hill (vocab), and phonemic and semantic fluency tasks.	ns	Longitudinal Follow up 3 to 4 yr Cog tests and OGTT at baseline and follow up.

Author (date)	N	GT measurement	Definition	Cognitive tests	Findings	Comments
Paike-Hyvärinen et al. (2009)	1279 subjects from the Helsinki Birth Cohort Study (average 64 yrs).	75 g OGTT	WHO criteria for NGT, IGT, IFG and DM.	Simple and choice RT Divided attention task Working memory Associate learning	ns Diabetics but nor IGT or IFG had poorer cognition.	Longitudinal 2-3 yr follow up - OGTT at baseline, cog tests at follow up.
Donohoe and Benton (2000)	46 female undergraduate volunteers from the University of Swansea, mean age 22 yrs.	50 g OGTT.	Baseline, AUC, Time to baseline, Area below baseline, Rate of rise, Time to peak, Zenith, Nadir, Rate of fall, Time to nadir.	Immediate recall, delayed recall, reaction time, and vigilance. Performed 1 week after OGTT. Glucose also measured during cognitive assessment	The quicker that blood glucose returned to baseline values from the nadir, the better was memory. Those with higher peak blood glucose performed worse on the vigilance task.	Cross-sectional. Cognitive tests 1 week after OGTT
Hiltunen et al. (2001)	Random sample from Finland, aged >69 years, n = 379. 141 males median age 75 and 238 females median 77 yrs.	75 g OGTT.	WHO criteria for DM, IGT, and NGT.	MMSE	IGT, was associated with impaired cognition, but not significant after controlling for CVD, age, sex, education, alcohol, and depression. Age and lower education most strongly related to poorer cognition.	Cross-sectional.
Kalmijn et al. (1995)	Recruited from Zutphen Elderly Study of chronic diseases in men, n = 462, aged 69-89, mean 75 yrs.	75 g OGTT	WHO criteria for NG, IGT, and DM	MMSE. A score of >25 indicated unimpaired cognitive function, 24 and 25 indicated borderline cognitive impairment, <24 indicates poor cognitive function	Larger percentage of those with IGT scored less than 24 on MMSE. However, overlap of confidence intervals suggests may not be any real differences.	Cross sectional.
Fuh et al. (2007)	28 women aged 40-54 (mean 48 years). 68 had IGT and 72 had diabetes.	75g OGTT	WHO criteria for IGT	Forward and Backward digit span, Continuous Recognition Paradigm, Trail Making Test A and B, Verbal Fluency, Rey Auditory Verbal Learning Test.	Ns trend for IGT to have a lower forward digit span.	Cross sectional.

Author (date)	N	Glucose measurement	Definition	Cognitive tests	Findings	Comments
Vanhanen et al (1997)	Recruited from study examining risk factors for myocardial infarction, <i>n</i> = 108, mean age 65 yrs.	75 g OGTT.	Participants split into 3 groups; DM, low risk, and high risk, the latter two groups being determined by a 2 h glucose level median split	MMSE Buschke Selective Reminding Task, the Visual Reproduction Test, the WAIS Block Design Test, the WAIS Digit Symbol Test, the Verbal Fluency Test on Category.	The group with increased risk (poor glucoregulation) had impaired performance on a variety of tests, compared with the low risk group. The normoglycaemic participants with increased risk for DM had more impaired cognition than those with DM	Cross sectional.
Convit et al. (2003)	30 elderly participants from a clinical population who were non-diabetic, mean age 69 yrs (range 53–89) 17 females and 13 males.	Intravenous GTT, 0.3 g/kg dose up to 25 g.	Three parameters were measured: baseline, 2 h glucose, and AUC during IVGTT	The Wechsler Paragraph Test was used to assess immediate and delayed recall and the MMSE for overall cognition	All three indices of glucose regulation were inversely associated with scores on the Wechsler Paragraph Recall Test. Higher baseline and 2 h post-infusion glucose levels as well as larger area under the curve had decreased immediate and delayed memory performance. In addition higher 2 h glucose levels and area under the curve were associated with poorer MMSE performance	Cross-sectional.
Rolandsson et al. (2008)	232 females and 179 males data pooled from a survey of health and a separate survey of cognition.	75 g OGTT	Fasting and 2hr	Episodic memory and semantic memory	Higher fasting glucose and 2 h-post OGTT levels were correlated with poorer episodic and semantic memory but only in women	Cross sectional
Lu et al. (2012)	813 males and 909 females Chinese (average 62.5 yrs)	75 g OGTT	WHO criteria for NGT, IGT, and DM.	MMSE	Impaired glucose tolerance and diabetes related to poorer cognition but only in females.	Cross sectional

Table 4. Longitudinal and cross sectional studies on the association between IGT and cognition. OGTT-oral glucose tolerance test, NGT-normal glucose tolerance, IGT-impaired glucose tolerance, DM-diabetes mellitus, MMSE-mini mental state examination, IVGTT - intravenous glucose tolerance test.

1.5.2.2 Glucose tolerance and cognition - longitudinal studies

Six studies were identified that provided longitudinal data (Table 4) all of which used the WHO criteria for the identification of participants with glucose intolerance. All studies examined episodic memory and the majority also measured attention, semantic fluency and the MMSE. Only 1 study (Paile-Hyvärinen et al, 2009) examined the effects of glucose tolerance on reaction times (RT) and working memory and one other study also measured reasoning (Kumari and Marmot 2005).

Findings from these studies vary and do not provide consistent evidence of an association between IGT and cognitive impairment. Three studies report no association between IGT and cognition (Scott et al. 1998; Kumari and Marmot 2005; Paile-Hyvärinen et al. 2009). The remaining studies provide some evidence that an association may exist in some but not all participants. Vanhanen et al. (1998) provided clear evidence that IGT was associated with poorer mini-mental state examination (MMSE) performance and poorer long-term verbal memory over a 3.5 year period; however, the correlation was only significant in men. In contrast, Kanaya et al. (2004) found after 4 years a major decline (25th percentile) in verbal fluency in females with IGT when there were no differences at baseline. The strength of these two studies is that GT was assessed at both baseline and follow up, allowing any participants who were no longer classified as having IGT to be removed from the analysis. This observation may explain the lack of association found in other longitudinal studies, for example, Scott et al (1999) gave participants an OGTT 3 years before cognitive tests were conducted. Similarly, Paile-Hyvärinen et al. (2009) examined cognition 2 to 3 years after GT was measured. Given that the reproducibility of an OGTT is open to question (Balion et al., 2007),

and that some participants may no longer have been considered as having IGT at follow-up, further longitudinal research is required in which cognition and glucose tolerance are assessed at both baseline and follow up. It is also worth noting that although all studies were conducted in older samples, the studies which report an association between cognition and IGT used participants that were a decade older than the studies that report no relationship. It is possible that some interaction occurs between the age of the subject and their gluco-regulatory status that negatively impacts upon cognition.

1.5.2.3 Glucose tolerance and cognition – cross sectional studies

Eight studies were identified that considered the relationship between IGT and cognition using a cross sectional design (Table 4). All studies gave a 75g OGTT with the exception of Donohoe and Benton (2000) who gave a 50g OGTT and Convit et al. (2003) who gave an intravenous GTT (0.3g/kg). Most studies used the WHO criteria to distinguish those with and without IGT again Donohoe and Benton (2000) were the exception in that they examined the predictive value of a number of glucose tolerance indices. A range of cognitive domains were considered including immediate and delayed episodic memory, semantic memory, various tests of attention and the MMSE. Only 1 study examined the effect of IGT on RT (Donohoe and Benton 2000) and one study considered effects on working memory (Fuh et al. 2007).

Overall, three studies did not find any association between IGT and cognition. (Fuh et al. 2007; Hiltunen et al. 2001; Kalmijn et al. 1995). These negative findings may be explained by the use of the insensitive MMSE to assess cognitive function,

although Fuh et al (2007) did also administer other tests (Table 4). The MMSE is useful for distinguishing clinical conditions such as dementia but is unlikely to be sensitive enough to distinguish small differences in cognitive function that might be expected between those with normal glucose tolerance (NGT) and those with IGT. A further two studies only found significant associations between glucoregulatory status and cognition in females. Rolandsson et al. (2008) reported that 2 hour post OGTT levels were correlated with poorer episodic and semantic memory, but only in women. Similarly, Lu et al. (2012) found that IGT and diabetes were related to poorer performance on the MMSE but only in females. As mentioned the MMSE may not be a reliable indicator of small differences so this latter finding should be viewed tentatively. One further study (Donohoe and Benton, 2000) only studied females in their sample. They found that those with the highest peak blood glucose values performed worse on the vigilance task, however, it should be noted that this was the only study to use a sample of young (average 22 years) females and all had NGT according to the WHO criterion.

Two studies report that both older males and older females with poorer GT have poorer cognition Vanhanen et al. (1997) found that those with the highest risk of diabetes (the higher half of a median split of glucose values 2 hours after the OGTT) had impaired performance on a variety of tests, compared with the low risk group. Again these participants did not necessarily have IGT according to the WHO criterion. Convit et al. (2003) reported that 2 hour glucose, fasting glucose and AUC all correlated inversely with memory performance. It should be noted that both Convit et al (2003) and Vanhanen et al (1997) did not consider the effect of gender in their analysis it is therefore not clear if the association exists in females,

males or both. Interestingly, in contrast to the longitudinal studies, cross sectional studies that found no significant relationship between IGT and cognition (Hiltunen et al. 2001; Kalmijn et al. 1995) had slightly older samples than those reporting a relationship (Vanhanen et al. 1997; Convit et al. 2003). It is possible that duration of IGT is important rather than age per se; more research is needed to elucidate the interactions between age, duration of IGT and cognition.

1.5.2.4 Glucose tolerance, cognition and glucose consumption

Nine studies have examined the effect of IGT on cognition either during the OGTT or following a subsequent glucose drink (Table 5). This approach has its advantages and disadvantages. On one hand it is possible that testing cognition following the consumption of glucose may ameliorate any negative effects of IGT and negate any differences in performance between those with or without IGT. On the other hand under these circumstances prior nutrition is controlled. The primary aim of most of these studies was to examine the effect of a glucose drink compared to a placebo on measures of cognition and thus the impact of GT was considered secondary. This has led to arbitrary definitions of GT that may or may not reflect meaningful physiological differences. Such heterogeneity also makes comparisons between studies difficult and hinders interpretation: therefore studies that have used similar GT definitions are grouped together.

Author (date)	N	Measures	Meals	GT definition	Findings
Riby et al. (2008)	33 M and 34 F mean 45 years.	Word recall (imm/del) Story recall (imm/del) National adult reading test Digit symbols Letter cancellation Trail-making Digit span forward and backward Category fluency	25g glucose 50g glucose Saccharin	Blood glucose 55 min post 50g glucose minus baseline. Median split.	Glucose enhanced episodic memory Good regulators completed trail making task faster after 50 g glucose. Poorer GT was related to poorer cognition.
Messier et al. (1997)	9 M, 6 F mean age 62 years.	Attention Working memory Immediate episodic memory Central executive functioning	50g glucose Saccharin	Blood glucose 75 min post 50g glucose minus baseline. Median split	Poorer GT was related to poorer memory: Ms with good glucooregulation performed better than Ms with poor regulation on memory tasks.
Craft et al. (1994)	27 younger mean 23.5 years. 32 older mean 67.5 years.	Paragraph recall Word list Pattern recall and recognition Procedural recall Working memory Verbal fluency Stroop	50g glucose Saccharin	Blood glucose 60 min post 50g glucose minus baseline. Median split.	Glucose improved memory in older adults with better GT but not poorer GT Glucose improved memory of younger adults with poorer GT but not better GT. Glucose decreased memory in younger adults with better GT.
Messier et al. (2003)	11M 46F mean 72 years.	Attention Working memory Immediate episodic memory Central executive functioning	75g OGTT - day 1 50g glucose or Saccharin – cog test day. 50 g glucose Saccharin	Blood glucose 60 min post 50g glucose minus baseline: median split. This measure was chosen because it correlated best with cognition. A median split determined good and poor glucooregulators based on subtracting baseline glucose from 60 min values after glucose	Older participants with poorer glucooregulation performed worse on tests of working memory, verbal memory, and executive functions. Participants with poor regulation had a poorer recall performance than participants with good regulation. This effect was absent when the participants were tested after drinking glucose.
Kaplan et al (2000).	10 M and 10 F mean age 72 years.	Immediate and delayed word and paragraph recall, Trails B, and Visual Attention Test. Performed during glucose measurement.	50 g glucose Saccharin	AUC after glucose was used to predict cognitive performance.	Poorer glucooregulation = poorer immediate recall and psychomotor performance after placebo.

Author (date)	N	Measures	Meals	GT definition	Findings
Messier et al. (2011)	57 M and 65 F mean age 20.5 years.	Arithmetic Digit Symbol coding Modified Brown Peterson task Symbol search Digit span (Forward and backward) Paragraph recall immediate and delayed) Figure copy Figure recall (immediate and delayed) Verbal free recall Letter-Number Sequencing Order reconstruction task	75g OGTT 50g glucose or Saccharin – cog test day.	PCA was used to determine indices: 1) evoked glucose levels 2) insulin sensitivity and beta cell function 3) evoked insulin indices 4) 2-h glucose and insulin indices 5) fasting glucose and c-peptide	Evoked glucose levels were significantly associated with the verbal memory performance: higher glucose predicted poorer memory.
Messier et al. (2010)	19 M 74F (ave 70 years).	Arithmetic, Digit Symbol Coding, Modified Brown–Peterson task, Digit Span, Spatial Span, Logical Memory immediate recall, Complex Figure Task copy, immediate and delayed recall, Verbal Free Recall, Logical Memory delayed recall, Letter–Number Sequencing, Order Recall.	75g OGTT 50g glucose or Saccharin – cognitive test day.	PCA was used to determine indices: 1) evoked glucose, 2) factors related to insulin 3) recovery	Evoked glucose measures were correlated with measures of working memory and executive function; the higher the glucose the poorer the performance (Arithmetic, Digit Span Backward, Letter–Number Sequencing, Spatial Span Forward, Spatial and Modified Brown–Peterson tasks).
Awad et al. (2002)	74, mean age 21 years.	Logical Memory (subtest of the Wechsler Memory Scale), verbal free recall (lists of words), Order Reconstruction Recall Test.	OGTT, 75 g	Several parameters were examined: insulin resistance and beta cell function. Recovery of glucose from 1 to 2 h was used to determine good and poor glucoregulators.	Glucose peak, evoked, and recovery indices were most associated with verbal declarative memory. Glucose recovery index from 1 to 2 h after consumption were most correlated with paragraph recall, verbal free recall, and the order reconstruction task. Poorer regulators had poorer memory at the second and delayed recall of story. For the reconstruction task worst regulators performed more poorly but only in the saccharin condition; glucose improved the worst regulators performance, although it had no effect on better regulators.

Table 5. The effect of glucose and glucose tolerance on cognition. OGTT-oral glucose tolerance test, NGT-normal glucose tolerance, IGT-impaired glucose tolerance, DM-diabetes mellitus, MMSE-mini mental state examination, IVGTT - intravenous glucose tolerance test.

Riby et al. (2008), Messier et al. (1997; 1999; 2003) and Craft et al. (1994) all defined glucose tolerance as glucose at 55-75 minutes minus fasting glucose, and used a median split to differentiate better and poor glucoregulators. Those with the largest increase in blood glucose were considered poorer regulators. All these studies reported that participants with poorer GT performed worse on at least one test, most often memory. However, how such a measure relates to other measures of GT should be considered. For example, in the study by Messier et al (1997) examination of the means suggested that those categorised as poorer GT actually had lower fasting levels, leading to their incorrect classification: that is those with lower fasting glucose may have artificially appeared to have the greatest increase when really after 60 minutes their glucose may have still been lower than others who had higher fasting glucose levels. All these studies employed sensitive tests of various aspects of memory (declarative, logical, immediate and delayed verbal memory) which may explain why effects were reported when they had not been for the IGT studies.

Interestingly, both Riby et al. (2008), in middle aged adults, and Messier et al (1997) in older adults, report that glucose improved performance only in those who had better glucose regulation. On the other hand Messier et al (1999), in a sample of undergraduates, and Messier et al (2003) in older adults, found that that glucose improved performance only in those classified as poor glucoregulators. It is possible that these discrepancies arise due to differences in the way of classifying participants as having better or poorer GT. Craft et al (1994) found that drinking glucose, rather than a placebo, improved memory in older men with better but not poorer GT. However, glucose also improved the memory of younger men with

poorer GT but decreased the performance of younger men with better GT. It is interesting is that older men with better GT, had GT comparable to younger men with poorer GT; thus there could be an optimal range of GT within which glucose has a facilitatory effect. Age may also have been an important moderator in Craft et al.(1994) effects.

Kaplan et al (2000), in a sample of older adults, used a regression analysis to determine whether glycaemic area under the curve (AUC), B cell function or fasting glucose levels predicted cognitive performance. Poorer glucose tolerance, as defined by a higher AUC, was associated with poorer memory, a higher AUC was related to a greater improvement in cognition from the placebo condition to the glucose condition, supporting the view that those with poorer GT may benefit more greatly from glucose. However, it should be noted that there were no differences between placebo and glucose condition and no interactions between treatment and glucoregulatory status when data were considered using ANOVA. Given this inconsistency these findings should be taken tentatively.

Messier et al. (2010), in older adults, and Messier et al. (2011), in young adults, also examined a number of GT indices, this time using principal component analysis (PCA) to determine meaningful clusters. These clusters were then entered into a regression equation to determine which factors best predicted cognition. In both studies higher 'evoked glucose levels'; that is, those that experienced a greater rise in blood glucose after a drink had poorer episodic memory (Messier et al. 2011) working memory and executive functioning (Messier

et al 2010). Neither study showed any facilitative effect of glucose relative to placebo in better or poorer gluco-regulators.

Awad et al. (2002), in a sample of undergraduates, found that nine indices of glucose tolerance, all representatives of higher plasma glucose levels, were related to cognition. Poorer gluco-regulators had poorer immediate and delayed paragraph recall and worse immediate word list recall after both glucose and saccharin consumption. Reconstruction recall was the only task in which glucose improved the worst regulators performance; there were no effects of glucose on better regulators.

1.5.2.5 Discussion and conclusions.

Overall, the studies that have used measures of GT within a normal range provide strong evidence of an association between GT and cognition. These studies also show that cognition may deteriorate at very early stages of poor GT, before individuals meet the WHO criterion for IGT. Interestingly, memory seems to be the cognitive domain most affected by decrements in GT (Messier et al. 1997; 1999; 2010; 2011; Awad et al. 2002; Kaplan et al. 2000; Riby et al. 2008). There is also some evidence that glucose may facilitate cognition, particularly in those with poorer gluco-regulation (Awad et al. 2002; Kaplan et al. 2000; Craft et al. 1994; Messier et al. 1999; 2003) although some studies have reported no effect (Messier et al. 2010; 2011) and others that there was a negative effect of drinking glucose (Messier et al. 1997). The research also shows that a wide range of GT indices may be related to cognitive performance; however, there is a need for systematic identification of which parameters are best associated with cognition. Such an

attempt may help to identify the mechanisms underlying the relationship between GT and cognition.

There is a discrepancy between those studies that have considered the relationship between poor GT and cognition using the WHO criterion to categorise participants as having IGT and those that have considered GT using alternative definitions, many of which fall within the normal GT range. As it is likely that the IGT (WHO criterion) studies have recruited participants with more severe levels of poor glucose tolerance than the NGT studies, it may be expected that there would be a stronger relationship between poor GT and cognition in these studies however, this was not observed. Some of this conflict may be caused by the types of cognitive tests used, with the IGT studies more often using tests which are unlikely to be sensitive enough to detect the small differences, for example, the MMSE. Another explanation may be the number of indices used to define GT in NGT studies (Table 5). Many studies used a few indices to determine which measure best correlated with cognition and this approach is more likely to produce significant correlations just by chance. This problem should be weighed against the need to identify which indices are important but indices should be chosen using insight into the mechanisms that may underlie each index. Despite the large number of indices used to date it is still not possible to identify which is the most strongly related to cognition and hence more research is needed.

1.6. A systematic review of the effect of energy drinks, cognition and mood.

1.6.1 Caffeine, energy drinks and postprandial glycaemia.

Chronically impaired glucose intolerance develops over time; however, acute changes in glucose tolerance also occur. For example, as previously discussed the consumption of low GL foods has been shown to attenuate blood glucose response during the postprandial period. In addition, positive metabolic effects can persist well beyond this period. One of these is known as the “second-meal effect,” whereby a previous meal affects the glycaemic response to a subsequent meal (Jenkins et al. 1981; Nilsson et al. 2007). In addition, these acute changes in GT may influence cognition (Lamport et al. 2011). In a similar fashion, consuming drinks that contain caffeine reduces glucose tolerance and insulin sensitivity, as assessed by postprandial plasma glucose concentrations (Dekker et al. 2007; Graham et al 2001; Robinson et al 2004; Petrie et al 2004). Interestingly, alkaloid caffeine such as that found in energy drinks (ED) may have a more pronounced effect on blood glucose control than caffeinated coffee. For example, Battram et al (2006) compared the effects of alkaloid caffeine, caffeinated coffee, and decaffeinated coffee when given before an oral glucose tolerance test and found that alkaloid caffeine elicited a 50% greater glucose area under the curve (AUC) than placebo, whereas the glucose AUC did not differ between the caffeinated coffee and placebo treatments.

Also noteworthy is that the effect of caffeine on glucose tolerance may be more pronounced when it is taken with a high rather than a low GL meal. For example, Moisey et al (2007) investigated the effect of caffeinated coffee (5mg/kg caffeine), compared to decaffeinated coffee, given with either a high (GL 65.75) or a low

glycaemic load (GL 30.75) meal. They found that caffeinated coffee was associated with a larger area under the curve (AUC) for both blood glucose and insulin, suggesting significantly impaired glucose management after the consumption of caffeine. Insulin sensitivity was also significantly reduced when caffeine was taken with a high GL meal but not when it was taken with a low GL meal. These findings raise particular concerns regarding the consumption of caffeinated ED that typically contain a significant quantity of high glycaemic carbohydrate. Given that poorer glucose tolerance may be related to declines in cognitive performance, as previously discussed, there is reason to suspect a detrimental effect of consuming caffeine and glucose simultaneously. The following section systematically reviews the evidence relating to the psychological effects of the co-consumption of high GL carbohydrates and caffeine in the form of EDs. It will address the cognitive effects of 'whole' EDs followed by a review of the studies that examined the interaction between individual components; glucose and caffeine.

1.6.2 Energy drinks cognition and mood.

During the last decade the consumption of EDs has grown exponentially (Reissig et al. 2009), with the claim that the combinations of ingredients can improve mood and cognitive performance (Alford et al. 2001). A typical energy drink contains a number of different ingredients, including but not limited to, glucose/sucrose and caffeine. There is a literature assessing the effects of these individual constituents (e.g. Smith 2002; Benton, 2002) and many randomized, placebo-controlled, crossover studies have documented the effectiveness of EDs as cognitive aids (Tables 6 -9). However, the predominant experimental design used to establish

the evidence-based support for EDs has involved a drink and placebo comparison, although this design does not make it possible to ascribe any positive effects to a single ingredient or to an interaction between ingredients. Recent reviews have concluded that caffeine is the ingredient that is responsible for the majority of EDs effects (McLellan and Lieberman 2012; van den Eynde et al. 2008). However, there is evidence that the context in which caffeine is consumed may modulate its effects. Caffeine may have different effects depending on whether it is consumed with a protein or a carbohydrate based meal (Smith et al. 1994a,b). As mentioned studies have demonstrated that an acute dose of caffeine can impair glucose tolerance in healthy individuals (Pizziol et al. 1998) and by modifying gastrointestinal hormone secretion altering the pattern of glucose uptake (Johnston et al. 2003). There is a need to systematically examine the interaction between ingredients.

1.6.2.1 Methods

For the present review, literature searches were conducted for RCT using Medline, Google 'scholar', Cochrane Library, Embase, psychINFO, and PubMed with keywords that included 'caffeine', 'glucose', 'energy drink', 'Red Bull' and 'cognitive performance' or 'mood'. Only English-language articles or abstracts were retrieved. The search primarily sought peer-reviewed publications, but some published dissertations were also evaluated. Studies were included or excluded based on the following criteria.

Inclusion/exclusion criteria

- Studies were included that investigated the effects of a combination of caffeine and carbohydrate on cognition and mood.
- Studies that considered the effect of only caffeine or only glucose were excluded.
- Studies examining the effect of a combination of carbohydrate and caffeine on neurophysiological measures were also excluded unless cognitive/subjective effects were simultaneously considered.
- Studies examining the effect of a combination of carbohydrate and caffeine on physical performance were excluded. Similarly, given the confounding influence of physical activity, studies examining effects on mood during/after exercise were not included.
- In addition, studies looking at an interaction between energy drinks and alcohol were excluded.
- Only studies examining affects in healthy adults were included.
- Survey type studies were excluded.

Figure 3 shows the selection process. A total of 17 studies met the inclusion criteria. Of these 11 examined the effect of 'whole' EDs (a combination of caffeine and carbohydrate) on subjective measures of mood and 12 considered cognition. A further 5 studies examined the effect of the interaction between caffeine and glucose on both cognitive performance and mood.

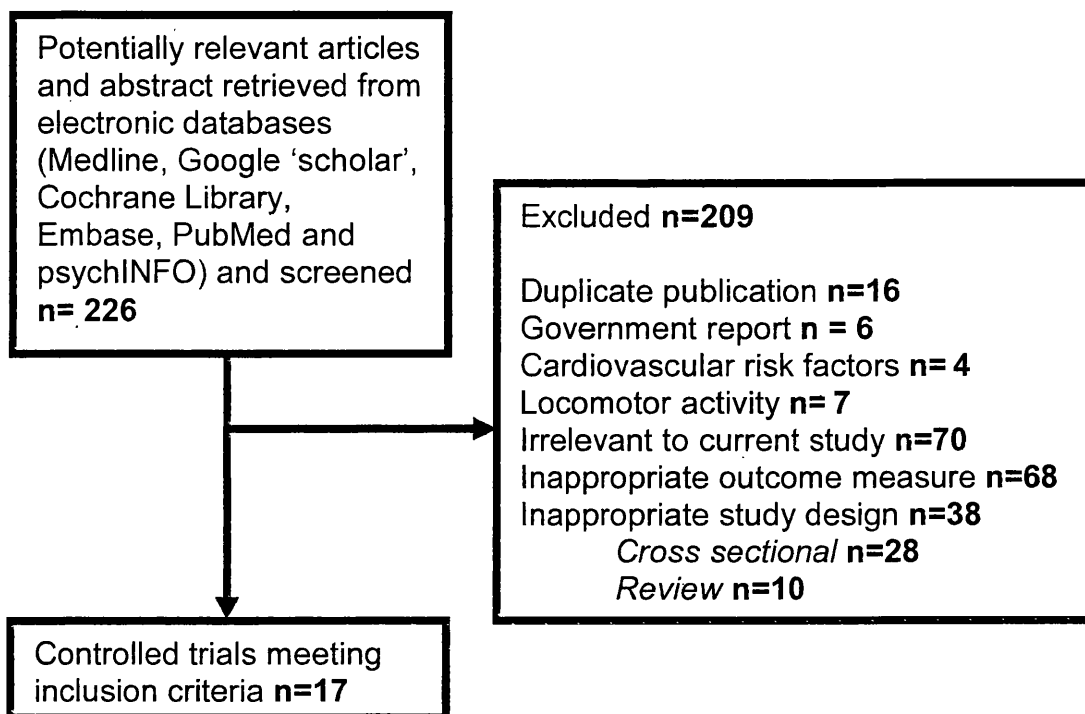


Figure 3. Flow diagram of the screening process. n - number.

1.6.2.2 The psychological effect of 'whole' EDs.

Table 6 lists studies that have determined the subjective effects of 'whole' EDs containing a combination of glucose, caffeine and other potentially psychoactive substances. Eight studies report that EDs when compared with placebo, within the first hour after drinking, either reduce mental fatigue (Kennedy and Scholey 2004a; 2004b; Howard and Marcziński 2010) or increase alertness (Warburton et al. 2001a; 2001b), energetic arousal (Smit and Rogers 2002; Smit et al. 2004a) and stimulation (Howard and Marcziński 2010). However, one reported no affect on either alertness or arousal, although the higher glucose/lower caffeine drink did reduce stress and anxiety (Sunram-Lea et al 2012). The two final studies examined only a single VAS; 'mood', and reported that participants were in a better

mood after they drank an ED rather than a carbonated mineral water. However, the comparison to water means that it is possible that the effects could reflect expectations associated with the drink.

Author (date)	Fast	Manipulation	N	Caffeine consumers	Measures	Test sessions	Results
Smit, et al. (2004) a	Food – no Caffeine - overnight.	1) 250ml Whole drink – 100mg taurine, 75mg caffeine, 37.5g CHO, 660kj, 2.4V/B carbonation. 2) 250ml 2.3V/B carbonated placebo, 25kj 3) Still water	13M 15F (ave age 24).	Ave 176.3mg/day	Tense arousal Hedonic tone Energetic arousal disorientation	Baseline, 5, 30, 60 and 90 min.	Energetic arousal (+ whole drink VS P and + carbonated P vs water immediately after drink) Hedonic tone (whole drink prevented decline seen in P) Overall mood (whole drink prevented decline seen in P)
Howard and Marczynski (2010)	Food – 2h Caffeine – 8h	1) 1.8ml/kg red bull (ave 45.6mg caffeine). 2) 3.6ml/kg red bull (ave 91.2mg caffeine). 3) 5.4ml/kg red bull (ave 136.7mg caffeine). 4) 3.6ml/kg placebo. 5) No drink.	34M 46F (ave age 20.1)	Range 3.2-7.1mg/kg per day.	Overall mental fatigue VAS Biphasic Alcohol effects Scale (stimulation and sedation)	Baseline 55min.	Stimulation (+ 1.8mg group VS P and no drink, + 5.4mg VS P). Mental fatigue (- 1.8mg, 3.6mg, 5.4mg VS P and no drink)
Seidi et al. (2000)	Caffeine – 24h	1) 80mg caffeine eine – 1g taurine – 6000mg glucuronolactone capsule. 2) Placebo capsule	10 (6F 4M) (age 20-28).	5 Non consumers. 5 Consumers.	Basler Befindlichkeit mood scale.	60min.	Consumers VS non consumers (ns) Mood (- placebo)
Kennedy and Scholey (2004) Study 1	Food – overnight Caffeine - overnight	1) 38 mg of caffeine and 68 g of glucose 2) 46 mg of caffeine and 68 g of glucose. 2) Vehicle placebo.	30 (age 18 – 25).	Not known	VAS 'mental fatigue'	Baseline 10, 20, 30, 40, 50 and 60min.	Mental fatigue (-) 46mg. 30, 40, 50 and 60min.
Kennedy and Scholey (2004) Study 2	Food – overnight Caffeine - overnight	1) 33 mg of caffeine and 60 g of glucose. 2) Vehicle placebo.	26 (18–24 years).	Not known	VAS 'mental fatigue'	Baseline 10, 20, 30, 40, 50 and 60min	Mental fatigue (- ED) at 30 and 40min.

Author (date)	Fast	Manipulation	N	Caffeine consumers	Measures	Test sessions	Results
Warburton et al. (2001) Study 2	Food – no Caffeine - no	1) 250 mg drink with 1 g taurine, 600 mg glucuronolactone, 80 mg caffeine, 5.25 mg glucose, 21.5 mg sucrose, 50 mg inositol, 20 mg niacin, 5 mg vitamin B6, 5 mg vitamin B5, 1.5 mg vitamin B2 and 0.005 mg vitamin B12. 2) Lucozade 6.4 mg glucose, ascorbic acid, 0.2 mg vitamin B2, 0.75 mg vitamin B5, 0.25 mg vitamin B6, 0.15 mg vitamin B12, 2.23 mg niacin and 22.5 mg caffeine.	22 (age 18 – 24).	> 2 cups coffee per day	Bond-Lader Visual Analogue Scales	Baseline 45 min	Alert (+ drink 1) Clearheaded (+ drink1) Attentive (+ drink1)
Warburton et al. (2001) Study 1	Food – no Caffeine - no	1) 250 mg drink with 1 g taurine, 600 mg glucuronolactone, 80 mg caffeine, 5.25 mg glucose, 21.5 mg sucrose, 50 mg inositol, 20 mg niacin, 5 mg vitamin B6, 5 mg vitamin B5, 1.5 mg vitamin B2 and 0.005 mg vitamin B12. 2) Sugar free placebo.	20 (age 18 – 24).	> 2 cups coffee per day	Bond-Lader Visual Analogue Scales	Baseline 45 min	Alert (+ drink) Quick-witted (+ drink) Clearheaded (+ drink) Attentive (+ drink)
Alford et al. (2000) study 1	Food – no Caffeine - no	1) Carbonated mineral water. 2) Red bull – 80mg caffeine, 21.5g sucrose, 5.25g glucose, 50mg inositol, 1000mg taurine, 600mg glucuronolactone, vit B6 and B12.	7M 7F (age 18-35).	Not known	A single mood VAS	Baseline -30 min	Mood (+ ED)

Author (date)	Fast	Manipulation	N	Caffeine consumers	Measures	Test sessions	Results
Alford et al, (2000) study 2	Food – no Caffeine - yes	1) Carbonated mineral water 2) Red bull – 80mg caffeine, 21.5g sucrose, 5.25g glucose, 50mg inositol, 1000mg taurine, 600mg glucuronolactone, vit B6 and B12.	5M 5F (aged 18- 30).	Not known	A single VAS.	Baseline -30 min.	Mood (+ ED)
Smit and Rogers (2002)	Not known	1) 75mg caffeine plus glucose and vitamins and ferrous gluconate - 150ml. 2) Water - 150ml 3) 75mg caffeine as guarana plus glucose – 250ml 4) Water – 250ml 5) Nothing	10M 13 F (age 19 – 56)	Not known	PCA analysis revealed an energetic arousal construct (revitalized, energetic, awake, alert, clearheaded, fatigued, tired).	Baseline 3, 38, 73 and 90min.	Energetic arousal (+ both caffeine drinks vs water @ 30,73 and 100 min). Bored (- both caffeine drink vs water). Relaxed (ns). Tense (-250ml caffeine drink vs nothing @ 73 and 100min). Overall mood (+ 250ml drink vs water). Thirsty (- both caffeine drinks vs water)

Table 6. Studies investigating the effects of Energy Drinks on Mood. VAS-visual analogue scale, P-placebo, ED-Energy Drink.

Although most often considered for their subjective benefits, energy drinks have also been acknowledged to benefit cognitive performance (Kim, 2003). Ten out of twelve studies presented in Table 7 reported improvements in tasks that involved an element of RT including: simple RT (Smit et al. 2004a; Seidl et al, 2000; Smit and Rogers, 2002), choice RT (Alford et al. 2000) sustained attention RT (Smit et al. 2004a; Sunram-Lea et al 2012; Warburton et al. 2001; Smit and Rogers, 2002), RT on a behavioral control task (Howard and Marcizinski, 2010) and RT on a reasoning task (Warburton et al. 2001). Two studies reported effects on the accuracy of attention (Kennedy and Scholey 2004a; 2004b), one reported improvements in delayed word recall (Sunram-Lea et al. 2012) and one reported benefits to the accuracy of working memory (Kennedy and Scholey, 2004a). However, it should be noted that whereas every study had some measure of RT, other domains were tested less frequently. For example, episodic memory was only considered 50% of the time and working memory was only measured in one study (Kennedy and Scholey 2004a).

Both mood and cognition effects were considered during the early postprandial period that ranged from 3 minutes post consumption (Smit and Rodgers 2002) to 90 minutes after a drink (Smit et al. 2004a). Longer term effects have not been considered.

Author (date)	Manipulation	Measures	Results
Sunram-Lea et al. (2012)	1) 50g glucose, 40mg caffeine. 2) 10.25g fructose/glucose (59% glu and 41% fru), 80mg caffeine. 3) Placebo	Immediate/delayed word recall. Grammatical reasoning. Letter cancellation. Letter/digit substitution task.	Delayed word recall (+50G glucose, 40mg caffeine & with P). Substitution task (+50G glucose, 40mg caffeine & 10.25g fructose/glucose (59% glu and 41% fru), 80mg caffeine & - P).
Howard and Marczinski (2010)	1) 1.8ml/kg red bull (ave 45.6mg caffeine). 2) 3.6ml/kg red bull (ave 91.2mg caffeine). 3) 5.4ml/kg red bull (ave 136.7mg caffeine). 4) 3.6ml/kg placebo. 5) No drink.	Cued go – no-go task (behavioural control)	Cued go – no go task RT (+ 1.8ml, 3.6ml & 5.4 ml drinks).
Warburton et al. (2001) Study 2	1) 250 mg drink with 1 g taurine, 600 mg glucuronolactone, 80 mg caffeine, 5.25 mg glucose, 21.5 mg sucrose, 50 mg inositol, 20 mg niacin, 5 mg vitamin B6, 5 mg vitamin B5, 1.5 mg vitamin B2 and 0.005 mg vitamin B12. 2) Lucozade 6.4 mg glucose, ascorbic acid, 0.2 mg vitamin B2, 0.75 mg vitamin B5, 0.25 mg vitamin B6, 0.15 mg vitamin B12, 2.23 mg niacin and 22.5 mg caffeine.	RVIP Verbal reasoning Verbal memory Spatial recognition	RVIP (RT - and number correct + with test drink) Verbal reasoning RT (+ test drink)
Warburton et al. (2001) Study 1	1) 250 mg drink with 1 g taurine, 600 mg glucuronolactone, 80 mg caffeine, 5.25 mg glucose, 21.5 mg sucrose, 50 mg inositol, 20 mg niacin, 5 mg vitamin B6, 5 mg vitamin B5, 1.5 mg vitamin B2 and 0.005 mg vitamin B12. 2) Sugar free placebo.	RVIP Verbal reasoning Verbal memory Spatial recognition	RVIP (RT - and number correct + with test drink) Verbal reasoning RT (+ test drink)

Author (date)	Manipulation	Measures	Results
Alford et al. (2000) study 1	1) Carbonated mineral water. 2) Red Bull – 80mg caffeine, 21.5g sucrose, 5.25g glucose, 50mg inositol, 1000mg taurine, 6000mg glucuronolactone, vit B6 and B12.	Choice RT.	Choice RT (+ Red Bull).
Alford et al. (2000) study 2	1) Carbonated mineral water. 2) Red Bull – 80mg caffeine, 21.5g sucrose, 5.25g glucose, 50mg inositol, 1000mg taurine, 6000mg glucuronolactone, vit B6 and B12.	Choice RT.	Choice RT (+ Red Bull).
Alford et al. (2000) study 3	1) Mineral water. 2) Red Bull – 80mg caffeine, 21.5g sucrose, 5.25g glucose, 50mg inositol, 1000mg taurine, 6000mg glucuronolactone, vit B6 and B12. 3) Artificially sweetened 'dummy' energy drink.	Word recall. Concentration task.	Word recall (+ Red Bull vs dummy drink). Concentration task (+ Red Bull vs dummy drink).
Smit and Rogers (2002)	1) 75mg caffeine plus glucose and vitamins and ferrous gluconate - 150ml. 2) Water - 150ml. 3) 75mg caffeine as guarana plus glucose - 250ml. 4) Water - 250ml. 5) Nothing	Simple RT. RVIP. Memory word list (immediate and delayed).	Simple RT (+ drink 1 and 3). RVIP (+ drink 1 vs nothing).
Seidl et al. (2000)	1) 80mg caffeine – 1g taurine – 6000mg glucuronolactone capsule. 2) P capsule.	RT task (part of EEG). D2 test (attention capacity under stress).	RT (- test drink). D2 test (+ test drink).

Author (date)	Manipulation	Measures	Results
Kennedy and Scholey (2004) Study 1	1) 38 mg of caffeine and 68 g of glucose. 2) 46 mg of caffeine and 68 g of glucose. 3) Vehicle P.	Serial subtractions. RVIP. Repeated 6 times consecutively.	Serial subtractions (+ acc with 46mg caffeine at 10 and 20min post drink). RVIP (+ acc with both caffeine drinks at 40, 50, 60 min post drink).
Kennedy and Scholey (2004) Study 2	1) 33 mg of caffeine and 60 g of glucose. 2) Vehicle P.	Serial subtractions. RVIP. Repeated 6 times consecutively	RVIP (+acc with 33mg drink at 40 and 60 min post drink).
Smit et al. (2004)a	1) 250ml Whole drink – 100mg taurine, 75mg caffeine, 37.5g CHO, and 2.4V/B carbonation. 2) 250ml 2.3V/B carbonated placebo. 3) Still water.	Simple RT. RVIP. Memory word list (immediate and delayed). Letter search task.	Simple RT (- whole drink vs carbonated P and carbonated P vs water). RVIP (info processing + whole drink vs water).

Table 7. Studies investigating the effect of Energy Drinks on cognitive performance. Where details concerning design, N, habitual consumption and deprivation are presented above these are not presented again, P-placebo, RT-reaction times, RVIP-rapid visual information task, glucose, CHO-carbohydrate. Acc-accuracy.

In conclusion, the evidence to date suggests that the consumption of a 'whole' EDs, compared to a placebo, can improve aspects of mood relating to energy/alertness and reaction times (RT). The nature of these effects suggests that caffeine may be the active ingredient as it is known to have an effect on alertness and RT (Smith, 2002). However, the design of these experiments means that it is not possible to elucidate the relative contribution of each component. It is possible that only one of the ingredients produced the effect, or on the other hand it could be that specific combinations of ingredients work together synergistically. Indeed it is possible that the components are antagonistic and that a more positive response may be obtained with the removal of one or more ingredient. Furthermore, the compositions of drinks in these studies vary to the extent that it is difficult to draw any clear comparisons between them.

The heterogeneity of the mood measures used in these studies means it cannot be determined whether they tap similar or different feelings. For example, do 'alertness' 'energetic arousal' and 'stimulation' represent similar or different dimensions? Similarly, more research is needed to determine the effect of EDs in different cognitive domains; studies have focused mainly on reaction times with limited attention to other domains such as executive function and memory. Perhaps most importantly, given the high GL of EDs and the potential for blood glucose levels to drop after 2-3 hours (Figure 1), the psychological effects of EDs need to be considered after a longer period of time.

1.6.2.3 The psychological effect of glucose, caffeine and their combination.

To determine the relative contribution to EDs, studies that systematically compare the individual and combined effects of each of the ingredients are needed. To date four studies have examined the relative contribution of glucose and caffeine (Scholey and Kennedy 2004; Smit et al. 2004b; 2004c; Adan and Serra-Grabulosa 2010; Table 8). Interestingly only two out of the four studies report a significant benefit of ED consumption on mood. This is in contrast with the aforementioned studies that examined the effects of 'whole' ED where eight out of ten studies reported mood benefits. Smit et al. (2004b) systematically varied the composition of experimental drinks; 75mg caffeine, 75mg caffeine plus 37.5g glucose, 37.5g glucose alone and a placebo and found that only the caffeine fraction improved energetic arousal. In a further study, Smit et al. (2004c) examined the contribution that carbohydrate makes to an energy drink by removing the carbohydrate component from one of the test drinks. This time there were no effects on reported energy levels but the addition of carbohydrate to caffeine reduced the feelings of tense arousal associated with taking the caffeine alone. Neither Adan and Serra-Grabulosa (2010), nor Scholey and Kennedy (2004), reported any effect of caffeine, glucose or their combination on measures of mood including the dimensions of alertness and energy levels.

Author (date)	Design	Fast	Manipulation	N	Caffeine consumers	Measures	Time	Results
Adan and Serra-Grabulosa (2010)	BS-DB	Food - 8h Caffeine-18h	<p>1) Water (150 ml).</p> <p>2) Water plus 75 mg of caffeine.</p> <p>3) Water plus 75 g of Glucose.</p> <p>4) Water plus 75 mg of caffeine and 75 g of glucose.</p>	72 (36 F; age 18-25)	<100 mg/day;	VAS: alert, sad, tense, energetic, satisfied, tired, calm, sleepy.	Baseline 30 and 60min	ns
Scholey and Kennedy (2004)	RM-DB	Food-overnight Caffeine -overnight	<p>1) Water with sweeteners.</p> <p>2) Water plus 75mg caffeine (from caffeine and guarana).</p> <p>3) Water plus 37.5g glucose.</p> <p>4) Water plus flavouring and herbs (ginseng and ginkgo biloba).</p> <p>5) Water plus 37.5g glucose, 75 mg caffeine; ginseng and ginkgo biloba at flavouring levels.</p>	14 F 6 M (age 18-32)	Not reported	Bond Lader VAS and POMS.	Baseline 30 min.	ns

Author (date)	Design	Fast	Manipulation	N	Caffeine consumers	Measures	Time	Results
Smit et al.(2004) ^b	BS	Food-no Caffeine - overnight	1) 250ml Whole drink – 75mg caffeine, 37.5g CHO, 660kj, 2.5V/B carbonation. 2) 250ml drink – 75mg caffeine, 25kj, and 2.4V/B carbonation. 3) 250ml drink – 37.5g CHO, 660kj, 2.5V/B carbonation 4) 250ml drink – 25kj and 2.3 V/B carbonation. 5) 250ml drink 75mg caffeine, 37.5g CHO, 660kj no carbonation.	73M 73F(23 +/-6)	Ave 164mg/day	Tense arousal Hedonic tone Energetic arousal Fullness	Baseline 5, 30, 60 and 90 min.	Energetic arousal (treatment X time interaction + caffeine vs no caffeine) (energetic arousal decreased over time when not given caffeine). Awake (+ carbonation vs no carbonation at 90 min).
Smit et al. (2004) ^c	BS	Food-overnight Caffeine - overnight	1) 250ml Whole drink – 75mg caffeine, 37.5g CHO, 660kj, 2.5V/B carbonation. 2) 250ml drink – 75mg caffeine, 25kj, 2.4V/B carbonation. 3) 250ml drink 75mg caffeine, 37.5g CHO, 660kj no carbonation	48M 49F (21+/-5)	Ave 246.4mg/day	Hedonic tone (euphoric) Hedonic tone (dysphoric) Energetic arousal Tense arousal Bored/ Restless/ Frustrated Confident/ Assertive	Baseline 5, 30, 60 and 90min.	Tense (- whole drink vs no CHO at 90min i.e. the addition of CHO to caffeine reduced the feelings of tense arousal associated with taking the caffeine alone) Assertive (+ carbonation at 3min) Cheerful (+ carbonation at 90min) Clearheaded (+ carbonation at 90min) Fatigued (- carbonation at 90min) Tense (- carbonation at 90min) Sluggish (- carbonation at 3min) Stomach full (+carbonation at 3min).

Table 8. Studies investigating the effect of 'whole' Energy Drinks and their individual components on mood. NC- non-consumer, C-consumer, VAS-visual analogue scale, P-placebo. CHO-carbohydrate, POMS-profile of mood states.

Again these four studies consistently report benefits on tasks involving RT (Table 9). Smit et al (2004b) reported that 75mg caffeine alone improved simple reaction times. However, Smit et al (2004c) found that RT on a letter search task was faster after the addition of 37.5g of carbohydrate to 75mg caffeine, than when considering caffeine alone. Adan and Serra-Grabulosa (2010) found that simple RT performance declined after drinking water but this was prevented by the consumption of either 75g glucose, 75mg caffeine or both. Similarly, sequential RT performance declined after water but this was only prevented by the drinking of both caffeine and glucose. Scholey and Kennedy (2004) noted that speed of attention only improved after the consumption of a 'whole' ED containing 37.5g glucose and 75 mg caffeine but not when either ingredient was drunk alone. Although all four studies considered effects on episodic memory only two found any effects. Scholey and Kennedy (2004) reported that memory improved after whole drink and when caffeine was taken alone. However, Adan and Serra-Grabulosa (2010) found that memory only improved when both glucose and caffeine were taken together but not when taken individually. Thus there is some evidence of a synergistic relationship between glucose and caffeine, which subsequently improves RT and possibly memory; although in some instances individual components act alone.

Author (date)	Manipulation	Task	Result
Smit et al. (2004)b	1) 250ml drink-75mg caffeine, 37.5g CHO. 2)250ml drink-75mg caffeine. 3) 250ml drink-37.5g CHO. 4) 250ml placebo.	Simple RT (SRT) Immediate/delayed word recall	SRT (treatment X time - whole drink vs CHO drink & vs caffeine drink) (main effect – caffeine vs no caffeine).
Smit et al. (2004)c	1) 250ml Whole drink – 75mg caffeine, 37.5g CHO. 2)250ml drink – 75mg caffeine. 3)250ml drink 75mg caffeine, 37.5g CHO.	Simple RT RVIP Immediate/delayed word recall Letter search task	Letter search task (hardest block RT + whole drink vs no CHO) i.e. drink better when CHO is added to caffeine than caffeine alone. RVIP – carbonation – performance @45min).
Adan and Serrab-Grabulosa (2010)	(1) Water. (2) Water plus 75 mg of caffeine. (3) Water plus 75 g of glucose. (4) Water plus 75 mg of caffeine, 75 of glucose.	RAVLT. Purdue Pegboard Benton line orientation task Wilcoxon card sorting task Computerised simple RT Sustained attention Digit span of WAIS	Simple RT (+ water vs caffeine, glu and caffeine plus glu) Sequential RT (+ water vs caffeine plus glu) Pegboard (+ glu vs water and caffeine) RAVLT (+ glu plus caffeine vs caffeine, glu and water).
Scholey and Kennedy (2004)	1) Water plus 75mg caffeine. 2) Water plus 37.5g glucose. 3) Water plus 37.5g glucose. 4) Water plus herbal flavouring. 5) Water plus 37.5g glucose, 75 mg caffeine.	Digit symbol substitution task Quality of memory Secondary memory Working memory Speed of memory Speed of attention Accuracy of attention Serial sevens	Quality of memory (+ whole drink & caffeine drink). Secondary memory (+ whole drink & caffeine drink). Speed of attention (- whole drink).

Table 9. Studies investigating the effect of 'whole' Energy Drinks and each of their components on cognitive performance.

Where details concerning design, N, habitual consumption and deprivation are presented above these are not shown again. P-placebo, RT-reaction times, RVIP, rapid visual information task, glu-glucose, CHO-carbohydrate. RAVLT - Rey Auditory Verbal Learning Memory test

1.6.2.4 Discussion and conclusions.

The moderating effect of fasting.

Evidence regarding the relative contribution to improved mood and cognition, of each ED fraction, is lacking. However one pattern emerged; when non-fasting participants consume a drink containing both caffeine and glucose caffeine emerges as the dominant ingredient (e.g. Smit, Cotton et al, 2004b; Scholey and Kennedy, 2004). In contrast, when fasting participants consume such a drink the addition of glucose appears to become important (e.g. Smit, Cotton, et al, 2004c; Adan, Serra-Grabulosa, et al, 2010). Although tentative, one hypothesis for these conflicting findings is that by restricting subject's food intake overnight, and denying them breakfast, their endogenous glucose supply may be reduced resulting in observable benefits when exogenous carbohydrates are consumed. It has been reported that the optimal glucose dose to improve cognition may differ depending on the level of depleted blood glucose resources (Owen et al, 2012). It is plausible that different doses of carbohydrate found in EDs may have different effects under fasting and non fasting situations; however, research is needed to test this hypothesis.

The moderating effect of caffeine withdrawal.

Similarly, the 'withdrawal reversal' hypothesis predicts that positive effects only occur in regular caffeine consumers when they are acutely withdrawn; a phenomenon now generally accepted to be at least partially responsible for the observed effects of caffeine (Rogers and Smith, 2011). Furthermore, with repeated use the effects of caffeine are reduced over time as tolerance builds (James and Rogers, 2005). Adan and Serra-Grabulosa (2010) was the only study to consider

low caffeine consumers: it therefore remains to be established if 'energy drinks' exhibit the same effects as in regular consumers. Indeed recent research has discovered associations between individual variations in the subjective anxiety produced by the ingestion of caffeine and polymorphisms in the A1 and A2a adenosine receptor genes (Alsene et al. 2003). This suggests that low consumers may be the select few in the population who choose not to consume caffeine as it exerts a negative effect. Whether habitual 'energy drink' consumption moderates their effects remains an important question, considering the targeted age group for these drinks. Individuals such as children and adolescents that do not consume caffeine daily may be at greater risk of intoxication from energy drinks containing high levels of caffeine than habitual caffeine consumers (Reissig et al. 2009).

The effect of dose.

It is also noteworthy that the doses of caffeine in the aforementioned studies ranged from 22.5mg to 80mg. This is a relatively narrow range and a low dose considering that some energy drinks can contain up to 505mg per bottle (Reissig et al. 2008). It will be worth considering the effect of higher caffeine doses, especially when taken in combination with other psychoactive substances.

The effect of time

Another important factor is the time scale over which the effect of energy drinks is studied. All the studies presented in Tables 5-9 examined only the immediate effects of drink consumption, taking measures from 10 minutes to 1 hour post consumption. The exception was Smit and Rogers (2002) who examined effects up to 100 minutes and Smit et al. (2004) who examined effects up to 90 minutes.

Both these studies found an effect of time; improvements in energetic arousal occurring from 30 min to 100min (Smit and Rogers, 2002) and feeling tense reduced from 73 min to 100min (Smit and Rogers, 2002) and at 90 min (Smit et al. 2004). However this still tells us relatively little regarding the longer term effects of consuming such drinks. The fact that no study on EDs has considered effects beyond 90 minutes is surprising given that caffeine is absorbed in about half an hour and has a half-life of five to six hours (Meyer et al., 1991), potentially offering relatively long-term benefits.

Evidence from studies conducted on the effect of glucose drinks on mood suggested that in the longer term (1h +) blood glucose has a tendency to fall rapidly, producing a reduction in mood and increased irritability (Benton 2002). If blood glucose levels fall too low, postprandial hypoglycaemia and its associated sympathetic nervous system and neuroglycopenia symptoms may be observed. Given that caffeine has a half life of up to 6 hours and has been shown to increase the perception of and sympathoadrenal responses to hypoglycaemia (Watson and Kerr, 1999; Watson et al. 2000), this combination could have negative consequences during the late postprandial period. The determination of any longer term effects is of obvious importance.

1.7 Literature review - summary

The brain relies on a continuous supply of glucose from the periphery in order to function. However, there are a number of factors, both dietary (high GL or caffeine) and physiological (IGT or PH), that can disrupt plasma glucose homeostasis and

hence hinder cognition. The general aim of this thesis is to investigate how changes in glycaemia affect cognition and mood.

1.8 Specific aims of this thesis

- To examine the influence of modulating the GL of meals on cognition and mood in children (chapter 2).
- To determine the impact of age, poor glucose tolerance and low blood glucose levels on cognitive decline, memory and mood in a sample of older adults (chapter 3).
- To investigate the influence of modulating the GL of breakfast on cognition and mood in older adults with or without poor glucose tolerance, low blood glucose and cognitive decline (chapter 4).
- To consider whether different aspects of diet, in particular caffeine which is known to cause declines in glucose tolerance, interact with the GL of a drink to effect cognition and mood of young adults (chapter 5).
- To present a theoretical statement that account for the findings presented in this thesis and to highlight possible areas for future research (chapter 6).

CHAPTER 2

Modulating the glycaemic properties of breakfast improves school children's cognition and mood.

2.1 Introduction

As reviewed in chapter 1 breakfasts that release glucose slowly (i.e. low glycaemic load, GL) are associated with better cognitive functioning in the late morning. Such effects have been reported in adults (Benton et al., 2003; Nabb & Benton, 2006a, b), adolescents (Cooper et al, 2012) and children (Benton et al., 2007; Ingwersen et al. 2007) but not in all instances (Dye et al. 2010). Although studies have focused attention on the glycaemic properties of the meals, to date this has typically been achieved by varying the type of food consumed or the nature and amount of various macro-nutrients, such that it is unclear whether it is the glycaemic response that is important rather than some aspect of the macro-nutrient composition of the meal. The major objective of the present study was to consider the influence of the glycaemic load of meals that are otherwise identical in terms of their macro-nutrient composition.

To vary the GL, while keeping the macro-nutrient composition constant, two meals were created that were identical in all respects other than one was sweetened with Isomaltulose and one with glucose. Isomaltulose is a disaccharide made from sucrose by the enzymatic rearrangement of the alpha 1,2 linkage, between glucose and fructose, to an alpha 1,6 linkage. It is fully digested and absorbed in the small intestine and provides the same energy as other sugars. In healthy volunteers, isomaltulose results in lower postprandial blood glucose and insulin responses than sucrose or glucose, while producing a prolonged blood glucose

delivery over the proceeding 3 hours (Holub et al, 2010). Thus the GI of isomaltulose is 32 compared with 65 for sucrose and 100 for glucose. The use of isomaltulose and glucose allowed the GL of the meal provided in this study to be varied whilst keeping the macro-nutrient contents identical.

Also considered was whether children's social background influenced the reaction to GL. At least in developing countries there are reports that those from a poorer background benefit more from a school breakfast programme (Pollitt and Mathews 1998; Powell et al., 1998; for a review see Hoyland et al. 2009). To date such matters have not been examined in industrialized countries, particularly in relation to GL. However, even in developed countries it is known that diet varies. For example, a survey of British children found that many aspects of diet varied with social background (Gregory & Lowe, 2000). Given the 'second meal effect' the glycaemic load of food eaten previously at home could influence the glycaemic response to the experimental breakfasts (Wolever et al. 1988; Lamport et al. 2011). The present study therefore examined whether children from a poorer social background preferentially benefitted from a lower, rather than a higher, GL breakfast.

2.2 Methods

2.2.1 Procedure

Children attending a school breakfast club were recruited and on two different days, at least one week apart, ate one of two meals designed to differ in their glycaemic load while being similar in macro-nutrient content. The procedure was double-blind and the order in which the meals were consumed was randomly generated. When the child had finished eating, the amount of the food items

remaining were recorded by weighing the plate before and after consumption, allowing estimates to be made of the nutritional composition of the breakfasts consumed. Breakfast was eaten between 0815 and 0845 and psychological testing took place twice between 0900 and 0945 and 1115 and 1200. On each occasion tests were administered on an individual basis. Initially immediate memory was assessed, followed by the Speed of Information Processing, reaction times, the ability to sustain attention, delayed memory, mood and appetite ratings. Due to time constraints the only task completed prior to breakfast was the Speed of Information Processing test.

2.2.2 Participants

Seventy-five children, aged five to eleven years, average age eight years eight months, were recruited from four schools. There were 28 boys and 47 girls in the sample. Their parents reported that participants were in good health and had never experienced an adverse reaction to food. Weight was not measured but no child was obviously over weight. Parents gave their consent to the child taking part (see appendix 1) and the study was approved by Swansea University ethics committee (reference number: 0402/2007/1). Irrespective of the study, all children attended a school breakfast club on a daily basis where they consumed a meal similar to the experimental meals: thus all habitually ate breakfast. The Welsh Index of Multiple Deprivation describes local areas in terms of a range of indices. Parents provided a home postcode from which an index was retrieved relating to their degree of deprivation. The sample came from amongst the most socially deprived areas of Wales.

2.2.3 Meals

The details of the meals are given in Table 11. They were designed to offer a similar intake of energy and macro-nutrients while differing in the glycaemic load (GL; amount of carbohydrate X glycaemic index of food items (Foster-Powell & Brand-Miller, 2002)). Children were asked to eat as much as possible of the food provided but were not forced to eat anything they did not like or more than they wanted to consume. When the child had finished eating, the weight of any food remaining was recorded. In practice, all children ate the majority of their breakfast such that the small amounts left were of limited practical significance. There were no systematic differences between treatments in the amount of food consumed (Table 10).

	Cereal	Yogurt	Orange drink	p
Higher GL	4.3 (4.9)	3.4 (18.5)	8.2 (8.9)	ns
Lower GL	4.1 (4.0)	1.2 (19.3)	6.1 (7.5)	ns

Table 10. The amount of food left over in the higher and lower GL conditions. Data are amount of food left over in grams.

	Higher GL						Lower GL					
	Kcal	CHO	Pro	Fat	GI	GL	Kcal	CHO	Pro	Fat	GI	GL
Cornflakes 20g	71	17.6	1.6	0.15	81	14.26	71	17.6	1.6	0.15	81	14.26
Milk semi-skimmed 100 ml	46	5.0	3.3	1.5	21	1.05	46	5.0	3.3	1.5	21	1.05
Glucose 5g	20	5.0	0	0	100	5.00	-	-	-	-	-	-
Palatinose 5g	-	-	-	-	-	-	20	5.0	0	0	32	1.60
Low calorie yoghurt 100g	41	6.0	4.3	0.25	19	1.14	41	6.0	4.3	0.25	19	1.14
Glucose sweetened fruit 20g	95	23.7	0	0	94.5	22.40	-	-	-	-	-	-
Palatinose in sweetened fruit 20g	-	-	-	-	-	-	95	23.7	0	0	35.7	8.61
Orange drink 200 ml (glucose 16 g)	64	16	0	0	100	16.00	-	-	-	-	-	-
Orange drink 200 ml (isomaltulose 16 g)	-	-	-	-	-	-	64	16	0	0	32	5.12
TOTAL	337	73.3	9.2	1.9	-	59.85	337	73.3	9.2	1.9	-	31.78

Table 11. The macro-nutrient content of the experimental meals.

2.2.4 Test battery

2.2.4.1 Memory

The approach was based on the Recall of Objects test of the British Ability Scale (Elliott, 1996). For 40 seconds the child viewed a card with pictures of 20 objects (Appendix 2), after which they had 60 seconds to recall as many as possible. On a second and third occasion the child viewed the card for a further 20 seconds and had 40 seconds to recall the items. The numbers of items recalled on the three occasions were added and are reported as the immediate memory score. After the third trial, spatial memory was assessed by placing a blank grid in front of the child and asking them to put a series of pictures in the place on the grid that corresponded to the original card. Delayed memory was assessed after performing the other tests, about 20 minutes after the initial memory test. The child was again asked to verbally recall as many pictures as possible. When the blank grid was again presented the child tried for a second time to recall the position of the 20 objects. In total four sets of objectives were presented, a different version on each of the four occasions the test was completed.

2.2.4.2 Speed of Information Processing

The test is taken from the British Ability Scale and measures how quickly simple mental operations can be performed. A row of circles was presented, each containing a number of small boxes. The task involved marking the circle containing the most boxes. On each of six pages there were five lines of circles. The time taken to complete each of the six pages was recorded. Six comparable forms of the test were used.

2.2.4.3 Reaction times

An auditory warning sounded and at the same time a light illuminated and a millisecond timer started. For ten trials the child as quickly as possible pressed a button that extinguished the light and stopped the timer. The reaction in milliseconds was recorded.

2.2.4.4 Ability to sustain attention

The paradigm of Shakow (1962) was used. An auditory warning sounded and after a delay of either three or twelve seconds, a light illuminated and a millisecond timer started. When the child saw the light a button was pressed that extinguished the light and stopped the timer. The test consisted of four blocks of six trials. The first and third block of trials had a delay of three seconds and the second and fourth block a delay of twelve seconds. The delay ensures that it is the ability to sustain attention that is measured rather than simple reaction times.

2.2.4.5 Appetite

Children were asked how many sandwiches do you think you would be able to eat 'at this moment' using an eight point scale that ranged from 1/4 of a sandwich to 2 whole sandwiches. In all cases a higher score indicated that they were hungrier.

2.2.4.6 Mood

Children were asked how they feel 'at this moment' using an eight point scale of smiley faces, that ranged from very unhappy to very happy. In all cases a higher score reflects a worse mood.

2.3 Statistical analysis

The data were analyzed using appropriate analysis of variance designs using SPSS version 19. For example: Type of Meal (glucose / isomaltulose) X One / three hours after eating X Immediate / delayed recall X Order of testing (was glucose / isomaltulose consumed on the first or second day of testing). The last variable was a between subjects factor and the others within subjects factors. Where significant interactions resulted they were further analyzed using post hoc tests. Due to time constraints speed of information processing was the only measure taken at baseline, and in the analysis of this one measure; baseline performance (for day 1) was entered as a covariate. Where in the results section higher order interactions are not mentioned it should be assumed that they were non-significant.

Although requested not to snack during the morning children were asked if they had in fact eaten after breakfast. The basic analysis of variance was carried out excluding those children who said that they had snacked, to maintain the integrity of the study that was designed to contrast two meals with identical macro-nutrient compositions. However, in addition an 'Intention to treat' analysis was performed that was identical except it used all participants, to exclude the possibility that a systematic bias had been introduced by excluding those who subsequently snacked. In practice, the response was similar in both the main and the 'Intention to Treat' analysis so the data presented are for those who did not eat after breakfast.

2.4 Results

To establish whether there was any effect of age children were split into three groups; five to seven, eight to nine and ten to eleven years of age. Preliminary analysis found that age influenced memory ($F(2,53) = 12.18, p < 0.001$), reaction times ($F(2,57) = 21.89, p < 0.001$), speed of processing ($F(2,54) = 27.69, p < 0.001$) and the number of lapses of attention ($F(2,56) = 14.84, p < 0.001$); those seven years or under were poorer than those aged eight to nine and ten to eleven. Similarly gender influenced memory ($F(1,61) = 6.84, p < 0.01$), speed of information processing ($F(1,56) = 11.08, p < 0.002$) and mood ($F(1,61) = 4.73, p < 0.03$); girls performed better and had a more positive mood than boys. The group was divided into two using a median split of the deprivation scores. Those from a more deprived background had a slower speed of information processing ($F(1,69) = 12.117, p < 0.001$) but did not differ in other respects. The effects of age, gender and deprivation did not, however, influence the effect of the meal on any measure of cognition and they are therefore not further reported.

2.4.1 Memory

When the immediate memory scores were analyzed the interaction Type of Meal X One / three hours after eating X Order of testing was non-significant ($F(1,63) = 0.44, n.s.$). However, the interaction Type of Meal X One / three hours after eating reached statistical significance ($F(1,63) = 10.89, p < 0.002$), and Figure 4 illustrates the interaction. When memory was assessed one hour after testing it was similar in the two groups. However, three hours after breakfast those who consumed the glucose based meal had poorer memories than those who ate the isomaltulose meal ($P < 0.01$).

After eating the glucose based meal memory was significantly poorer three compared to one hour following consumption ($p < 0.001$). That is performance declined across the morning. However, after eating the isomaltulose based meal, children's memory was similar regardless of whether was tested one hour or three hours after breakfast; in fact there was a trend for it to be slightly better later in the morning (Figure 4).

Ten participants had either been observed or had admitted snacking during the morning, for example eating an apple or a chocolate bar. Although they were removed from the sample for the above analysis an 'Intention to treat' analysis was carried out to exclude the possibility that bias had been introduced into the study. The above effects were identical when this second analysis was carried out. With the immediate memory scores the interaction Type of sugar X One / three hours after eating reached statistical significance ($F(1,73) = 12.47, p < 0.001$), although the interaction Type of Meal X One / three hours after eating X Order of testing was non-significant ($F(1,73) = 0.73, n.s.$).

The interaction Type of Meal X Order of testing was also significant ($F(1,63) = 16.11, p < 0.001$). Figure 5 illustrates the phenomenon. Memory was significantly better when the isomaltulose rather than the glucose based meal was consumed on the second rather than first day of testing ($p < 0.04$). The nature of the meals did not influence memory on day 1. The 'Intention to treat' analysis produced a similar significant Type of Meal X Order of testing interaction ($F(1,73) = 15.57, p < 0.001$).

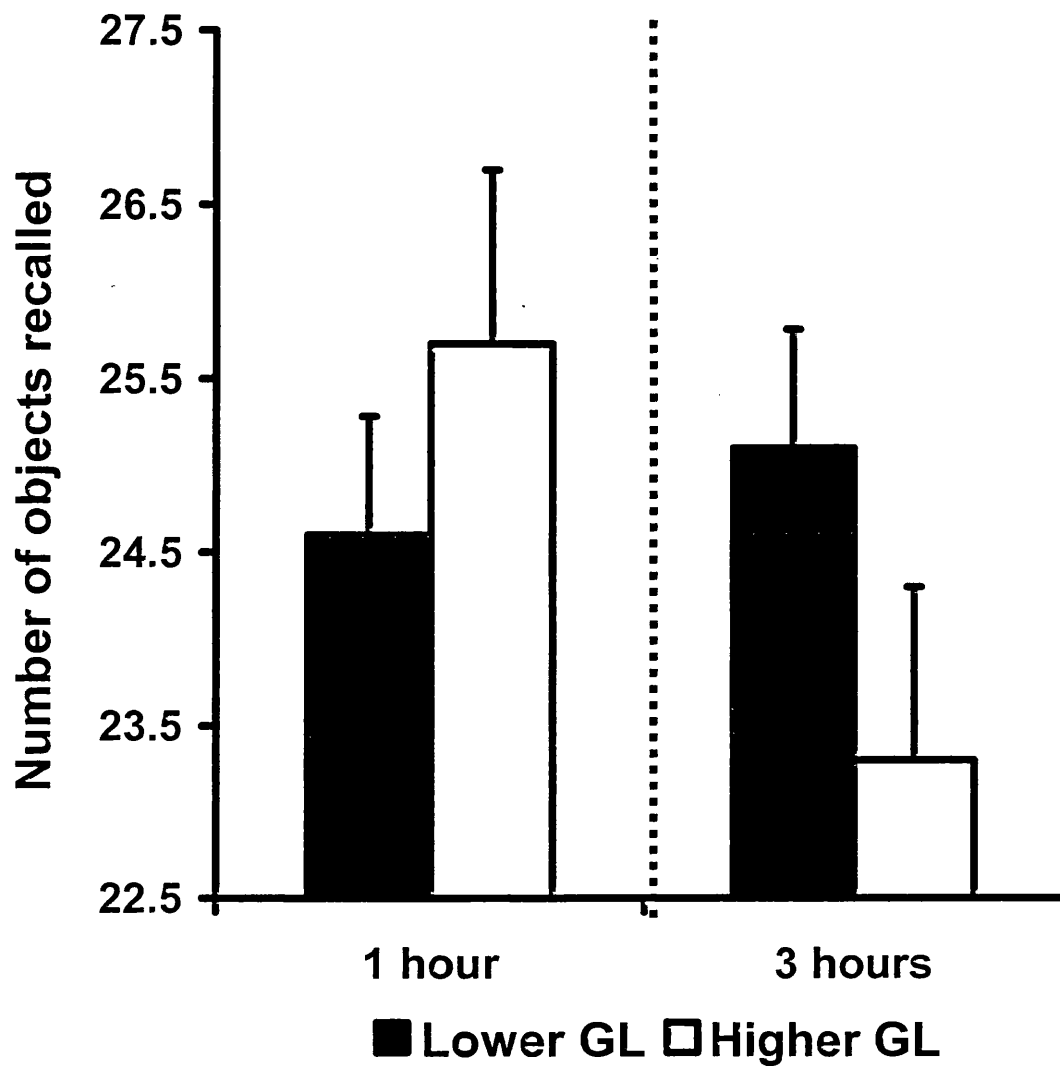


Figure 4 - Immediate memory one and three hours after breakfast. The data are mean number of words recalled +/- standard error. The two breakfasts did not differ after one hour but after three hours those eating the lower GL had significantly better memories ($p < 0.01$). The memory significantly declined in those eating the higher ($p < 0.001$) but not lower GL meal.

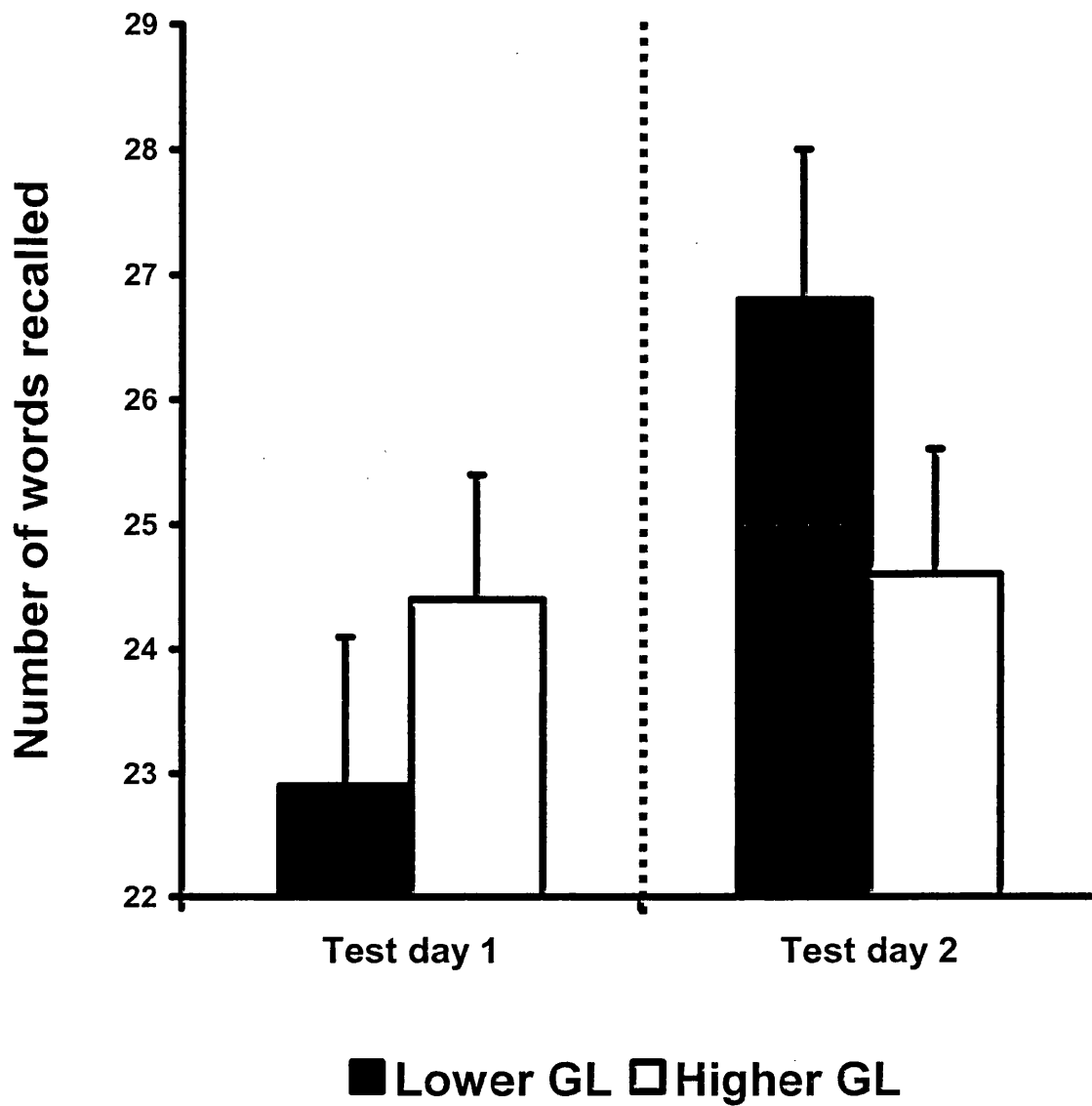


Figure 5 - Immediate memory depending on whether the test was taken on the first or second occasion. The data are mean number of words recalled +/- standard error. After eating the lower GL meal memory was significantly better when tested for a second rather than first time ($p < 0.04$).

2.4.2 Memory retention

When the possible influence of meal on the retention of memory was considered the analysis Type of Meal X Immediate (number recalled at the third test of immediate memory) / delayed recall ($F(1,63) = 0.027$, n.s.) however the interaction Type of Meal X Immediate / delayed recall X One / three hours after eating was significant ($F(1,63) = 7.402$, $p < 0.008$). Post hoc tests revealed that children were able to retain more words later in the morning after they had eaten the isomaltulose based meal compared to when they had eaten the glucose based meal ($p < 0.05$). Furthermore children's ability to retain words decline significantly over the morning when they ate glucose (-13.8 to -15.3; $p < 0.02$) but stayed the same across the morning when they ate isomaltulose (-14.6 to -14.0).

The interaction Type of Meals X Immediate / delayed recall X Order of meals also reached significance ($F(1,63) = 12.771$, $p < 0.001$). Post hoc tests showed that children were able to retain more words if they ate the isomaltulose meal on day 2 compared to when they ate the glucose based meal on day 2 ($p < 0.001$). Although there was a significant interaction involving order, post hoc comparisons also showed that children were also able to retain more words if they ate the isomaltulose meal on day 1 compared to if they ate the glucose based meal on day 1 ($p < 0.05$). The glucose based meal caused more of a decline in children's ability to retain words if they ate it on the second day compared to if they ate it on the first day ($p < 0.02$). The ability of isomaltulose to prevent this decline did not differ between days. The intention to treat analysis produced similar interactions; Type of Meals X Immediate / delayed recall X Order of meals ($F(1,73) = 15.068$,

$p < 0.001$) and Type of Meal X Immediate / delayed recall X One / three hours after eating was significant ($F(1,73) = 7.752, p < 0.007$).

2.4.3 Spatial memory

When spatial memory was considered the interaction Type of Meal X Order of testing reached statistical analysis ($F(1,63) = 4.187, p < 0.04$). Post hoc tests showed that when children ate the isomaltulose based meal on day 2 their spatial memory was better than if they ate the glucose based meal on day 2 ($30.5(1.2)$ compared to $28.0(1.0)$; $p < 0.03$). Children's spatial memory did not depend on the type of breakfast that ate on day 1 (Iso $29.0(1.3)$ Glu $30.0(1.4)$; ns). Intention to treat analysis revealed a similar Type of Meal X Order of testing interaction ($F(1,73) = 5.397, p < 0.02$).

2.4.4 Speed of information processing (SIP)

Prior to consuming breakfast the groups who subsequently ate one of the two meals did not differ ($F(1,61) = 0.46, n.s.$) illustrating that prior to eating the groups were well matched. However, irrespective of the order in which meals were consumed, children's performance improved from baseline on day 1 to baseline on day 2 ($t(64) = 4.268, p < 0.001$). Performance at baseline on day 2 did not depend on which meal had been consumed on day 1 ($F(1,69) = 0.009, ns$). Day 1 baseline SIP was entered into the analysis and significantly predicted performance after breakfast ($F(1,63) = 7.940, p < 0.006$) but there were no Baseline X Treatment interactions. After breakfast, although the Type of Meal X One / three hours after breakfast interaction was non-significant ($F(1,61) = 0.51, n.s.$), the Type of Meal X Order of testing interaction reached significance ($F(1,61) = 52.97, p < 0.001$). The

nature of the interaction is illustrated in Figure 6. There was a general improvement in performance from day one to day two ($p < 0.0001$). There were, however, no significant differences in speed in those eating the two meals when tested on the first day of testing although the consumption of the isomaltulose, rather than glucose based meal, was associated with faster performance when tested after consuming a meal for a second time ($p < 0.01$).

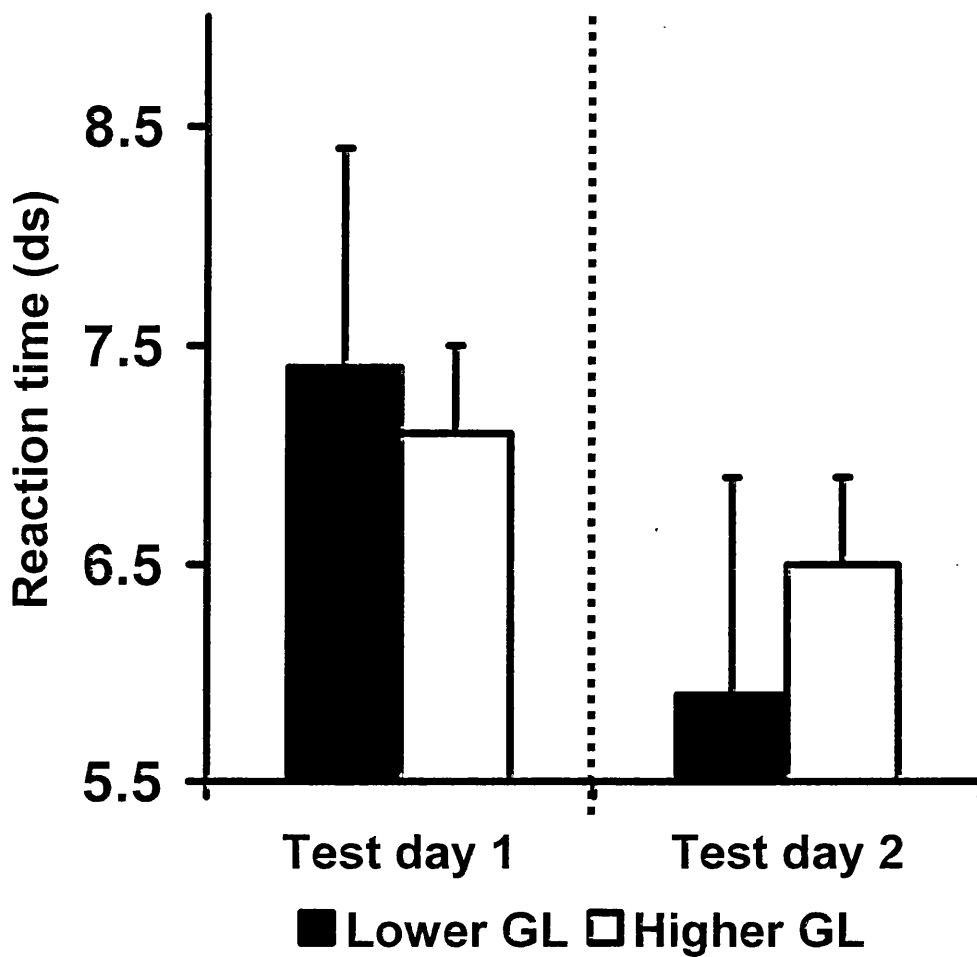


Figure 6 – Speed of information processing depending on whether the tests were taken on a first or second occasion. The data are the times taken in seconds +/- standard error. On the first day of testing those eating the two meals did not differ but when tested on a second occasion those who had eaten the lower GL meal were significantly quicker ($p < 0.01$)

2.4.5 Reaction times

When simple reaction times were considered there were no significant differences between meals or changes over time ($F(1,62) = 1.12$, n.s.). The Type of Meal X Order of testing interaction ($F(1,62) = 3.48$, n.s) was also non-significant.

2.4.6 Attention (3 second delay)

Initial examination of the ability to sustain attention found that on occasions there were very long delays before responding. Thus the mean response times did not reflect the data as they were distorted by a few extreme values. It was decided to look at the instance of long response times; that is the incidence of lapses in attention. The median response time was about 600 ms when there was a three second delay before the light illuminated. Therefore the number of trials were analyzed when individual's responses were in the bottom quintile of the distribution, that is responses were longer than 800 ms.

Overall the number of lapses in attention was not influenced by the type of meal eaten (Type of Meal X One/three hours after breakfast ($F(1,62) = 0.02$, n.s.)), however, the Type of Meal X Time after breakfast X Order of testing ($F(1,62) = 6.66$, $p < 0.01$) interaction reached significance (Figure 7). Although the number of lapses of attention were greater three rather than one hour after breakfast ($p < 0.03$), the meal consumed did not influence the number of lapses on the first day of testing. However, on the second test day those who had consumed the isomaltulose rather than the glucose based meal tended to have fewer lapses of attention although post hoc tests did not achieve statistical significance.

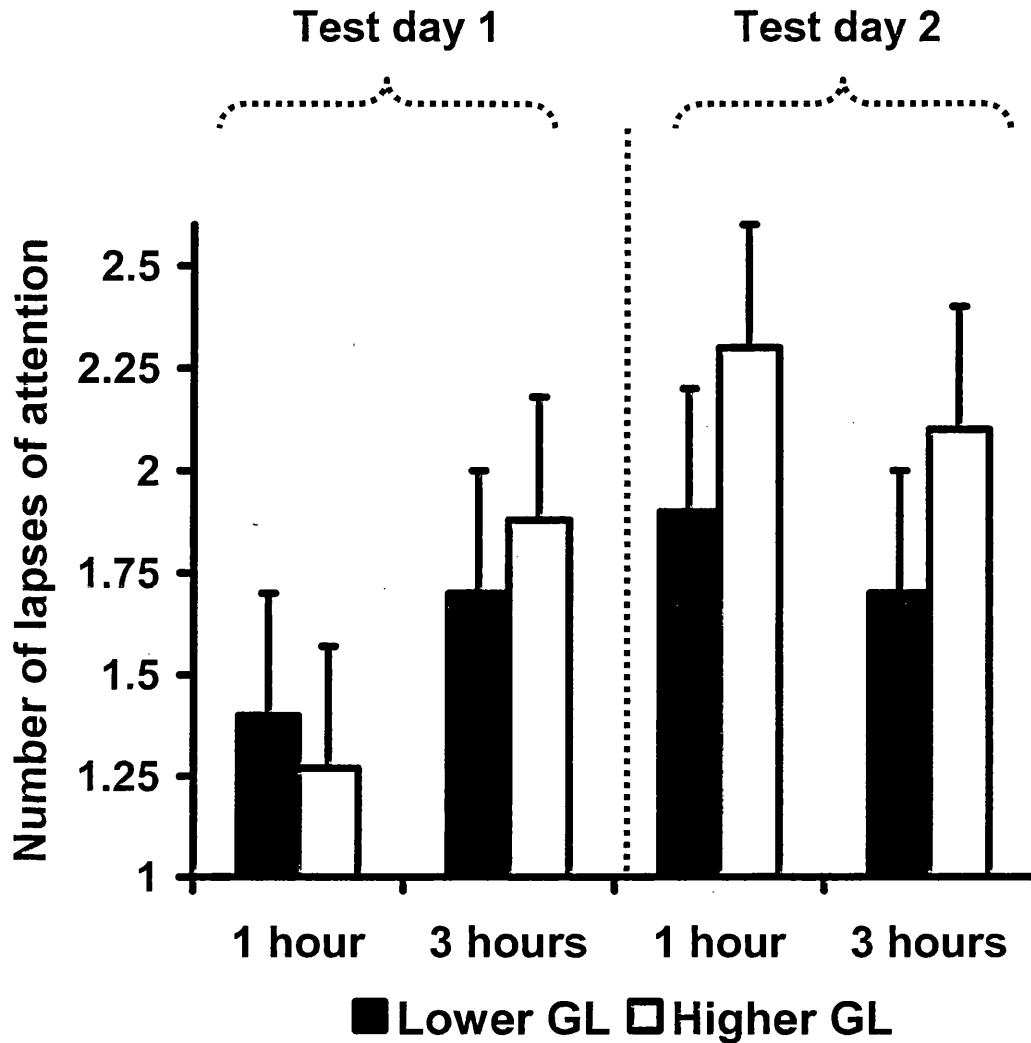


Figure 7 –Lapses of attention with a three second delay depending on whether the tests were taken on a first or second occasion. The data are the number of lapses of attention +/- standard error. On the second test day children who had consumed the isomaltulose rather than the glucose based meal tended to have fewer lapses of attention.

2.4.7 Attention (12 second delay)

The median response time for the twelve second delay was about 750ms. Those responses in the bottom quintile, that is more than 1000 milli-seconds, were distinguished. When the lapses in attention associated with the longer delay were considered, although the Type of Meal X One / three hours after breakfast interaction ($F(1,62) = 0.19$, n.s.) was non-significant, the interaction Type of Meal X Time after breakfast X Order of testing ($F(1,62) = 4.46$, $p < 0.04$) (Figure 8) reached significance. On day one of testing the number of lapses of attention was greater three rather than one hour after breakfast ($p < 0.04$), although the type of breakfast consumed was not influential. However, when tested on the second occasion there was again a trend for those consuming the isomaltulose based meal to have fewer lapses of attention although no post hoc reached statistical significance.

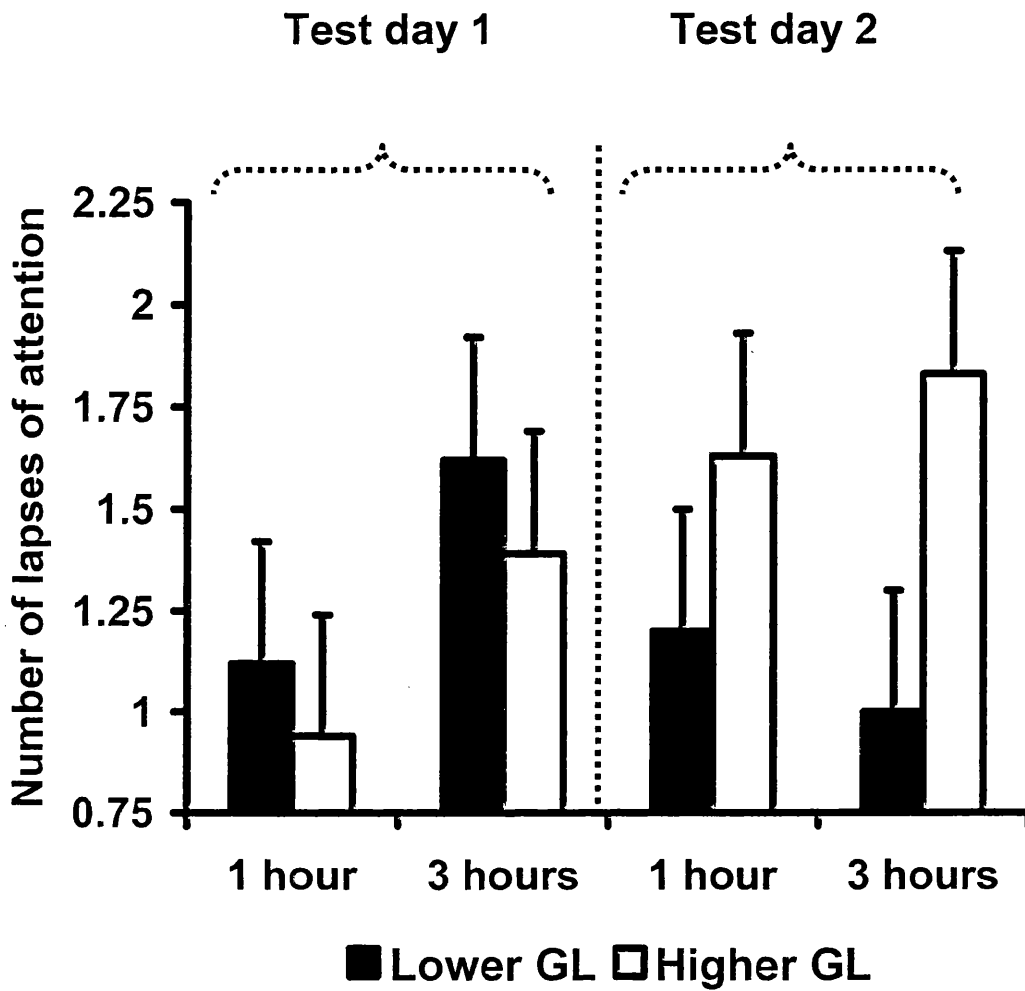


Figure 8 –Lapses of attention with a twelve second delay depending on whether the tests were taken on a first or second occasion. The data are the number of lapses of attention +/- standard error. Children consuming the isomaltulose based meal on day 2 tended to have fewer lapses of attention.

The possibility that the number of lapses of attention increase during the second half of the test (i.e. trial 7-12 rather than trial 1-6) and that the nature of the meal may influence this effect was considered. The interaction Type of Meal X Time after breakfast X Trials 1-6 / 7-12 also reached significance ($F(1,61) = 7.61, p < 0.01$). There was a trend for those eating the glucose based meal rather than the isomaltulose meal to display more lapses of attention during the second half of the test, although the post hoc tests failed to achieve statistical significance.

2.4.8 Appetite

The interaction Type of meal X Time after breakfast X Social background reached significance ($F(1,51) = 5.839, p < 0.02$). Inspection of the means showed that those from a poorer social background were hungrier later in the morning when they consumed the glucose based meal. However, post hoc tests failed to find any significant differences ($p < 0.2$ n.s). No other effects were significant when considering those that did not snack, however, when an intention to treat analysis was conducted it revealed a similar of Type of meal X Time after breakfast X Social background interaction ($F(1,65) = 4.105, p < 0.05$) and the interaction Type of meal X Time after breakfast X Order reached significance ($F(1,65) = 5.375, p < 0.03$). Initial inspection of the means showed that those who consumed the glucose based meal on day 2 were hungrier later in the morning than those who ate the isomaltulose based meal on day 2. However, follow up comparisons failed to find any significant differences ($p = 0.4$ n.s). There was also a main effect of Time ($F(1,68) = 49.607, p < 0.0001$). Children were hungrier later in the morning. Although older children were hungrier than younger children ($F(1,65) = 12.532,$

$p < 0.001$), and boys were hungrier than girls ($F(1,65) = 11.649, p < 0.001$), these factors did not influence the reaction to the sugar consumed.

2.4.9 Mood

The interaction Type of meal X Time reached significance ($F(1,56) = 4.807, p < 0.03$). Follow up tests found that when children ate the glucose based meal they had a worse mood after 3 hours ($p < 0.03$) compared to when they ate the isomaltulose based meal (Figure 9). The nature of the meal made no difference 1 hour after eating. There was also a main effect of gender ($F(1,61) = 4.729, p < 0.03$); girls reported better moods than boys. Intention to treat analysis showed a similar Type of meal X Time interaction ($F(1,61) = 4.151, p < 0.05$).

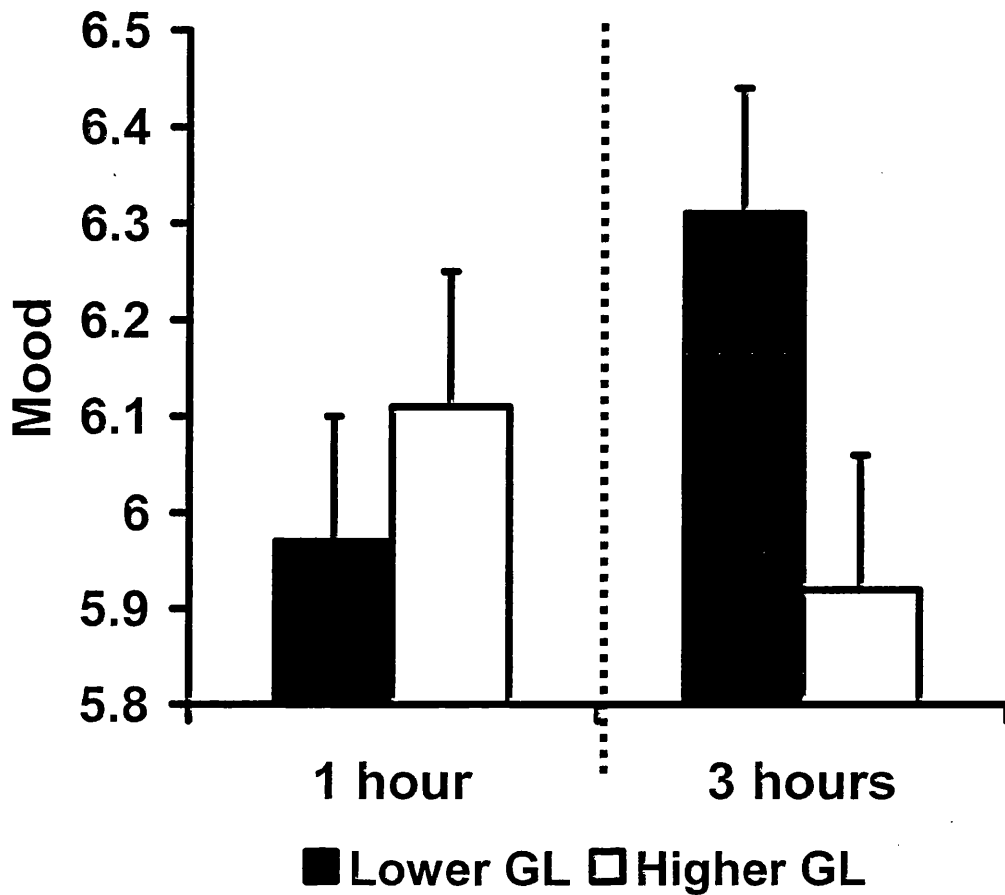


Figure 9 - Mood one and three hours after eating. The data are mean mood scores +/- standard error where a higher score indicates better mood. There were no significant differences after one hour although mood was significantly better three hours after isomaltulose consumption

2.5 Summary

- Irrespective of the day on which it was consumed, a lower, rather than a higher, GL breakfast improved children's memory and mood during the late postprandial period.
- A lower, rather than a higher, GL breakfast increased children's speed of information processing, but only if it was consumed on day 2.
- The nature of breakfast did not significantly influence children's simple reaction times, attention or appetite.
- Age, gender and social background did not influence the children's response to breakfast.

2.6 Discussion

Previously studies have varied the breakfasts of children and interpreted beneficial influences on cognition as reflecting the glycaemic properties of the meals. On different days Ingwersen et al. (2007) gave children two breakfast cereals that differed in their speed of absorption (GL 15 vs 27). The lower GL breakfast was associated with a slower decline in memory and attention throughout the morning. Similarly, Benton (2007) found in children (5 to 8 years) that memory, attention and the time spent on task were better in those consuming a meal with a GL of 6 rather than 15 or 39, although the meals had a different macronutrient composition. However, not all studies have reported a beneficial response to varying the GL of breakfast. In 10 to 12-year-old children Brindal (2012) replaced carbohydrate with protein (GL 18, 24, 33) and failed to find changes in cognitive functioning over a three hour period. Whereas any positive outcomes that occurred in these studies were interpreted as reflecting differences in GL, differences in the actual foods

consumed, and in the macro-nutrient composition, prevented the conclusion that GL was necessarily the mechanism. The present findings, in contrast, reflected differences resulting from meals that were identical in the foods consumed and the macro-nutrient composition, although the GL differed.

Although the isomaltulose based meal benefitted memory (Figure 4) and mood (Figure 9), regardless of the day on which it was consumed, there were effects observed only on the second day of testing. On the second day children processed information more quickly, and had better spatial memory later in the morning, if they consumed the low GL breakfast. In addition where a difference was found on the first day it was less than if it occurred on the second day of testing. These observations suggested a possible mechanism by which isomaltulose may be influential. It is important that although the use of a cross-over design is common when investigating the effects of nutrition on cognition, few studies include order of presentation in their analysis (e.g. Nabb and Benton, 2006; Cooper et al, 2012; Ingwersen et al. 2007; Taib et al. 2012; Jones et al, 2012, Fischer et al. 2001). Such findings are difficult to interpret as effects that occurred on only the first or second testing occasion may have been hidden. Similarly, if a parallel design is used, and testing occurs only once, effects may be missed if they occur only with repeated testing.

It cannot be assumed that those taking a test battery will be similar on a second rather than first occasion, or alternatively that repeated testing will produce results comparable to a second testing session. When first tested the situation is novel and attention grabbing and therefore motivation is likely to be greater; there is a

need to find out what is required and to develop ways of working. The effect on mood in the present study is consistent with the pattern of results: isomaltulose resulted in children reporting feeling happier (Figure 9). It is possible that on day one, when children were exposed to a novel and interesting situation, they had the motivation needed to overcome the presumed physiological disadvantage that resulted from the glucose based meal. However, by day two when they had become accustomed to the monotonous nature of the tasks, and were likely to be getting a little bored, the lower GL meal was helpful. Thus a hypothesis that accounts for the present findings is that the benefit of a lower GL meal is that it helps an individual persevere with an uninteresting task. That is isomaltulose improved mood and motivation with a resulting better attitude, a change that would be less likely to be influential when the tests were novel. If this proves to be the mechanism then a low GL meal may potentially have a greater and longer term impact in monotonous situations, such as school, where many tasks are repeated. In fact, if a response to a meal occurs only when a task is novel it would have little practical importance, although it is to be demonstrated that the present effects last longer than two days of testing. Some studies give a practice session prior to experimentation this offers only one of a series of situations under which an intervention needs to be assessed. The present study did not have a familiarisation session, and it is possible that had the children had an opportunity to practise the tasks, order effects may have been avoided.

Previous research has found benefits with very low GL values, for example Benton et al. (2007) found a positive response to a meal that provided a GL of 6 and benefits have been reported after meals composed entirely of fat or protein that

have a GL of zero (Fischer et al. 2001). In contrast, the present meals provided a GL of 32 or 60 such that a very low GL was not necessary to observe a positive response.

To our knowledge only two studies have examined the cognitive effects of varying the GL within a higher range. In 11 to 14 year olds Micha (2011) investigated the effects of four meals differing in their glycaemic properties, varying both the GI and GL of meals in a systematic way. They found that the greatest facilitation of learning resulted from a low-GI (GI=48) / high-GL (GL=41) breakfast such that both the quantity and nature of carbohydrate were important. However, an important consideration was that the meals that provided a low GL were much smaller (275 and 281 kcal) than those that provided a high GL (469 and 468 kcal). Given that the physical size and caloric intake of breakfast may influence cognition (Michaud et al. 1991) the underlying mechanism is unclear. In addition, as the macronutrient composition of the meals in the Micha (2011) study differed, the response may not have been due to GL. In adolescents, Cooper (2012) found that the decline across the morning in reaction times and various measures of cognition was less after a lower GL meal. They compared a high GL breakfast (cornflakes and white bread, GL=54) and a low GL breakfast (muesli, apple, GL=36) that, although they supplied the same amount of carbohydrate, differed in the amounts of other macronutrients consumed. The present findings, however, are consistent with a beneficial response being to GL rather than the macro-nutrient profile of the meal.

When comparing studies the developmental stage of the children may be important. It may be critical that the ages of the present sample varied from five to

eleven years, as it is this age range when the metabolic rate of the brain is twice that of the adult (Chugani et al. 1998). Hence potentially younger children may be more susceptible to the GL of a meal. However, although both Cooper (2012) and Micha (2011) considered older children (13 and 12.6 years respectively), similar benefits have been reported in those with an average age of 6 (Benton et al. 2007) and 9 years (Ingwersen et al. 2007). The present study, that recruited children aged 5-11, found that age did not modulate the effect of GL on cognition. That is all children in this age range benefited from a lower GL.

Similarly, previous evidence suggests that children from a more deprived background, who may be more cognitively or nutritionally vulnerable, preferentially benefit from consuming breakfast (Hoyland et al. 2009). To our knowledge this is the first study to investigate whether a lower, rather than a higher, GL breakfast differentially affects cognition of children from more or less deprived backgrounds. There was no evidence that this was the case; although children from a more deprived background had slower speed of information processing, on no occasion did the background of children interact with the nature of breakfast to affect cognition.

The possibility remains that there is specific response to isomaltulose that is unrelated to its glycaemic consequences, although such a possibility will be logically impossible to exclude in any study that uses this sugar. For example, there is evidence that consuming isomaltulose, rather than sucrose, increased the rate of postprandial fat oxidation (van Can et al. 2009; 2012); effects attributable to an attenuated rise in glucose and insulin concentrations. It is possible that an

increase in fatty acid oxidation in the periphery may result in a relative sparing of glucose, which can subsequently be utilised by the brain. Isomaltulose has been shown previously to improve cognitive performance. For example, Kashimura et al (2003) found that the consumption of a drink containing 40g of isomaltulose (GL 12.8) improved middle aged adults ability to concentrate on a mathematical task after 90 and 120 minute, however, statistical contrasts between the two sugars were not taken and thus these findings should be interpreted with this in mind. Similar benefits were seen following a drink of 40g of sucrose (GL 26); however, improvement was not as great as that following isomaltulose. Much the same effect was observed in a follow up experiment using only 5g and 10g of isomaltulose. Although these contrasts provided indirect evidence that isomaltulose can aid cognitive performance more than sucrose as no direct statistical comparison was drawn between the two sugars it is not possible to determine if isomaltulose was significantly more beneficial than sucrose.

More recently, Dye et al. (2010) examined the effects of 50 g palatinose (GL 16), 50 g of sucrose (GL 32.5) or water on measures of cognitive performance in a group of young adults. They found that although isomaltulose produced a lower blood glucose profile than sucrose, there were no differences observed for memory or psychomotor performance. It may be critical that the final testing session took place 115 minutes after the drink, whereas the present study did not observe benefits until 180 minutes after breakfast. Taib et al (2011) conducted the only study that investigated the effects of isomaltulose on cognitive performance in school children (age 5-6 years). Participants received either isomaltulose enriched milk (4.48g isomaltulose, 11g lactose, 6.8g glucose, 2.3g sucrose GL ~ 14),

standard growing up milk (7.6g lactose, 12.8g glucose, 4.2g sucrose GL ~20), reformulated growing up milk (11g lactose, 9.4g glucose, 4.2g sucrose GL ~ 14) or glucose (GL ~ 40). They found children's cognitive performance declined across the morning but that after the drink containing isomaltulose, children's focused attention was better than after the standard milk, in addition after drinking isomaltulose there was less of a decline in numeric memory than after the reformulated milk or glucose. Surprisingly, after the glucose drink, spatial memory declined less compared to all other drinks. In addition drinking glucose also resulted in a lower decline in speed of picture recognition compared to the reformulated milk. After drinking the reformulated milk children's speed of spatial working memory was better than after drinking the standard milk.

Given the inconsistency, Taib et al's findings are not easy to interpret, but given the relatively small dose of isomaltulose (4.48g) and the small differences in GL (14 compared to 20) it is unlikely that these factors can account for their findings. The present study supported the hypothesis that isomaltulose provided in isocaloric meals with the same macro-nutrient composition, consumed in a dose sufficient to produce a significant difference in GL may improve children's mood and cognition (Figures 4 and 9).

The present study compared isomaltulose with glucose rather than sucrose, producing a relatively large difference in the GLs of the meals (32 vs 60; table 9). In contrast, both Dye et al. (2011) and Kashimura et al. (2003) compared isomaltulose to sucrose resulting in smaller differences in GL (16 vs 32; 12.8 vs 26; table 1). It may be that larger differences in GL are more likely to produce

significant differences. In addition, Taib et al. (2012) and Dye et al. (2011) used a milk-based vehicle, a potentially critical variable. Milk is known to increase insulin secretion and both an insulin-induced decrease in the levels of blood glucose, and insulin itself, are known to influence cognition (Shemesh et al. 2012). Thirdly, given that children of the age presently tested have a higher rate of brain glucose utilisation than adults (Chugani et al. 1998) they may be particularly vulnerable to fluctuations in peripheral glucose levels and hence GL.

The aim of this chapter was to examine the effect of modulating the GL of meals on cognition and mood in children. For the first time the present findings report the beneficial responses of children to meals of an identical macro-nutrient composition that varied in GL. It is concluded that lowering the GL of children's breakfast could potentially increase the ability of children to benefit from their schooling by improving their memory and mood.

CHAPTER 3

Low blood glucose reduces cognitive decline and improves mood in older adults with poorer glucose tolerance.

3.1 Introduction

As glucose is the primary fuel of the brain a continuous supply is required to maintain cognitive functioning (Amiel, 1994), and both poorer glucose tolerance (Lamport et al, 2009) and low blood glucose (Warren et al, 2005) are disruptive. Furthermore both glucose intolerance (Cheng et al, 2012) and hypoglycemia (Whitmer et al. 2009) have been related to an increased incidence of mild cognitive impairment (MCI) and dementia. Therefore the aim of the present study was to relate, in a healthy older sample, the ability to control blood glucose to mood, cognitive functioning and cognitive decline.

As discussed in chapter 1, a reduced ability to regulate blood glucose is associated with poorer attention (Donohoe et al, 2000), slower reaction times (Yaffe et al, 2012; Donohoe et al, 2000) and poorer memory (Yaffe et al, 2012; Awad et al, 2002). Furthermore these decrements in cognitive functioning, as a result of poorer glucose tolerance, may be more pronounced in older age (Ryan and Geckle, 2000). Although the mechanism is unclear, one suggestion is that the disruption of cognition in glucose intolerant individuals reflects a reduced ability to transport glucose into the brain (Convit, 2005). Thus the brain is deprived of the fuel it requires to function optimally and in the long term such deprivation may play a role in the development of cognitive decline.



Given that reductions in cerebral glucose metabolism are often observed in dementia, and emerge in at risk individuals even before cognitive symptoms develop (Mosconi et al, 2007), it has been argued that reductions in brain glucose metabolism may play an important role in the development of cognitive disease (Costantini et al, 2008). This implies that any factor that compromises neuronal glucose supply, such as poor glucose regulation, has the potential to exacerbate age related cognitive decline. On the other hand any factor that increases the supply of glucose to the brain or its utilisation may have the potential to aid cognitive performance.

Despite much research into the effects of poor glucose tolerance on cognition, little research has explored the role of glucose excursions on cognitive decline. Individuals in the early stage of glucose intolerance often experience hyperinsulinaemic compensation (Polonsky 2000); that is increased plasma insulin levels. However, if too much insulin is produced blood glucose levels may fall to low levels leading to substantial fluctuations in blood glucose levels and the possibility of developing mild hypoglycaemia (Brun et al, 2000). Although during frank hypoglycaemia (<3.0 mmol/L) cognition and mood suffer (McCrimmon et al, 1997; Kindgren et al, 1996), there are large individual differences in susceptibility (Gonder-Frederick et al, 1994). One possible explanation is that in some brain adaptations occur so that if hypoglycaemia re-occurs cognition is preserved. For example, in animals following hypoglycaemia (2.5-3.0mmol/L) once glycaemia is restored hippocampal glucose concentrations are increased (McNay et al, 2006). In healthy humans Boyle (1994) used a central venous catheter to study the effects

of recurrent episodes of hypoglycaemia (2.9mmol/L) on the uptake of glucose by the brain, and related this to cognition and hypoglycaemic symptoms. The rate of glucose uptake by the brain was initially impaired when blood glucose was reduced to 3.6 mmol/L, although after 56 hours of intermittent hypoglycaemia (3.0 mmol/L) the normal rate of uptake of glucose by the brain was preserved, even when blood glucose levels were reduced to 2.5mmol/L. Similarly, after exposure to recurrent hypoglycaemia cognitive performance was preserved and hypoglycaemic symptoms reduced when low glucose levels were experienced.

Previous studies have tended to examine these effects under, experimental conditions that artificially lower blood glucose to levels that will rarely be found in everyday life. As the levels of blood glucose needed to induce adaptation are unclear one aim was to see if comparable effects occur in those whose physiology predisposes to the development of moderately low levels of blood glucose. The present study therefore examined for the first time, in healthy older adults, the association between the ability to control blood glucose, in particular glucose intolerance and the tendency for blood glucose to fall to low levels, mood and memory. In particular we believe this to be the first study to relate a tendency to develop moderately low levels of blood glucose to cognitive decline.

3.2 Methods

3.2.1 Participants

One hundred and fifty five adults (58 males; 97 females) aged 45 to 85 years (average age 56 (12.3)), were recruited following an appeal in the local media. Exclusion criteria included anyone diagnosed with type 1 or type 2 diabetes,

chronic liver disease, or gastrointestinal problems that may interfere with absorption, for example Crohn's disease. Participants who had a current diagnosis of a mood disorder, dementia or other mental disorder that may affect cognition were also excluded. Eyesight and hearing was normal or corrected to normal. Of the participants 16.1% were taking drugs to control blood pressure, 3.8% thyroxine, 3.2% statins and 2.7% asthma controlling drugs. The procedure was followed with the approval of the Swansea University ethics committee (reference number: 0825/2009/1) and only after the participants had given written informed consent. (Appendix 3 and 4)

3.2.2 Procedure

After fasting over-night, 1.75 grams of glucose per kilogram of body weight was consumed, to a maximum of 75 g. Every 30 minutes for 150 minutes blood glucose was monitored from finger pricks using an ExacTech sensor (Medisense Britain Limited) that used an enzymic method, coupled with microelectronic measurement (Matthews et al, 1987). Capillary blood samples have been shown to be sufficiently sensitive to postprandial changes in systemic glucose concentrations (Brouns et al. 2005). In addition to the oral glucose tolerance test (OGTT) participants completed a cognitive test battery in the order presented below. In studies of blood glucose inevitably the food that has been eaten, and how recently, are confounding factors. The test battery was therefore started 10 minutes after the consumption of a standard glucose drink to ensure that all participants were comparably nourished and were tested at a time when blood glucose levels were similar and individual differences in physiology had little time to be influential (Figure 10). Evidence suggests that the consumption of glucose

may reduce the differences in cognition that are observed between those with better or poorer glucose tolerance (Awad et al, 2002; Kaplan et al, 2000), thus it was considered that this approach would bias against rather than in favour of detecting differences. Participants completed the tasks in the following order; Immediate episodic recall, Working memory, Reaction times, Sustained attention, Delayed episodic recall, Semantic memory, NART. The cognitive test session lasted approximately 30 minutes. Participants then sat quietly whilst they completed the questionnaires.

3.2.3 Test Battery

3.2.3.1 Episodic memory

Episodic memory was measured by recalling a word list. Using the MRC Psycholinguistic Database a list of thirty words was constructed matched for the number of syllables, image-ability and the frequency with which they occur in English (appendix 5). Using a recorder words were presented one every 2 seconds. Immediately after the presentation, as many words as possible were written down (immediate recall). Approximately 25 min later, after completion of the other tasks, participants again recalled the words (delayed recall).

3.2.3.2 Semantic memory

Semantic memory reflects knowledge that is unrelated to specific experiences and the meaning of words and knowledge. Verbal fluency was assessed as a measure of semantic memory. In a one minute period participants were asked to provide as many words as possible beginning with a particular letter of the alphabet: words beginning with C, F and L. In the set of letters words beginning with the first letter

occur frequently, with the second letter less frequently and with the third letter even less often. The scores excluded proper nouns such as people's names, place names or the same word with a different suffix.

3.2.3.3 Working memory

Working memory that temporarily holds and manipulates information was assessed with a computerized version of serial sevens. A series of numbers between 800 and 999 was given and subject indicated whether a second number was seven less than the previously observed number. The responses to 28 sequences were recorded and the number of errors made, and the time to respond to the second number, was reported.

3.2.3.4 Cognitive impairment

The National Adult Reading Test (NART) (Nelson et al, 1982) estimates premorbid intelligence (Bright et al. 2001; Maddrey et al. 1996). As the ability to recognize words is preserved with age, phonologically irregular words that occur progressively less frequently in English are pronounced. The NART correlates with both premorbid episodic and working memory (Frick et al, 2011). The NART and the memory scores were both transformed to Z scores (differences from the population mean in terms of standard deviations). The NART Z scores were then subtracted from the immediate memory Z scores; such that a negative value indicated that the current memory was poorer than predicted by the intelligence score; that is there was evidence of cognitive impairment.

3.2.3.5 Vigilance

A computer generated a series of digits at the rate of 100 digits per minute. Participants pressed the space bar when they detected three consecutive odd or consecutive even digits. Eight target sequences were presented every minute. Following the presentation of the third consecutive digit, 1500 msec was allowed for a correct response. Responses made at any other time were recorded as errors. A minimum of 5 and a maximum of 30 digits separated any two target sequences. The task was performed for five minutes. Data analysed were the number of correct responses and reaction times for the correct responses for each minute of the test, allowing for the detection of changes in performance over time.

3.2.3.6 Reaction Times

The reaction time procedure was based on Jensen (1987). On a panel eight lamps were arranged in a semicircle, each 5.5 inches from a central button (the home key). The index finger was placed on the home key. Within one to two seconds an auditory warning signal sounded and after a random interval of one to four seconds one of the lamps illuminated. The subject then extinguished the light by depressing a button directly below the lamp, using the finger initially on the home key.

All participants completed a practice session of 20 trials using all eight lamps. Simple reaction times were then measured for 20 trials using one lamp. Choice reaction times were then measured over three sets of 20 trials when one of 2, 4 or 8 lamps could potentially illuminate. Decision times, the time taken to lift the finger from the home key, were analyzed. Similarly movement times, the time from the

hand leaving the home key to pressing the button under the illuminated light, were considered.

3.2.3.7 General Health Questionnaire (GHQ)

The GHQ-30 (Goldberg et al, 2008) (appendix 6) identifies minor psychiatric disorders when participants responded on a 4 point scale (not at all, same as usual, more than usual, much more than usual). As well as an overall scale, based on a factor analysis by Chan (1985) the questionnaire was divided into subscales representing depression and anxiety.

3.2.3.8 Hypoglycaemia

Neuroglycopenic and sympathetic nervous systems symptoms (SNS) were assessed using symptoms listed by Hepburn (1991) (appendix 7). Each were assessed using visual analogue scales where 100mm lines were anchored with the labels 'Exactly like me' and 'Not at all like me'. Seven questions made up the neuroglycopenic scale: for example "I have difficulty concentrating", "I am confused" or "I have blurred vision". The SNS scale consisted of eight questions; for example "I experience a pounding heart", "I blush" or "I tremble". The responses were added to produce single scores for the two dimensions.

3.3 Statistical analysis

Participants were divided into two groups according to their blood glucose level after two hours of the OGTT. To allow sufficient sample sizes in the various groups, as near a median split as possible was made. If their blood glucose was 7.0mmol/L or higher they were described as having poorer glucose tolerance (GT)

and if their blood glucose was less than 7mmol/L they were described as having better GT. Of those with poorer glucose tolerance 66% would be classified as clinically glucose intolerant in so much that values were greater than 7.9mmol. Individuals were also divided into two groups depending on the tendency for blood glucose to remain above or fall below fasting values. Those whose levels fell below baseline all had glucose levels <5.0mmol/L at the end of the test. Importantly, 50% of these final values fell below 4.0mmol/L and 30% were <3.6mmol (a suggested threshold for adrenal discharge in healthy humans (Auer, 2004); this variable was labelled Lowest Blood Glucose (LBG).

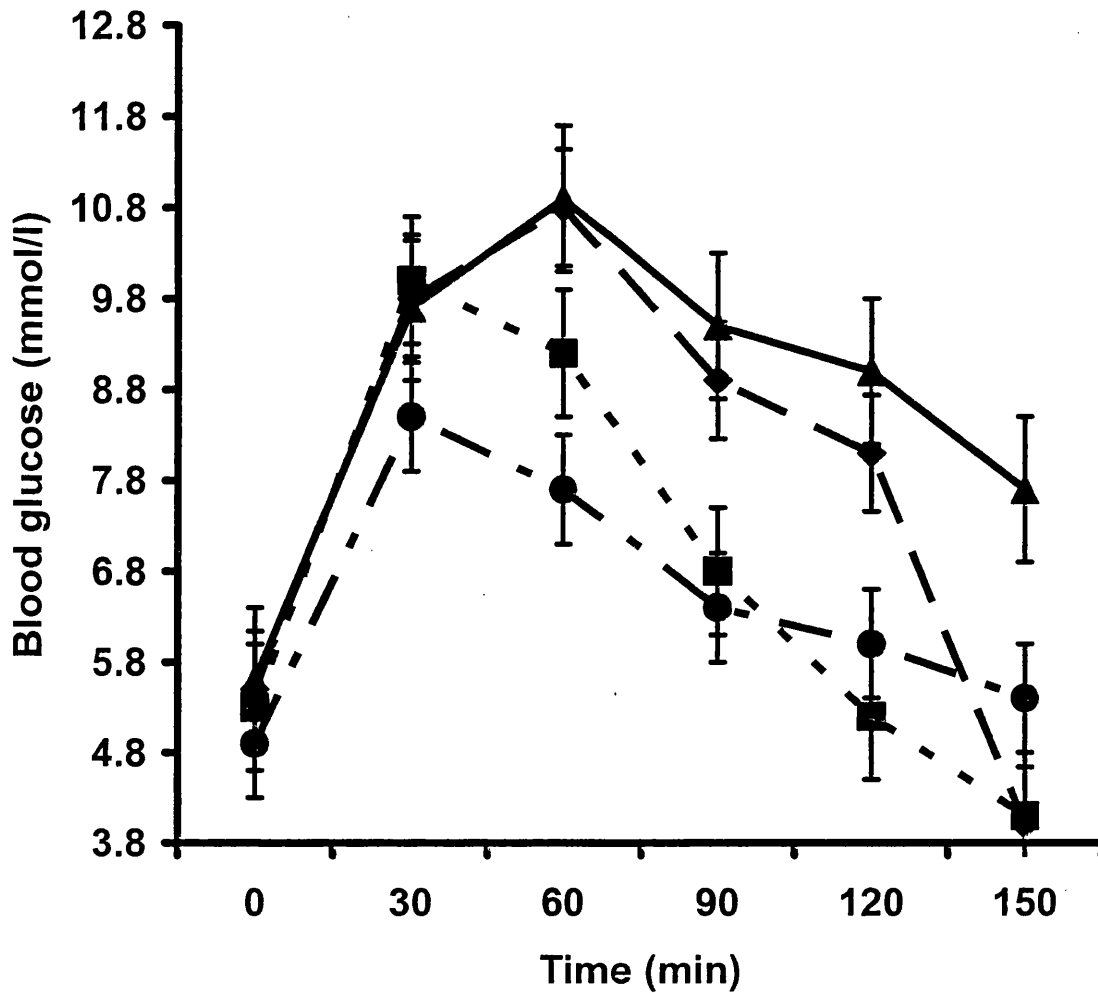


Figure 10 - Oral glucose tolerance profiles of four groups of participants. The data are mean blood glucose values as mmol/dl for four groups defined in terms of poorer and better glucose tolerance (above and below 7 mmol/dl at 120 minutes) and either staying above or falling below baseline values.

- ▲ — ▲ Poorer glucose tolerance – Above baseline N = 67
- ◆ - - ◆ Poorer glucose tolerance – Below baseline N = 41
- ····· ■ Better glucose tolerance - Below baseline N = 21
- — · ● Better glucose tolerance – Above baseline N = 25

To ensure the independence of each glucoregulatory group preliminary analysis tested whether blood glucose profile differed depending on group. The interaction Time (0, 30, 60, 90, 120 and 150 minutes) X Glucoregulatory group reached significance ($F(10.2,511.7)= 20.019, p<0.0001$). Table 12 shows the significant differences in blood glucose at each time point of the OGTT.

	After 0 minutes	After 30 minutes	After 60 minutes	After 90 minutes	After 120 minutes	After 150 minutes
PGT, LBG vs BGT, LBG	ns	ns	ns	p<0.01	p<0.0001	ns
PGT, LBG vs PGT, no LBG	ns	ns	ns	ns	p< 0.05	p<0.0001
PGT, LBG vs BGT, no LBG	p<0.001	p<0.001	p<0.001	p<0.006	p< 0.0001	p< 0.0001
BGT, LBG vs PGT, no LBG	ns	ns	ns	p<0.001	p< 0.0001	p< 0.0001
BGT, LBG vs BGT, no LBG	p<0.003	ns	ns	ns	p< 0.0001	p< 0.0001
PGT, no LBG vs BGT, no LBG	p<0.005	p<0.02	p<0.0001	p<0.0001	p< 0.0001	p< 0.0001

Table 12. Differences in blood glucose, at each time point, depending on glucoregulatory group. PGT-Poorer glucose tolerance. BGT- Better glucose tolerance. LBG-Lowest blood glucose.

Table 13 shows the demographic data for the participants in each group. There were no significant differences in BMI between any of the glucoregulatory groups ($F(3,152) = 1.736, ns$). Similarly, BMI was not related to any measure of cognition or mood and so was not considered further. Gender did not determine glucoregulatory status $\chi^2(3) = 5.177, p = .158$. Gender significantly predicted memory ($F(1,154) = 4.118, p< 0.05$); females had better memory than males ($M 9.4(0.4) F 10.6(0.3)$). However, there were no interactions between gender and glucoregulatory profile.

	Poorer GT LBG above baseline	Poorer GT LBG below baseline	Better GT LBG above baseline	Better GT LBG below baseline	P
BMI	28.1(0.8)	29.5(1.6)	25.0(1.4)	26.8(1.0)	ns
Gender M	20	17	10	11	ns
F	47	26	15	8	
Age	58(11.4) * **	56.2(9.6)	53.0(10.7)*	54.2(10.8) **	* p<0.01 ** p<0.005

Table 13. Demographic data for the four glucoregulatory groups. Adults with better GT and LBG above baseline were significantly younger than those with poorer GT and LBG above baseline. Similarly, adults with better GT and LBG below baseline were significantly younger than those with poorer GT and LBG above baseline.

Adults with better GT and LBG above baseline were significantly younger than those with poorer GT and LBG above baseline ($p<0.01$). Similarly, adults with better GT and LBG below baseline were significantly younger than those with poorer GT and LBG above baseline ($p<0.01$). It was considered whether age should be entered in the analysis as a covariate. A premise of ANCOVA is that the effects of the covariate should be statistically independent of the effects of group (Miller and Chapman, 2001). The main reason for this is that if the treatments differentially affect the covariate scores, then ANCOVA would remove from the treatment sum of squares part of the treatment effect (Maxwell and Delaney, 1990), hence reducing rather than increasing power to detect group effects (Miller and Chapman, 2001). Given the relationship between age (potential covariate) and glucoregulatory status (IV) it was clear that this assumption would have been violated. In addition, the assumption of homogeneity of slopes states that the groups should not differ in their regression of the dependent variable on the covariate. Given that age was not only related to glucoregulatory group but also expected to predict cognition this assumption would also have been violated.

Therefore, the effects of GT and LBG were considered using appropriate analysis of variance designs. Typically they took the form of GT (Better/poorer) X LBG (Above/below baseline) X Age. Age was considered by distinguishing those 60 years and below from 61 years and above. This was chosen as the cut off because it had been argued that there may be a critical period, which begins at around 60 years, during which glucose intolerance has the greatest impact on cognition (Biessels et al, 2008). Table 14 shows cell frequencies for each level of each factor included in the analysis. Given the uneven sample sizes sum of squares type III were used which uses an unweighted mean and is therefore more robust to the effects of uneven sample sizes. Interactions were probed using appropriate post hoc tests. Where Levene's test for equality of variances was significant adjusted p values were reported. Where interactions are not mentioned it should be assumed that they were non-significant.

LBG below baseline				LBG above baseline			
Better GT		Poorer GT		Better GT		Poorer GT	
Younger	Older	Younger	Older	Younger	Older	Younger	Older
N = 12	N = 9	N = 25	N = 16	N = 18	N = 7	N = 32	N = 35

Table 14. Cell frequencies for each level of each factor included in the analysis. GT-Glucose tolerance. LBG – Lowest blood glucose.

3.4 Results

3.4.1 Memory

A four way ANOVA was calculated, GT (Better/poorer) X LBG (Above/below baseline) X Age (45 – 60 yrs / 61 – 85yrs) X Immediate / delayed recall. The interaction GT X Age X Immediate / delayed recall reached significance ($F(1,146) = 7.02, p < 0.009$). Post hoc tests revealed that older participants (61 – 85yrs) with poorer GT forgot more words (difference between immediate and delayed recall)

than both older participants with better GT (6.8(2.8) vs 5.3 (2.1); $p < 0.02$) and younger participants (45-60 yrs) with poorer GT (6.8 (2.8) vs 4.9 (2.3); $p < 0.01$). The nature of GT did not influence the forgetting of younger participants. LBG did not affect the number of words forgotten (6.2(2.4) vs 5.9(2.3); $F(1,146) = 0.56$, ns).

3.4.2 Semantic Memory

Semantic memory was considered in a GT X LBG X Difficulty level (letters C, F or L) X Age. The main effect of difficulty reached statistical significance ($F(2,292) = 2.94$, $p < 0.05$); participants retrieved fewer words with L the most difficult letter. However, age ($F(1,146) = 0.69$), ns), GT ($F(1,146) = 0.04$, ns) and LBG ($F(1,146) = 1.41$, ns) did not influence semantic memory.

3.4.3 Working Memory

When the response times with the serial sevens test were examined the interaction GT X LBG reached significance ($F(1,139) = 5.57$, $p < 0.02$). Post hoc tests showed that with those with better GT responses did not differ depending on LBG. However, in those with poorer GT, if LBG fell below baseline, mental arithmetic was performed more quickly ($p < 0.02$). No other post hoc tests reached significance (Figure 11). When the number of correct responses was examined the GT X LBG X Age interaction was not significant ($F(1,139) = 0.326$ ns). All main effects and other interactions were similarly non-significant.

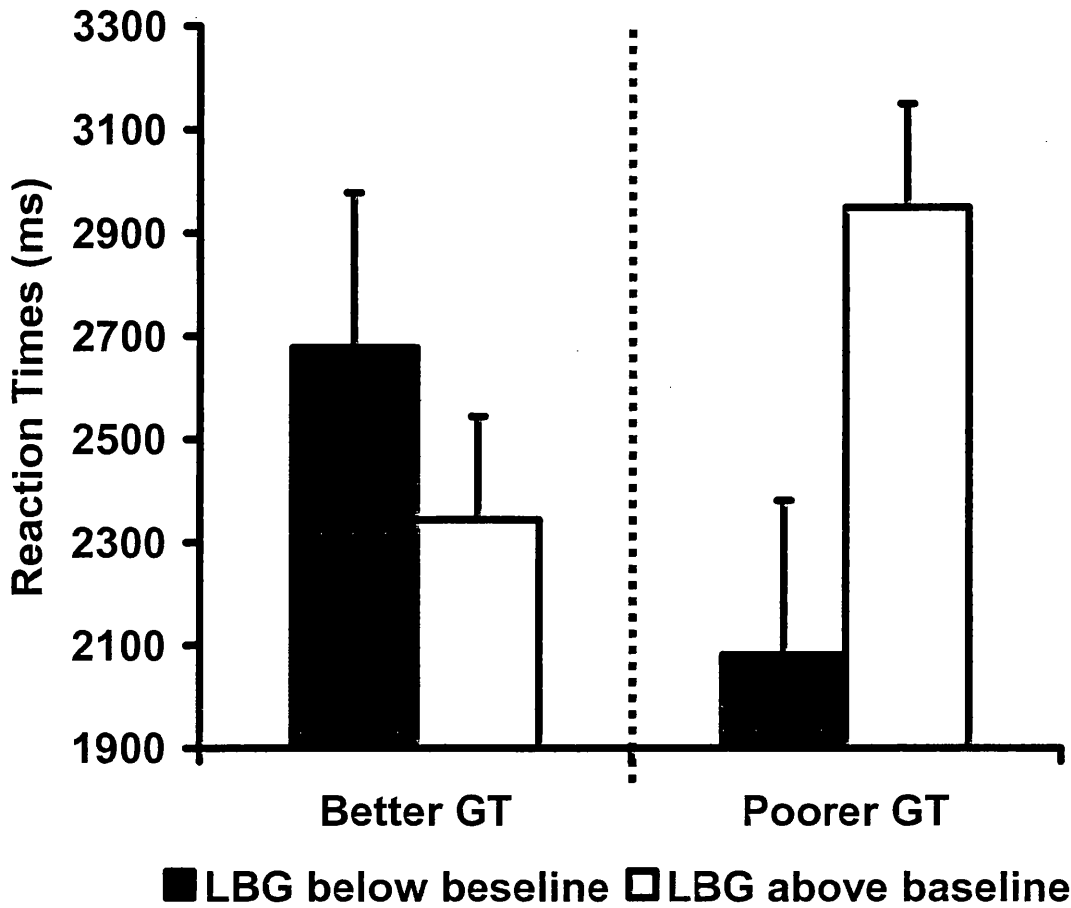


Figure 11. The effect of glucose tolerance and of staying above or falling below baseline glucose values on working memory reaction times. Data are the mean (standard error) in milliseconds (ms) for working memory reaction times in those with better or worse GT and LBG above or below baseline. A lower score indicates better performance. Those with poorer GT and a LBG below baseline had significantly faster responses than those poorer GT and LBG above baseline * ($p < 0.02$).

3.4.4 Cognitive decline

There was a main effect of age on cognitive decline ($F(1,147) = 7.961, p < 0.002$). Participants over the age of 61 showed greater cognitive decline than those aged 60 or under ($-0.86(0.268)$ vs $+ 2.32(1.78)$), however, the effect of age did not influence the effect of either GT or LBG. When a three way analysis was calculated, GT X LBG X Age, the GT X LBG interaction achieved statistical significance ($F(1,147) = 7.331, p < 0.008$; Figure 12).

When participants who's LBG fell below baseline were considered, those that had poorer GT had less cognitive impairment than those with better GT ($p < 0.05$). However, when those who's LBG remained above baseline were examined, a poorer GT was associated with greater cognitive impairment than with those with better GT ($p < 0.006$). When those with poorer GT were considered, those with a LBG above baseline had significantly greater cognitive decline than those LBG fell below the baseline ($p < 0.0001$). When those with good GT were considered there were no differences depending on LBG. Thus in those with poorer GT the tendency for LBG to fall below baseline was associated with less cognitive decline.

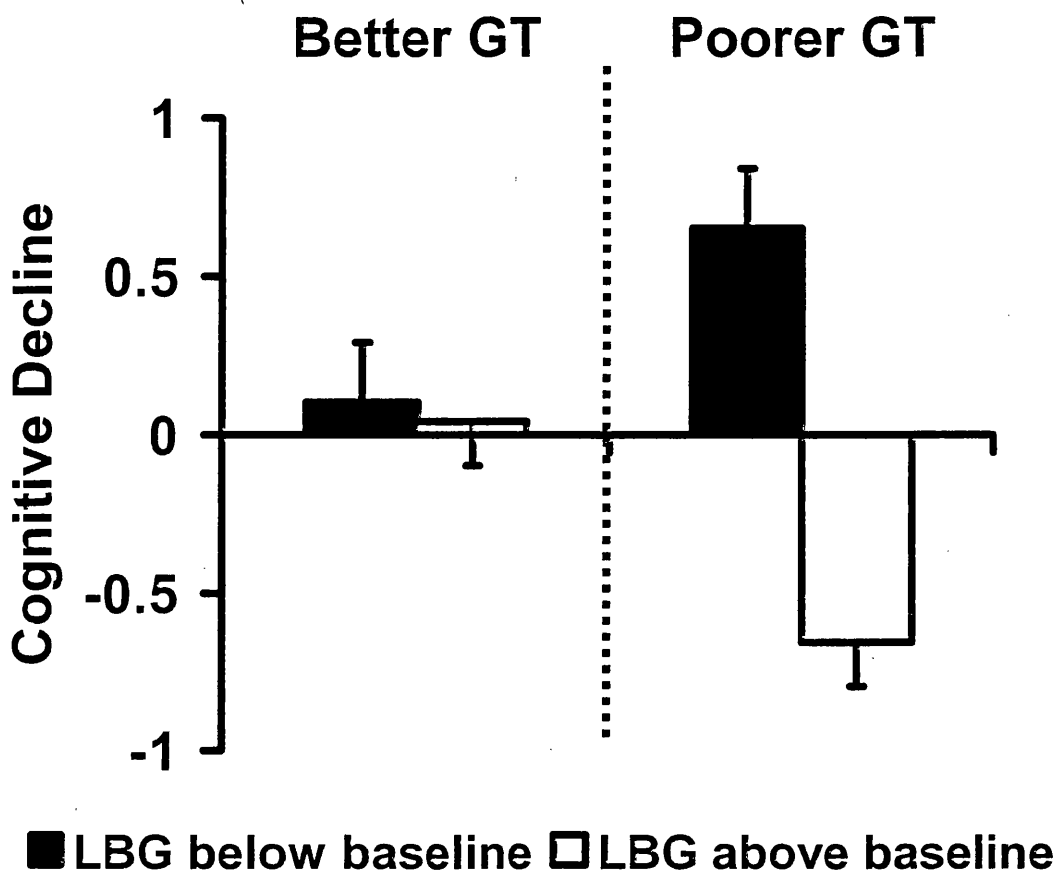


Figure 12. The effect of glucose tolerance and of staying above or falling below baseline glucose values on Cognitive decline. Data are the mean (standard error) for level of cognitive impairment in those with better or worse GT and with LBG above or below baseline. A negative score indicates more impairment. Those with poorer GT and LBG above baseline had significantly more cognitive decline than those poorer GT and LBG below baseline * $p < 0.001$.

3.4.5 Vigilance

When the number of correct responses were analyzed the interaction GT X LBG X Age X Min of test was non-significant ($F(4,528)=0.642$, ns) and similarly all main effects and other interactions failed to reach statistical significance. Similarly with the speed of responding the GT X LBG X Age X Min of test was non-significant ($F(4,536)=1.01$, n.s.).

3.4.6 Reaction Times

With decision times the GT X LBG X Age X Number of lamps interaction was significant ($F(3,432) = 2.84$, $p < 0.03$). There were, however, no differences when those under the age of 60 were selected ($F(3,171) = 0.17$, n.s.). However, in the older individuals there was a main effect of GT ($F(1,57) = 12.90$, $p < 0.001$); the responses of those with poorer GT were slower (1266.9(84.6) vs 843.8 (81.9)). LBG did not influence decision times (1019.1(92.2) vs 991.7(73.22); ns). In contrast, with decision times, movement times were not influenced by either GT or LBG.

3.4.7 GHQ

With the overall GHQ score there were no interactions involving either GT ($F(1,146) = 0.23$, n.s.) or LBG ($F(1,146) = 0.30$, n.s.). However, there was a main effect of age ($F(1,146) = 5.030$, $p < 0.02$); participants over rather than under the age of 61 reported poorer mental health (58.3(1.24) vs 53.2(1.88)).

However, with the depression sub-scale there was a significant GT X LBG interaction ($F(1,146) = 7.039, p < 0.009$; Figure 13). When a LBG that fell below baseline was combined with poorer GT, depression was less than when it was combined with better GT ($p < 0.02$). However, when those with LBG above baseline were considered those with poorer GT were more depressed than those with better GT ($p < 0.01$). When those with poorer GT were selected, those with LBG below the baseline had a less depressed mood than those without that tendency ($p < 0.007$). When those with better GT were selected, those with a LBG below baseline had a more depressed mood than those whose values remained above baseline ($p < 0.04$). Thus, a LBG below baseline was associated with less depression in those with poor GT, whereas it increased the depression of those with better GT. Age did not influence levels of depression ($F(1,146) = 0.30, n.s.$).

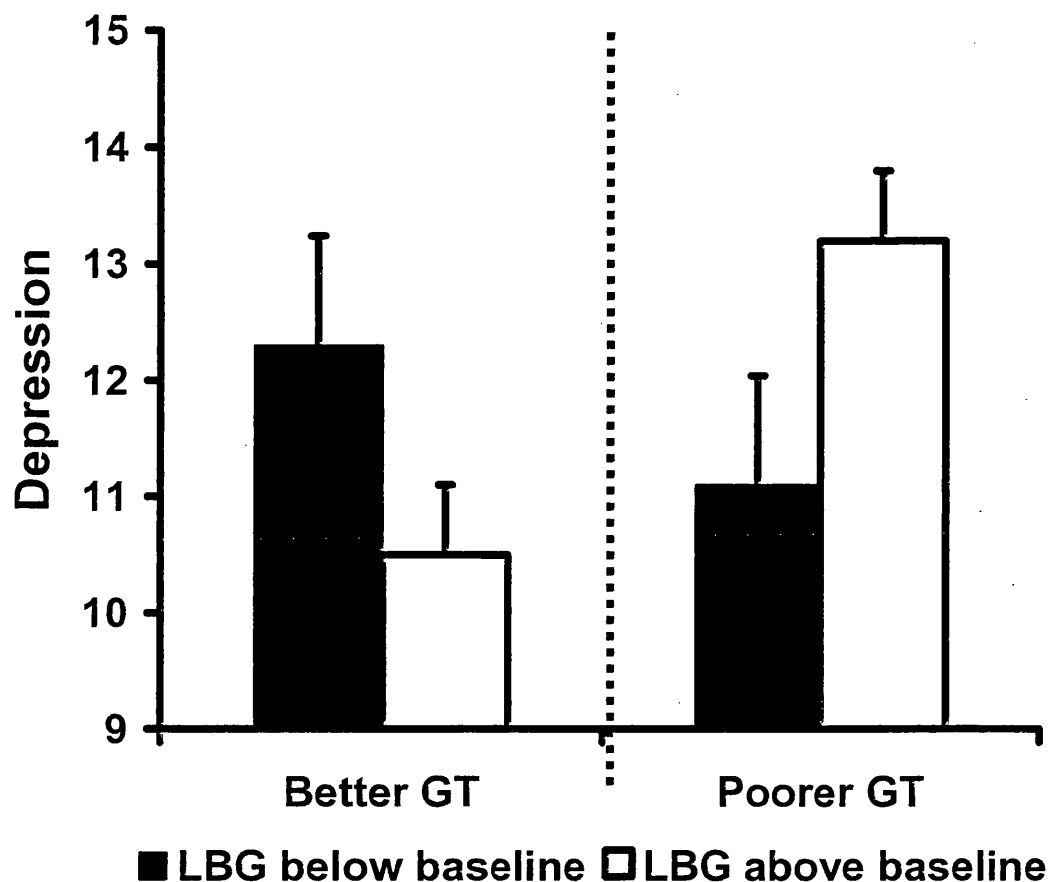


Figure 13. The effect of glucose tolerance and of staying above or falling below baseline glucose values on depression ratings. Data are the mean (standard error) for level of depression in those with better or worse GT and with LBG above or below baseline. A higher score indicates more depression. Those with poorer GT and LBG above baseline had a more depressed mood than those with poorer GT and LBG below baseline ($P < 0.007$). Those with better GT and LBG above baseline had a less depressed mood than those with better GT and LBG below baseline ($p < 0.04$). Those that had LBG below baseline and had better GT had a more depressed mood than those that had LBG below baseline and had poorer GT ($p < 0.02$). Those that had LBG above baseline and had better GT had a less depressed mood than those that had LBG below baseline and poorer GT ($p < 0.01$).

When the anxiety subscale was similarly considered the interaction GT X LBG was non-significant ($F(1,146) = 0.29$, n.s.) and there was no effect of age ($F(1,146) = 1.03$, n.s.). However, the main effect of GT reached significance ($F(1,146) = 4.67$, $p < 0.03$), those with poorer rather than better GT were more anxious (22.2 (0.53) vs 20.7 (0.51)).

3.4.8 Sympathetic nervous system symptoms (SNS)

There were no effects of LBG on SNS symptoms ($F(1,146) = 0.56$, n.s.). However, the interaction GT X Age reached significance ($F(1,146) = 9.38$, $p < 0.003$). In participants under 60 years there were no differences in SNS symptoms depending on better or poorer GT. However, in those over the age of 61, poorer compared with better GT, was associated with more SNS symptoms ($p < 0.003$); 327.62 (148.92) vs 206.52 (135.92)). In addition, when those with poorer GT were selected there were no differences between older or younger participants. However, when those with better GT were considered, those over the age of 61 reported fewer symptoms than those under 60 years ($p < 0.0001$); 206.50 (135.9) vs 360.59 (159.60)).

3.4.9 Neuroglycopenic symptoms

With neuroglycopenic symptoms the interaction GT X Age approached significance ($F(1,146) = 2.92$, $p < 0.07$). There were no differences in neuroglycopenic symptoms, depending on GT, in those 61 years or over. In contrast those under the age of 60 with poorer rather than better GT reported more neuroglycopenic symptoms (492.2(168.4) vs 416.3(161.0); $p < 0.04$). LBG did not effect neuroglycopenic symptoms ($F(1,146) = 0.08$, ns).

3.5 Summary

- Older adults' aged 61 years or over, who had poorer, rather than better, GT had poorer memory, slower decision times, and more SNS symptoms.
- Older adults' with poorer GT and LBG below baseline had less cognitive decline, rated themselves less depressed, and had faster working memory than those with poorer GT and no LBG (Figures 12 and 13).

3.6 Discussion

The pattern of findings is consistent with the literature dealing with the effects of aging on cognitive performance (Verhaeghen et al, 1997). Those 61 years or over had poorer episodic memory, greater cognitive decline, slower decision times and poorer mental health although vigilance and semantic memory did not differ. In addition, the negative effects of poor GT were more pronounced in those over 61 years; in older rather than younger individuals, poorer GT was associated with slower decision times, forgetting more words and a higher frequency of SNS symptoms. Although previously those considering the relationship between GT and cognition have tended to report a stronger association in older (Messier et al, 2003; Convit et al, 2003; Kaplan et al, 2000) rather than young adults (Lamport et al, 2009; Awad et al, 2002; Kaplan et al, 2000; Messier et al, 1999; Yaffe et al, 2004), this is the first study to directly compare younger and older adults with a similar level of impaired GT. Although the exact mechanism is to be determined, the present data supported the idea of a synergistic interaction between age-related changes in the brain and the metabolic consequences of impaired GT (Ryan and Geckle, 2000).

However, given the relationship between glucose tolerance and age (Tables 13 and 14) and the use of dichotomisation the present findings should be interpreted cautiously. Median splits are highly sample dependant and thus present difficulties in generalisation. In addition, they are unrealistic, with individuals close to but on opposite sides of the cutpoint characterized as having very different rather than very similar outcome. Further, the central tendencies of two dichotomised groups may be artificially shifted further apart by a small number of extreme observations at either end of a continuous measure. This latter point has the potential to strongly bias results. In fact, it has been argued that dichotomizing continuous predictor measures may lead to overestimates of strength of relationship accompanied by an increase in Type I errors (Maxwell and Delaney 1993). This is particularly true when testing for interactions and may be partially explained by an inability to distinguish nonlinear effects from interaction effects when artificially dichotomizing variables (Maxwell and Delaney, 1993).

On the other hand, as discussed by Cohen (1983), dichotomization can lead to a loss of one-fifth to two-thirds of the variance that may be accounted for by the original continuous variables, and a concomitant loss of power equivalent to that of discarding one-third to two-thirds of the sample; thus it is possible that the present approach is a conservative one that would likely bias against significant results.

An alternative approach is to consider the interaction between two continuous variables using moderated regression (aka simple slopes). Essentially, the effect of an IV on a DV is not a single number but, rather, a function of a MV (moderator

variable). However, the creation of a cross-product term may result in substantial collinearity with its constituent parts, making it difficult to detect main and interaction effects and increasing the chances of type 2 errors. The commonplace response to this problem is to mean-center (Aiken and West, 1991), however, it has been illustrated that this is not always successful (Echambadi and Hess 2004). In fact, a comparison of centered and raw score analyses demonstrated the two methods to be equivalent; yielding identical hypothesis tests associated with the moderation effect and regression equations (Kromrey and Foster-Johnson 1998).

Given evidence of interaction between IV and MV, investigators typically probe that interaction by estimating the conditional effect of IV at various values of MV, and testing whether it is statistically different from zero (Aiken & West, 1991). If MV is continuous, the dominant approach is to set MV to various values that represent low, medium, and high, such as a standard deviation below the mean, the mean, and a standard deviation above. This approach, has been coined the 'pick-a-point' approach (Bauer & Curran 2005) due to its arbitrary nature and falls pray to many of the criticisms of artificial dichotomisation.

The Johnson-Neyman technique for probing interactions avoids the need to arbitrarily select values of the moderator at which to estimate the conditional effects of IV and identifies the value or values within the measurement range of the moderator where the conditional effect of IV transitions between statistically significant and not (Hayes 2012). These values identify the boundary or boundaries of regions of significance. However, determining the significance region involves comparing the slopes at each unique predictor value, thus yielding statistical tests for each IV value. It is well-known that if each null hypothesis is

tested at nominal level α , the overall Type I error rate will be substantially inflated (Lazar and Zerbe 2011).

In order to confirm the present findings a slope analysis was conducted as detailed above using the SPSS macro PROCESS (Hayes 2012). Memory was entered as the dependant variable, age as the independent variable and blood glucose levels after two hours as the moderator; all variables were continuous. As expected both age ($\beta = 0.2$, $p < .01$) and glucose tolerance ($\beta = 2.0$, $p < .003$) predicted memory. The interaction was also significant ($\beta = 0.03$, $p < .002$) (see appendix 8 for a table and scatter plot of these effects). The Johnson-Neyman technique identified 7.2 mmol/l as the region of significance; age predicted memory in those with blood glucose greater than 7.2 mmol/l, therefore supporting the validity of our median split (7 mmol/l) for this factor. Similar effects occurred when decision times were plotted against age for those with better or poorer GT (Appendix 9).

The most noteworthy finding of the present study was that in those with poorer GT, having blood glucose that fell below baseline values was associated with reduced cognitive decline (Figure 11), improved working memory (Figure 12) and decreased depression (Figure 13). Although the brain adaptation to low levels of blood glucose has been little considered in the context of the cognitive decline of healthy humans, similar phenomena have been described in diabetics and animal studies. In animals low blood sugar levels (2.5 - 3.0 mmol/l) have been reported to induce brain adaptation such that subsequently cognitive functioning was improved (Jacob et al, 1999; McNay and Sherwin, 2004; McNay et al, 2006 Fruehwald-Schultes et al, 2000; Mellman et al, 1994; Boyle et al, 1994). For example studies

have reported that a single episode of low blood glucose resulted in increased hippocampal interstitial glucose concentrations (Jacob et al, 1999; McNay and Sherwin, 2004; McNay et al, 2006; Oz et al, 2009; Criego et al, 2005; Boyle et al, 1994). You can speculate that this increase in brain glucose levels may enhance subsequent cognitive performance by providing a greater supply of glucose during periods of demand. For example, maze learning by rats, a task that is associated with a drop in hippocampal interstitial glucose levels (McNay et al, 2000; 2001), was performed better after three days of exposure to hypoglycaemia (McNay and Sherwin, 2004): there were smaller falls in interstitial glucose than when animals had not been exposed to hypoglycaemia.

Although the mechanisms underlying these findings remains unclear, GLUT 1 at the endothelium and the neuronal glucose transporter GLUT 3 are both up regulated following hypoglycaemia (Kumagai, 1995; Koranyi et al.1991; Boado and Pardridge,1993; Uehara et al 1997) and these adaptations lead to an increased level of brain glucose (Lei and Gruetter, 2006). The glucose transporter GLUT 1, that transports glucose across the BBB, is highly expressed in the vascular endothelial cells (Poitry-Yamate et al, 2009) and maintains a constant glucose concentration gradient from blood to brain; (approximately 5–1 during euglycemia) (Lund-Andersen, 1979). Thus factors that compromise the integrity of the BBB are likely to have a significant impact on glucose concentrations within the brain. Endothelial dysfunction is a commonly observed in those with hyperglycemia and insulin resistance (Cohen, 1993; Johnstone et al, 1993; Caballero 2012) and reduced endothelial vasodilatation may decrease the number of GLUT 1 transporters in contact with the blood. Convit (2005) proposed that endothelial

dysfunction may lead to a state of 'functional hypoglycaemia' within the brain; that is region specific low levels of glucose develop during times of increased activation. It is interesting that localized drops in brain interstitial glucose during activation have been reported to be greater, and to last longer, in aged rather than young animals (McNay and Gold, 2000) and that exposure to hypoglycaemic episodes modified the effects of aging on cognitive decline (McNay, 2005).

These observations suggest possible mechanisms that may underlie the present finding that older adults were more susceptible to the deleterious effects of impaired glucose tolerance. If similar mechanisms as those found in rats occur in humans, periods of low levels of blood glucose may stimulate adaptations that compensate for the impaired glucose transport across the blood brain barrier (BBB) seen in people with poor GT. In this way 'functional hypoglycaemia' could be alleviated leading to improved memory and reduced cognitive decline.

It is also interesting that the tendency for blood to fall below baseline values had few effects in the absence of poor GT. It is possible that the beneficial effects associated with LBG are more easily demonstrated in those with poorer GT because performance had already deteriorated; a reflection of lower levels of brain glucose. Consistent with this analysis the SNS and neuroglycopaenic symptoms were related to GT rather than LBG. It may be hypothesised that if LBG leads to an adaptive response that increases cerebral interstitial glucose, then those with a tendency for LBG would have fewer neuroglycopaenic symptoms than those without this tendency, a phenomenon only observed in those with poorer GT.

The question arises as to why the blood glucose levels of some with poor GT remained high while in others there was a sharp decline after 120 and 150 minutes (Figure 8): it was this fall in blood glucose that was beneficial (Figures 10-13). It is probable that this effect reflected increased late insulin secretion in those with the tendency for blood glucose to fall below baseline levels. Glucose induces insulin secretion in a biphasic pattern; the first phase is a rapid release that lasts only a few minutes followed by a much more steady sustained secretion (second phase; Seino et al, 2011). The usual response to insulin resistance is compensatory hyperinsulinaemia (Polonsky, 2000), therefore hyperglycaemia and glucose intolerance do not occur until insulin secretion is impaired (Seino et al, 2011). The first phase of insulin secretion is attenuated in those with poor GT, while the second phase is only usually reduced when diabetes ensues (Luzi et al, 1989; Seino et al, 2011; Triplitt, 2012; Weir et al, 2004). Thus it is possible that the adults with poorer GT whose blood glucose values subsequently fell may have developed hyperinsulinaemia to compensate for insulin resistance. In contrast those with poorer GT whose values did not subsequently fall rapidly, appeared not to have developed hyperinsulinaemia and therefore lacked the ability to compensate for poor GT. This consideration of the role played by insulin in controlling blood glucose in those with poor GT raises the question as to whether it may more directly play a part in improving the cognitive function of those with poorer GT whose levels fell below baseline values. Although the current consensus is that glucose transport into the brain is not dependent on insulin, the hormone does cross the BBB (Banks et al 2012) and is thought to modulate memory (McNay, 2010; Benedict et al, 2004; Zhao et al, 2004). As such

differential rates of insulin secretion could be part of the mechanism underlying the association between falling blood glucose and enhanced cognitive functioning.

Poorer GT was also related to higher levels of anxiety, regardless of age. People with glucose intolerance show hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis (Anagnostis et al, 2009) and increased tissue sensitivity to cortisol (Andrews et al, 2002). Cortisol profiles that deviate from the 'normal' pattern of release have been associated with higher levels of stress and anxiety (Vedhara et al, 2003); responses that potentially underlie the association between have poor GT and being more anxious. In addition, at least in those over the age of 61, poorer GT was associated with more SNS symptoms. Future studies in this area would thus benefit from monitoring the cortisol response.

In conclusion, this chapter sought to determine the effect of poor glucose tolerance and low blood glucose levels on cognitive decline, memory and mood in a sample of older adults. Findings include that older rather than younger adults with poor GT had poorer cognitive performance. In addition, the tendency to develop LBG was associated with better cognitive performance and mood in those with poorer glucose tolerance. This observation was obtained in a sample without diabetes and extends to an earlier stage than previous observations that diabetes increases the chances of developing dementia (Cheng et al, 2012). As those differing in their GT have previously been found to respond differentially to meals differing in their glycaemic load (Nabb and Benton, 2006a,b) the question arises as to whether cognitive decline in older adults may be influenced by dietary interventions. This question is addressed in the next chapter.

CHAPTER 4

Modulating the glycaemic load of meals affects older adults' cognition and mood depending on their glucose tolerance.

4.1 Introduction

In chapter 3 it was reported that an inability to control the level of postprandial blood glucose is related to cognitive decline, particularly in adults over the age of 60; those with glucose intolerance have poorer cognitive performance and mood. In addition, a tendency to develop LBG was related to having a better mood, more accurate working memory and less cognitive decline (Figures 11 to 13), however, it is not clear whether such benefits are maintained during subsequent LBG. Acute LBG, as sometimes occurs following a high GL meal (Figure 1), is associated with temporary decrements in performance and mood (Taylor and Rachman, 1988); a lower GL meal may avoid this consequence. However, how previous LBG interacts with the GL of a meal has not been examined. The present study compared the influence of three breakfasts that differed in the speed with which they release glucose into the blood stream on the cognitive functioning and mood of older adults. In addition, the interaction between manipulating the GL of meals and pre-existing LBG was examined.

The evidence supporting benefits to older adult's cognition of consuming a low GL meal is limited and inconsistent (Gilsenan et al. 2009). As discussed in chapter 1 one possibility is that the response to a meal depends on pre-existing individual differences in glucose tolerance (GT). Glucose tolerance becomes poorer with age and it has been argued that meals with a lower rather than a higher GL may "reduce the cognitive differences between better and worse glucoregulators"

(Lamport et al, 2009. p. 408). Thus the hypothesis presently tested was that those with poorer rather than better GT would differentially respond to meals differing in GL.

An important consideration is the time scale over which effects were examined, a dimension that has varied considerably (Table 1, 2 and 5). Although there may or may not be a transient benefit from consuming a high GL within the first hour, high GL meals may generate a hypoglycaemic reaction two to three hours later that disrupts cognitive functioning. Alternatively, a lower GL meal that releases glucose more slowly may not confer an immediate benefit but may prevent a decline later on. The finding that a low GL meal did not benefit the memory of children up to sixty minutes after consumption, but it did particularly after three hours (Chapter 2), illustrates the need to examine the prolonged response to a meal. This study therefore examined the cognitive effects of meals differing in GL up to one hundred and eighty minutes post consumption.

Although there is a relationship between poor glucose regulation and cognitive decline, particularly in adults over the age of sixty (Chapter 1 and 3), the mechanisms mediating this relationship remain to be determined. Interestingly the disruption of cerebral glucose metabolism that is observed in dementia also occurs in at risk individuals before cognitive symptoms develop (Mosconi et al, 2007). Therefore, any factor that compromises neuronal glucose supply has the potential to exacerbate the problem. Conversely any factor that increases the supply of glucose to the brain or its utilisation could aid cognitive performance. Thus this study also tested whether a lower rather than a higher GL meal, that provides a

consistent supply of energy, may benefit cognition in older adults with early cognitive decline, especially during the late postprandial period.

4.2 Methods

4.2.1 Participants

As described in section 3.2.1.

4.2.2 Procedure - Day 1

As described in section 3.2.2

4.2.3 Procedure - Day 2

On a second testing day, a minimum of three days later, after again fasting overnight, participants completed a baseline mood measure and randomly consumed one of the three test meals (0830-0900) and took the test battery on three occasions 0930-1000; 1045-1130; 1215-1245 (Table 15). Different but comparable versions of each test were used at each session. Participants were previously exposed to the tests (different versions where appropriate), on an earlier day, and thus had an opportunity to practise. These data were analysed separately (see chapter 3) and were not entered as covariates due to their statistical relationship with glucoregulatory group (Miller and Chapman, 2001) (see section 3.4). They consumed the entire breakfast and did not consume any other food throughout the morning, although water was consumed if requested. Each test

battery included the tests in the same order: mood, immediate recall of words, serial sevens, reaction times, sustained attention, delayed recall of word list, mood.

08:30-09:00 (0 min).	Moods measured / eat breakfast.
09:30-10:00 (+30min).	1st testing session. <ul style="list-style-type: none"> • Mood – 2 minutes. • Immediate recall of word list – 5 minutes. • Serial sevens - 3 minutes. • Reaction times / sustained attention – 15 minutes. • Delayed recall of word list – 2 minutes. • Mood – 2 minutes.
10:45-11.15 (+105 min).	2nd testing session (as above).
12:15-12:45 (+195 min).	3rd testing session (as above plus verbal fluency).

Table 15. Procedural timeline for the study of GL and cognition on older adults.

4.2.4 Breakfast

Each meal consisted of two small slices of whole meal toast (60 kcal) topped with reduced sugar jam, 100g of plain low fat yogurt that was sweetened with either 30g of glucose, sugar or isomaltulose. Participants also received an orange flavoured drink that was sweetened with 25g of one of the three sugars; Sucrose, Glucose or Isomaltulose. Table 16 presents the nutritional composition of the three test meals that were identical in terms of macro-nutrients but produced a GL of 24.3, 34.9 or 45.4.

	Higher GL							Medium GL							Lower GL						
	Kcal	CHO	Pro	Fat	GI	GL		Kcal	CHO	Pro	Fat	GI	GL		Kcal	CHO	Pro	Fat	GI	GL	
2 slice wholemeal bread.	110	19.4	5.0	1.2	69	13.3		110	19.4	5.0	1.2	69	13.3		110	19.4	5.0	1.2	69	13.3	
Low carbohydrate jam * 25g	11	2.4	0.2	0.00	*	*		11	2.4	0.2	0.00	*	*		11	2.4	0.2	0.00	*	*	
Low calorie yoghurt 100g	41	6.0	4.3	0.25	19	1.14		41	6.0	4.3	0.25	19	1.14		41	6.0	4.3	0.25	19	1.14	
15g glucose.	60	15	0	0	100	15		-	-	-	-	-	-		-	-	-	-	-	-	
15g sucrose.	-	-	-	-	-	-		60	15	0	0	67	10.0		-	-	-	-	-	-	
15g isomaltulose.	-	-	-	-	-	-		-	-	-	-	-	-		60	15	0	0	32	4.8	
Drink 25g Glucose.	64	16	0	0	100	16		-	-	-	-	-	-		-	-	-	-	-	-	
Drink 25g Sucrose.	-	-	-	-	-	-		64	16	0	0	67	10.7		-	-	-	-	-	-	
Drink 25g Isomaltulose.	-	-	-	-	-	-		-	-	-	-	-	-		64	16	0	0	32	5.12	
TOTAL	275	56.4	9.3	1.45	-	45.4		275	56.4	9.3	1.45	-	34.9		275	56.4	9.3	1.45	-	24.3	

Table 16. The macro-nutrient content of the experimental meals. * The low carbohydrate jam was specially made for the study from strawberries sweetened with sucralose, an artificial sweetener that is not broken down by the body so it offered no calories. The GI of the jam is unknown so the jam is not included in the summated nutritional data. However, the GI of strawberries is 40 so the jam, that was eaten by all subjects, might be expected to have added about 1 to each total GL.

4.2.4.1 Test battery

The test battery included the following tests administered in the order; Mood, Episodic Memory - Word list recall (immediate and delayed), Working memory - Serial sevens, Vigilance - Sustained attention, Simple and Choice Reaction Times, Semantic memory - Verbal fluency (letters P, R and W, only administered during the last testing session; after 180 minutes), Mood. Participant also took the National adults reading test. These tests are described in section 3.2.3.

4.2.4.2 Mood (POMS)

Participants were asked to report how they felt "at this moment" using visual analogue scales with pairs of adjectives at the ends of 100 millimetre lines; Composed / Anxious; Hostile / Agreeable; Elated / Depressed; Unsure / Confident; Energetic / Tired; Confused / Clearheaded. Mood was measured both before and after each of the three testing sessions (appendix 10).

4.2.4.3 Glucoregulatory profile

As those with both poorer glucose tolerance (GT) are more likely to display mild cognitive decline and those with the tendency to develop lower blood glucose levels (LBG) are less likely to display mild cognitive decline (Chapter 3), the sample was distinguished depending on the results of the day one OGTT. Participants were divided into four groups according to their blood glucose levels after 120 and 150 minutes.

To allow a sufficient sample size, if their blood glucose was 7.0mmol/l or higher they were considered to have poorer GT; if their blood glucose was less than

7mmol/l they were considered to have better GT. The lowest blood glucose value (LBG) was determined and individuals were divided into two further groups, according to whether at any point during the test they either fell, or not fall, below the fasting blood glucose value. Those whose blood glucose fell below fasting values all had capillary glucose levels <5.0mmol/l by the end of the test (after 150 min). Importantly, 50% fell below 4.0mmol/l and 30% had glucose levels <3.6mmol, the level about which an adrenal discharge begins to be observed in healthy humans (Auer, 2004).

By classifying participants depending on whether they had poorer or better GT and whether the LBG did or did not fall below the baseline value, four Glucoregulatory groups were created: better GT and LBG above baseline (N=25); better GT and LBG below baseline (N=21); poorer GT and LBG above baseline (N=67); poorer GT and LBG below baseline (N=41) (Figure 10).

4.2.4.4 Calculation of cognitive decline

Older adults experiencing the early stages of cognitive impairment have marked disruption in cerebral glucose metabolism (Mosconi et al, 2007) and hence it was predicted that they might differentially react to the GL of meals. The NART scores and the memory scores during baseline testing were transformed to Z scores (a score transformed into differences from the population mean in terms of a standard deviation). The NART Z scores were then subtracted from the day one memory Z scores. A negative value indicated that the current memory was poorer than that predicted by the intelligence score. This continuous measure was then divided according to whether performance was less or more than would be predicted by their NART score. This measure of cognitive decline was then entered into the

analysis of co-variance as a between participants factor. According to this definition 53.4% of the sample had poorer memory scores than would be predicted by the NART.

4.3 Statistical analysis

The data were examined using appropriate analysis of variance designs (ANOVA) with performance on the first day of testing as the covariate. Typically they took the form of Meal (Glucose, Sucrose, Isomaltulose) X Time (Test session 1-3) X Glucoregulatory group (Poorer GT and LBG below baseline / Better GT and LBG below baseline / Poorer GT and LBG above baseline / Better GT and LBG above baseline). For clarity follow up tests are grouped according to glucoregulatory profile (Figure 10). If on occasion a glucoregulatory group is not mentioned it should be assumed that all follow up tests for that group were not significant. As an alternative the third factor was Cognitive decline (Memory below or above the level predicted by NART). When interactions were significant appropriate post hoc tests were conducted to determine the nature of the interaction.

4.4 Results – effect of meal depending on glucoregulatory profile.

4.4.1 Episodic Memory

The data were analysed using a four-way ANOVA; Meal (Glucose, Sucrose, Isomaltulose) X Short or Long term memory (ST/LT) X Time (Session 1-3) X Glucoregulatory Group. Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(2) = 2.707$, $p = .258$ (for time) and $\chi^2(2) = 1.362$, $p = .506$ (for time X ST/LT). There was a significant Meal X Time X

Glucoregulatory group interaction ($F(12,280) = 2.23, p < 0.01$; Figures 14a and 14b).

Better GT/LBG below baseline

Better GT/LBG above baseline

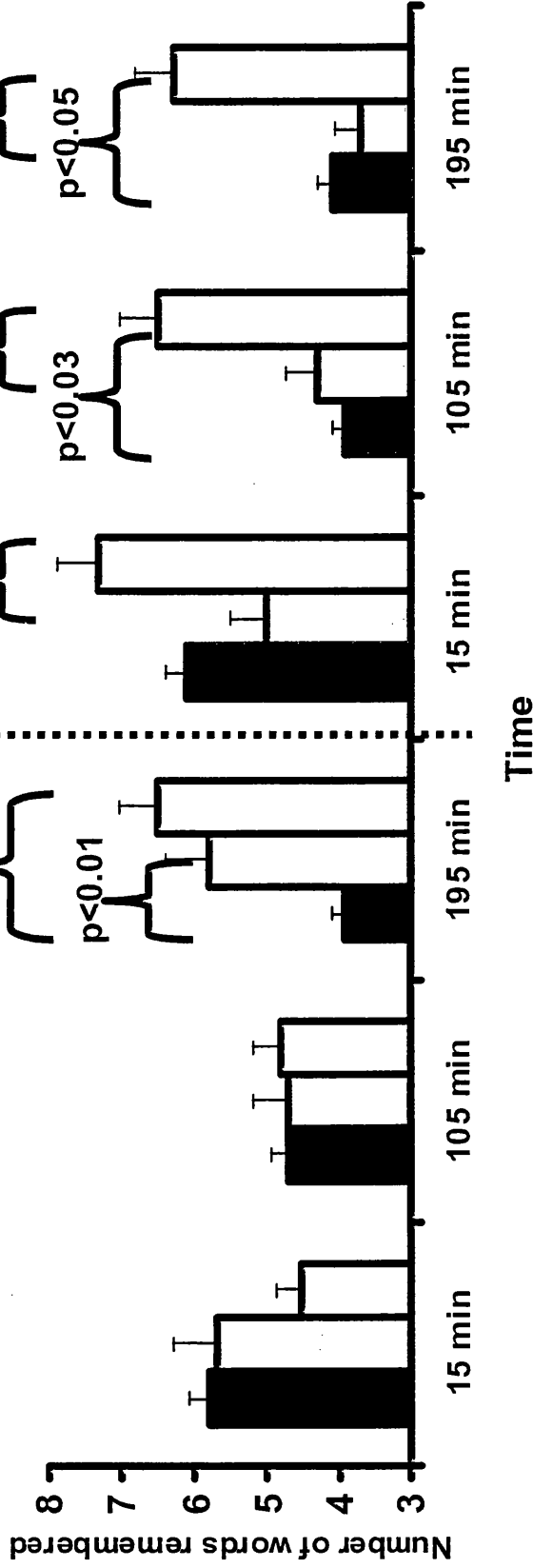
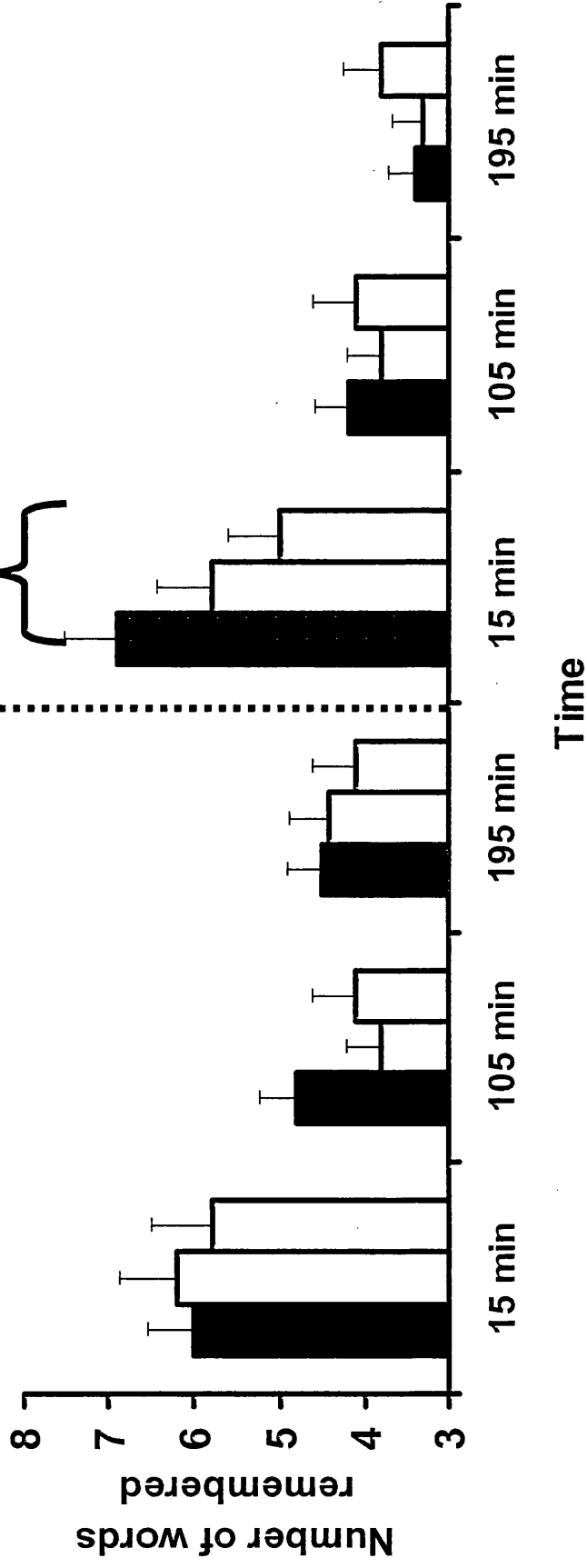


Figure 14a. The influence of type of meal on memory in those with better GT and LBG above or below baseline. Data are mean number of words remembered. Older adults with better GT and LBG above baseline remembered more words if they ate isomaltulose than if they ate glucose after 105 ($p < 0.03$) and 195 min ($p < 0.05$). Older adults with better GT LBG above baseline remembered more words if they ate isomaltulose than if they ate sucrose after 15 min ($p < 0.02$), 105 min ($p < 0.04$) and 195 min compared to those that ate sucrose ($p < 0.01$) and those that ate isomaltulose ($p < 0.004$).

Poorer GT/LBG below baseline

Poorer GT/LBG above baseline

p < 0.02



■ Glucose □ Isomaltulose

Figure 14b. The influence of type of meal on memory in those with poorer GT and LBG above or below baseline. Data are mean number of words remembered. Older adults with poorer GT and LBG above baseline had a better memory after 15min if they had glucose rather than isomaltulose. The number of words remembered by those with poorer GT and LBG below baseline did not depend on the type of meal they had eaten.

Better GT and LBG above baseline

When those with better GT and LBG above the baseline were considered those who ate isomaltulose had better memories after 30 minutes ($p < 0.02$), 105 minutes ($p < 0.04$) and 195 minutes ($p < 0.03$) compared to those that had eaten sucrose. Those who consumed isomaltulose also had better memories compared to those who ate glucose after 105 ($p < 0.03$) and 195 minutes ($p < 0.05$), but not earlier (Figure 14a).

Better GT and LBG below baseline

When those with better GT and LBG below the baseline were examined, those that ate glucose had poorer memories after 195 minutes compared to those that had eaten sucrose ($p < 0.01$) and isomaltulose ($p < 0.004$). There were, however, no effects of meal after 30 or 105 minutes (Figure 14a).

Poorer GT and LBG above baseline

With the group with poorer GT, and LBG above the baseline, the consumption of glucose rather than isomaltulose resulted in better memories after 30 minutes ($p < 0.02$), although not during the two later testing sessions. There were no differences between those who consumed sucrose and those who ate isomaltulose at any time. Similarly there were no differences in those eating glucose or sucrose (Figure 14b).

4.4.2 Semantic Memory

The analysis Meal (Glucose, Sucrose, Isomaltulose) X Glucoregulatory Group was non-significant ($F(6,140) = 1.62, n.s.$).

4.4.3 Working memory

Incorrect responses

Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(2) = 3.697$, $p = .158$ (Time). The Meal (Glucose, Sucrose, Isomaltulose) X Time (Sessions 1-3) X Glucoregulatory Group interaction was not significant ($F(12,264) = 0.624$, n.s.). However, the interaction Meal X Glucoregulatory Group approached significance ($F(6,136) = 1.98$, $p < 0.06$).

Better GT and LBG above baseline

Older adults with better GT, and LBG above the baseline, that ate glucose rather than isomaltulose made more errors after 195 minutes ($p < 0.05$). There was a similar pattern of results after 105 minutes (6.2(7.1) vs 1.8(0.7)) and after 30 minutes (6.7(5.1) vs 2.5(2.1)), however, these effects just missed statistical significance.

Reaction times

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(2) = 52.725$, $p < .001$ (time) therefore epsilon was estimated and the degrees of freedom were adjusted using the Greenhouse-Geisser correction. The analysis Meal (Glucose, Sucrose, Isomaltulose) X Time (Sessions 1-3) X Glucoregulatory Group was not significant ($F(7.829,177.457) = 1.10$, ns).

4.4.4 Mood

Mood was measured upon arrival, prior to consumption of breakfast (baseline). Initially baseline values (for total mood) were checked to ensure no significant

differences at baseline; The interaction Meal X Glucoregulatory group was not significant ($F(6,140) = 1.025$ ns) and neither were the main effects of Meal ($F(2,140)=1.603$, ns) and Glucoregulatory group ($F(3,140)=0.939$, ns) (Table 17). Mood was also measured before and after each of the three testing sessions. To determine the effect of the type of meal on mood, change from baseline scores were calculated by subtracting the mood ratings at baseline from subsequent scores. A positive score indicated an improvement in mood and a negative score indicated a decline in mood from baseline. These scores were then entered in the analysis Meal (Glucose, Sucrose, Isomaltulose) X Time (Session 1-3) X Glucoregulatory Group X Before/after testing. Where Mauchly's Test of Sphericity was significant Greenhouse-Geisser correction was reported.

		Agreeable/ Hostile	Clearheaded/ Confused	Composed/ Anxious	Elated/ Depressed	Confident/ Unsure	Energetic/ Tired
Better GT, LBG	GLU	58.0(7.6)	73.1(8.3)	75.1(8.8)	55.8(7.7)	71.3(8.6)	53.6(9.9)
	SUC	78.4(8.3)	77.4(9.0)	75.0(9.6)	60.6(8.5)	66.0(9.4)	60.8(10.9)
	ISO	88.8(7.6)	81.8(8.3)	87.1(8.8)	73.5(7.7)	85.1(8.6)	70.5(9.9)
Poorer GT, LBG	GLU	78.3(4.9)	75.2(5.4)	74.5(5.7)	62.7(5.1)	72.5(5.6)	66.0(6.5)
	SUC	76.9(5.4)	70.1(4.9)	69.7(5.2)	57.7(4.6)	68.2(5.1)	51.2(5.9)
	ISO	87.5(5.3)	75.3(5.8)	83.5(6.2)	66.5(5.5)	81.2(6.1)	65.0(7.0)
Poorer GT, No LBG	GLU	74.2(3.9)	70.6(4.3)	67.7(4.5)	60.4(4.0)	72.1(4.5)	56.5(5.2)
	SUC	83.0(4.2)	77.8(4.6)	71.8(4.9)	61.2(4.3)	70.4(4.8)	56.9(5.5)
	ISO	80.9(3.6)	70.6(3.9)	77.4(4.3)	58.5(3.7)	72.1(4.1)	60.1(4.7)
Better GT, No LBG	GLU	77.0(6.2)	76.2(6.7)	73.7(7.1)	57.5(6.3)	72.8(7.0)	62.0(8.1)
	SUC	86.2(6.2)	74.0(6.7)	72.0(7.1)	59.2(6.3)	66.4(7.0)	53.3(8.1)
	ISO	73.4(7.0)	63.7(7.6)	75.0(8.1)	56.7(7.2)	71.1(8.0)	56.7(9.2)

Table 17 Baseline mood for the four glucoregulatory groups prior to consuming one of the three test meals. Data are mean (standard error) for the six VAS mood scales taken at baseline. GT – Glucose Tolerance. LBG – Lowest Blood Glucose. GLU – Glucose based meal. SUC – Sucrose based meal. ISO- Isomaltulose based meal.

Initially the six mood dimensions were considered independently, resulting in one main effect of the influence of the type of sugar. With the Agreeable – Hostile dimension there was a main effect of Meal ($F(2,140) = 2.88$, $p < 0.05$). Those who consumed isomaltulose (Isomaltulose) were more agreeable than those who

consumed glucose ($p < 0.03$). In addition, sucrose rather than glucose consumption increased ratings of being agreeable ($p < 0.03$). There were no significant differences between those who had eaten sucrose or isomaltulose (Figure 15).

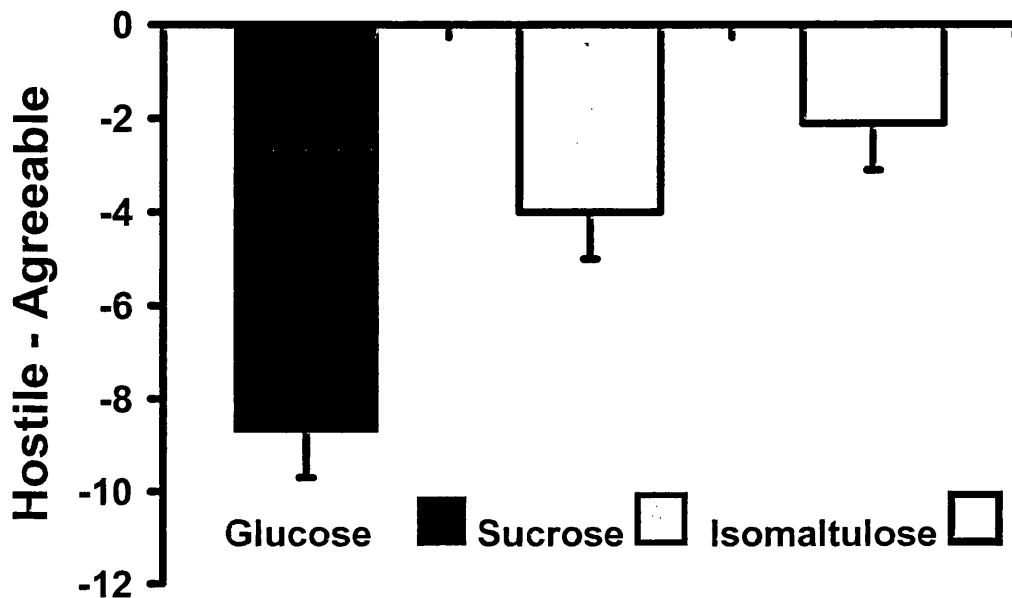


Figure 15. The effect of Meal on agreeableness. Data are mean (standard error) for decline in VAS ratings of agreeableness across the morning. Older adults that consumed isomaltulose were more agreeable (less decline) than those that consumed glucose ($p < 0.03$). In addition older adults that ate sucrose were more agreeable than those that consumed glucose ($p < 0.03$).

There were also a series of significant interactions, for example, with Clearheaded – Confused ($F(11.4,266.3) = 2.33, p<0.009$) and Elated – depressed ($F(11.4,266.2)=2.65, p<0.05$) the Meal X Glucoregulatory group X Time interaction reached statistical significance. The effects of the type of sugar were, however, similar with the different mood dimensions. Rather than generating considerable complexity by reporting a series of three way interactions for the six mood dimensions, for brevity and clarity all six were added to produce an overall mood score that illustrates the effects that were observed (see appendix 11) for the interactions for each individual mood scales). The interaction Meal X Glucoregulatory group X Time was statistically significant ($F(10.8, 254.2)=2.177 p<0.02$) (Figure 16a and 16b) and is representative of the types of effects found with specific mood dimensions. Post hoc tests found a series of differences in the response to meal.

Better GT and LBG above baseline

A profile of better GT with no LBG was associated with differential responses to the type of meal. After both 105 ($p<0.003$) and 195 minutes ($p<0.001$) having consumed sucrose was associated with a better mood than after a glucose containing meal. The consumption of isomaltulose, rather than sucrose, resulted after 105 minutes in a better mood ($p<0.01$). Similarly, after 195 minutes the isomaltulose rather than glucose meal resulted in a more positive mood ($p<0.03$).

Poorer GT and LBG below baseline

Those with the poorer GT and LBG profile again responded to the nature of sugar consumed. After 105 minutes having eaten the glucose containing meal resulted

in a poorer mood than when sucrose ($p < 0.04$) was eaten with a trend for difference between glucose and isomaltulose ($p < 0.07$).

Poorer GT and LBG above baseline

When those with poorer GT and LBG above baseline were considered those that ate glucose rather than isomaltulose ($p < 0.05$) reported better moods after 30 minutes. There was a similar trend for those who ate glucose rather than sucrose ($p < 0.07$) to report better moods after 30 minutes but this just missed significance.

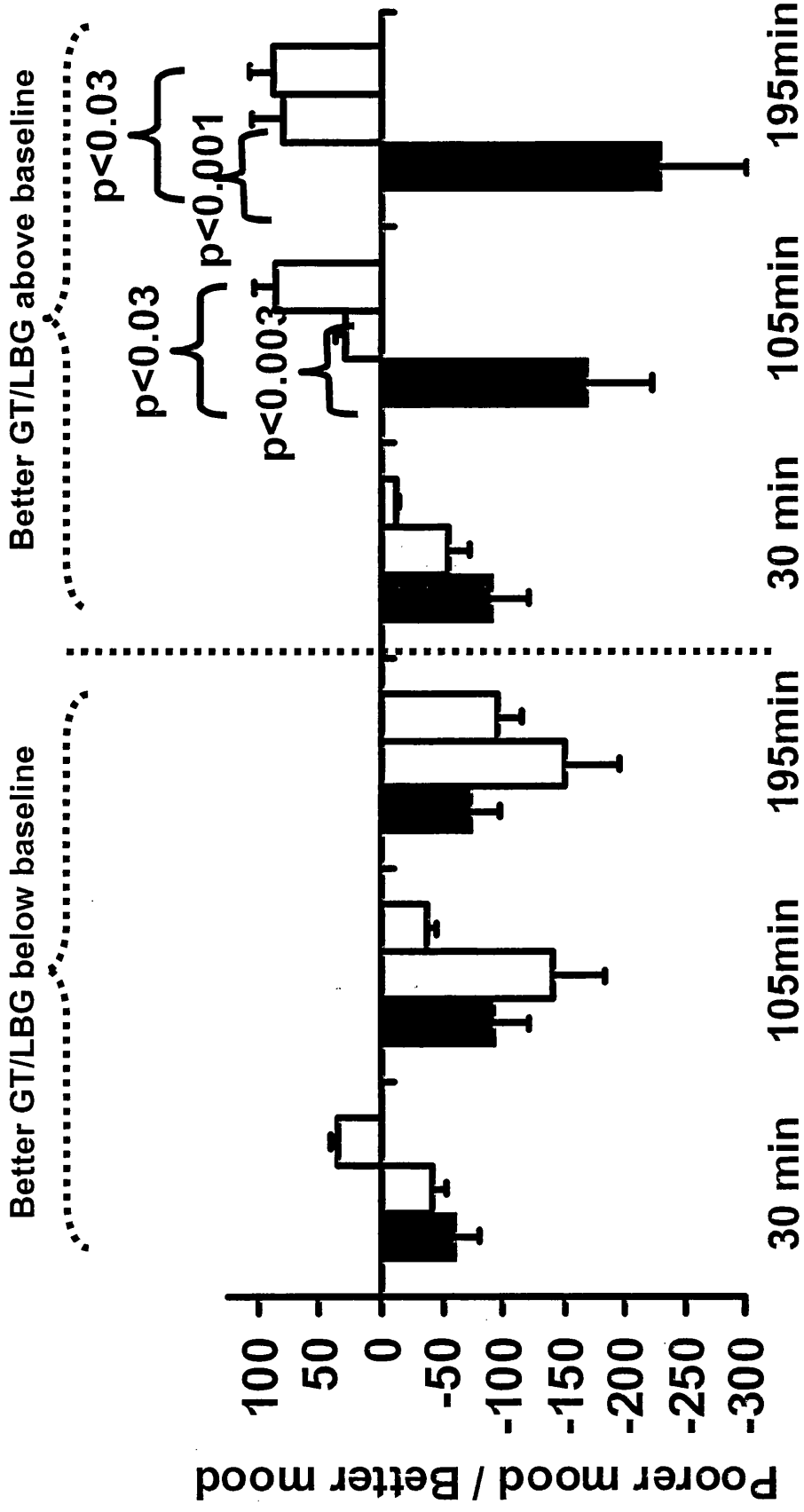


Figure 16a. The influence of type of meal on mood in those with better GT and LBG above or below baseline. Data are mean change in mood from baseline. Older adults with better GT and LBG above baseline had better moods if they ate isomaltulose than if they ate glucose after 105 (p<0.03) and 195 min (p<0.03). Older adults with better GT LBG above baseline had better moods if they ate isomaltulose than if they ate sucrose after 105 min (p<0.003) and 195 min (p<0.001). The nature of the meal consumed did not affect the mood of older adults with better GT LBG below baseline.

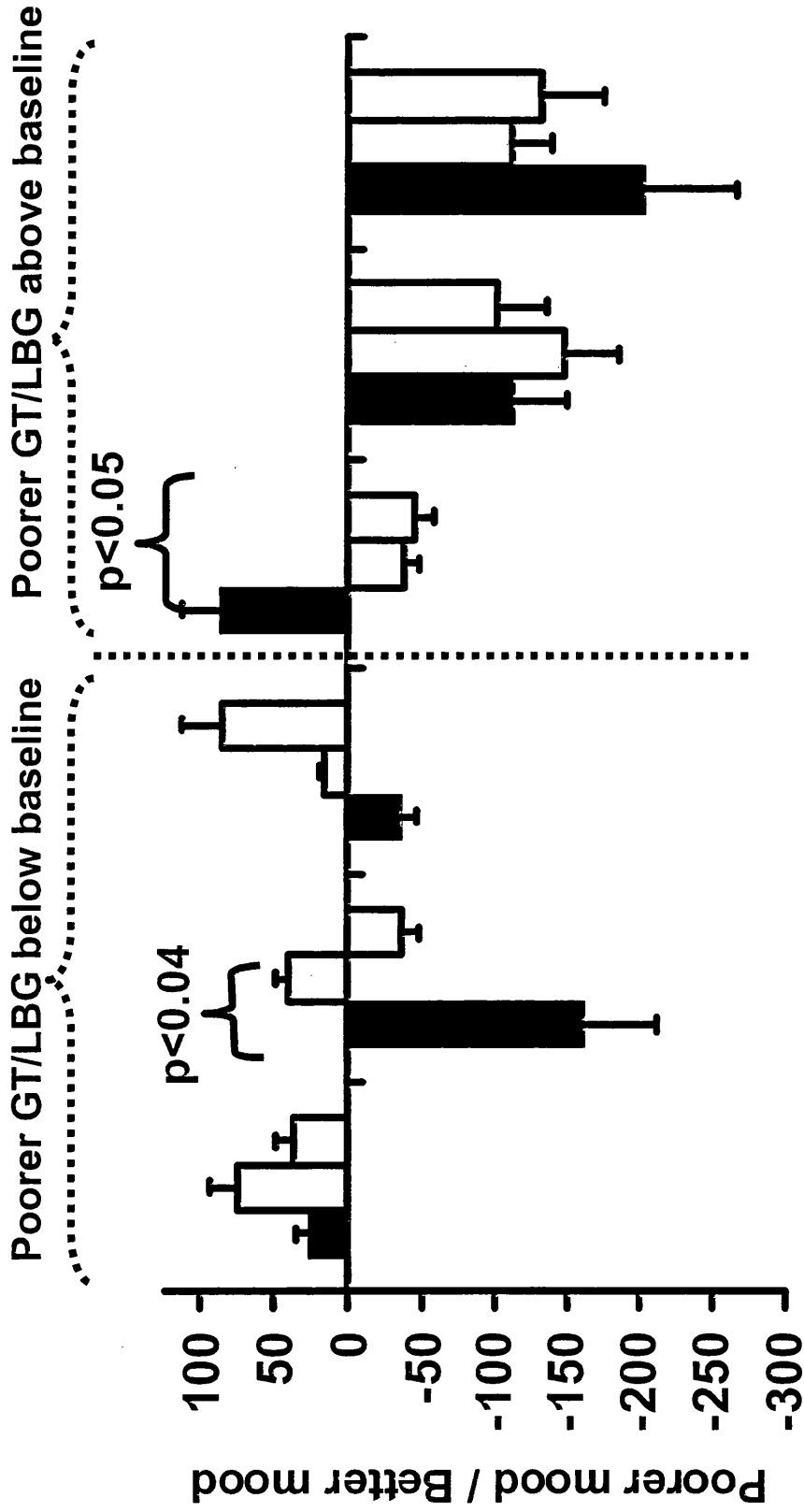


Figure 16b. The influence of type of meal on mood in those with poorer GT and LBG above or below baseline. Data are mean change in mood from baseline. Older adults with poorer GT and LBG above baseline that ate glucose rather than isomaltulose ($p < 0.05$) reported better moods. After 105 minutes having eaten the glucose containing meal resulted in a poorer mood than when sucrose ($p < 0.04$) for older adults with poorer GT and LBG below baseline.

4.4.5 Vigilance

Reaction times

Mauchly's Test of Sphericity for the factor min (1st, 2nd, 3rd, 4th, and 5th minute of the test) indicated that the assumption of sphericity had not been violated, $\chi^2(2) = 0.540$, p ns (Time), $\chi^2(9) = 8.164$, p ns (Min), $\chi^2(35) = 36.045$, p ns (Time X Min). The interaction Meal (Glucose, Sucrose, Isomaltulose) X Time (Sessions 1-3) X Glucoregulatory Group X Min (1st, 2nd, 3rd, 4th, and 5th minute of the test) was not significant ($F(48,1016)=0.85$, n.s.). The interaction Time X Minute X Group reached significance ($F(24,1016)=1.95$, $p<0.004$), however, follow up testing showed no clear pattern and given the number of contrasts this effect was considered to be the result of chance variation in a particular cell.

Accuracy

Mauchly's Test of Sphericity for the factor min (1st, 2nd, 3rd, 4th, and 5th minute of the test) indicated that the assumption of sphericity had been violated, $\chi^2(9) = 33.918$, $p < .0001$ therefore epsilon was estimated and the degrees of freedom were adjusted, for interactions involving min, using the Greenhouse-Geisser correction. Mauchly's Test of Sphericity for the factor Time (Session 1-3) indicated that the assumption of sphericity had not been violated, $\chi^2(2) = 3.125$, p ns. Similarly, Mauchly's Test of Sphericity for the interaction Time X Min was not significant, $\chi^2(35) = 40.366$, p ns. When the accuracy during the vigilance task was considered again the meal was without effect (Meal X Time (Sessions 1-3) X Glucoregulatory Group X Minute, ($F(7.4, 1003.6)=1.06$, n.s.).

4.4.6 Reaction times

Decision times

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(20) < 79.203$, $p < .0001$ (time X lamps) and $\chi^2(5) < 74.977$, $p < .0001$ therefore epsilon was estimated and the degrees of freedom were adjusted using the Greenhouse-Geisser correction. The interaction Meal (Glucose, Sucrose, Isomaltulose) X Time (Sessions 1-3) X Glucoregulatory Group X Number of lamps (1,2,4,8) was not significant ($F(30.037,670.817) = 0.70$, n.s.). The interaction Meal X Time reached significance ($F(3.747,5.620) = 2.71$, $p < 0.03$), however, post hoc tests failed to find any significant differences between meals at any time.

Movement times

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(20) = 58.802$, $p < .0001$ (time X lamps) and $\chi^2(5) = 71.013$, $p < .0001$ therefore epsilon was estimated and the degrees of freedom were adjusted using the Greenhouse-Geisser correction. The interaction Meal (Glucose, Sucrose, Isomaltulose) X Time (Sessions 1-3) X Glucoregulatory Group X Number of lamps (1,2,4,8) was not significant ($F(31.288,698.771) = 1.02$, n.s.).

4.5 Results – The effect of meal depending on cognitive decline.

4.5.1 Episodic memory

Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(2) = 4.775$, $p = .092$ (Time) and $\chi^2(2) = 0.979$, $p = 0.613$ (time X ST/LT). The interaction Meal (Glucose, Sucrose, Isomaltulose) X Short or Long term memory (ST/LT) X Time (Sessions 1-3) X Cognitive decline (yes/no) was not significant ($F(4,286)=0.66$, n.s.). However, there was a main effect of Cognitive decline ($F(1,143) = 8.79$, $p<0.004$); older adults with cognitive decline remembered fewer words than those with no cognitive decline (4.4 (0.1) vs 5.2 (0.1)).

4.5.2 Semantic memory

The analysis Meal (Glucose, Sucrose, Isomaltulose) X Time (Session 1-3) X Cognitive decline (yes/no) X Difficulty (three letters of increasing difficulty) was not significant ($F(4,286) = 0.22$, n.s.).

4.5.3 Working memory

Reaction times

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(2) = 58.504$, $p < .0001$ (time) therefore epsilon was estimated and the degrees of freedom were adjusted using the Greenhouse-Geisser correction. The analysis Meal (Glucose, Sucrose, Isomaltulose) X Time (Session 1-3) X Cognitive decline (yes/no) was not significant ($F(2.5,179.9) = 1.017$, n.s.).

Accuracy

Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(2) = 3.288$, $p = .267$ (Time). The analysis Meal (Glucose, Sucrose, Isomaltulose) X Time (Session 1-3) X Cognitive decline (yes/no) was not significant ($F(4,278) = 2.17$, n.s.). There was, however, a significant Time X Cognitive decline interaction ($F(2,278) = 5.08$, $p < 0.007$). Those with cognitive decline had a larger increase in errors across the morning (-0.2(3.7) vs -1.7 (4.0)). To determine whether the nature of the meal effected decline in performance, in those with and without cognitive decline, a two way ANOVA was conducted, Meal X Cognitive decline, with changes in the number of errors produced from 30 to 180 minutes as the dependent variable. This interaction was significant; ($F(2,144) = 3.01$, $p < 0.05$). Inspection of the means found a trend for those without cognitive decline to have a smaller increase in errors if they had a lower rather than a higher GL meal, although no comparisons achieved statistical significance.

4.5.4 Mood

Mauchly's Test of Sphericity indicated that the assumption of sphericity, for the factor Time, had been violated, $\chi^2(2) = 12.789$, $p < 0.0001$. When the overall mood score was examined the interaction Meal X Glucoregulatory group X Before/after testing X Cognitive decline was non-significant ($F(3.9,278.8) = 0.76$, n.s.).

4.5.5 Vigilance

Reaction times

The interaction Meal (Glucose, Sucrose, Isomaltulose) X Minute (1-5 minute of test) X Time (Session 1-3) X Cognitive decline (yes/no) was not significant ($F(14.9,976.4)=0.77$, n.s.).

Accuracy

Mauchly's Test of Sphericity indicated that the assumption of sphericity, for the interaction Time X Min, had been violated, $\chi^2(35) = 55.679$, $p < 0.01$. The interaction Meal (Glucose, Sucrose, Isomaltulose) X Minute (1-5 minute of test) X Time (Session 1-3) X Cognitive decline (yes/no) was not significant ($F(14.6,958.2)=1.00$, n.s.).

4.5.6 Reaction times

Decision times

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(20) = 61.635$, $p < .0001$ (time X lamps) and $\chi^2(5) < 74.319$, $p = .0001$ therefore epsilon was estimated and the degrees of freedom were adjusted using the Greenhouse-Geisser correction. The interaction Meal (Glucose, Sucrose, Isomaltulose) X Lamps (1, 2, 4, or 8 lamps) X Time (Session 1-3) X Cognitive decline (yes/no) was not significant ($F(10.4,712.4)=1.91$, n.s.).

Movement times

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(20) = 77.442$, $p < .0001$ (time X lamps) and $\chi^2(5) < 79.551$, $p = .0001$

therefore epsilon was estimated and the degrees of freedom were adjusted using the Greenhouse-Geisser correction. The interaction Meal (Glucose, Sucrose, Isomaltulose) X Lamps (1, 2, 4, or 8 lamps) X Time (Session 1-3) X Cognitive decline (yes/no) was not significant ($F(10.0,688.5)=0.105$, n.s.).

4.6 Summary

- The influence of meals differing in GL depended on pre-existing individual differences in glucose tolerance.
- Although it has been assumed previously that it was those with poor glucose tolerance that would benefit from a low GL meal, it was those with better glucose tolerance who responded to a meal containing isomaltulose with better mood and memory.
- It has been suggested that a tendency to develop low levels of blood glucose will disrupt neural functioning, a phenomenon that can be reduced by consuming low GL meals. However, no evidence of this mechanism was found as there was no interaction between the nature of the meal and a pre-existing tendency for blood glucose to fall to low values.
- There was no evidence that those with the early signs of cognitive decline differentially responded to meals that varied in GL.

4.7 Discussion

The major finding of this paper is that on a number of occasions individual glucoregulatory status interacted with the type of meal that was consumed (Figures 14 and 16). Interestingly, older adults with better GT and no tendency for blood glucose to fall below baseline value, which is those who were best able to manage

their blood glucose levels, benefited most from consuming a lower rather than a higher GL meal. In particular, those that ate the isomaltulose based meal performed mental arithmetic more accurately, were able to remember more words and had a better mood. These effects are consistent with the findings in chapter 1 and chapter 2 that suggested that a lower rather than a higher GL meal may improve cognitive performance and mood in children, adolescents and young adults. Given their young age, it is reasonable to assume that such populations would have relatively better GT compared to older adults. It may be the case that the inconsistencies in the literature dealing with older adults (summarised in chapter 1) reflects a failure to establish their prior ability to regulate blood glucose levels.

From this perspective it may not be surprising that the literature addressing the effects of GL on mental performance in older adults, with or without poor GT, is limited and the findings inconsistent. On one hand Papanikolaou et al (2006) reported in older diabetics that a lower GL (50g pasta), rather than a higher GL meal (50g white bread) improved cognition sixty to one hundred and forty minutes after consumption. However, it was unclear whether poor glycaemic state interacted with the nature of the meals as there was no healthy control group. Also the majority of participants were taking oral hypoglycaemic medication (metformin or sulphonylureas) that are known to influence insulin secretion, insulin resistance and gluconeogenesis such that medication may interact with the GL of a meal. Similarly, Nabb and Benton (2006) found that meals that were low in carbohydrates and high in fibre resulted in better memory thirty and one hundred and five minutes after consumption; an effect that was only observed among those

with higher fasting glucose levels. However, when interpreting these findings one must consider that although impaired fasting glucose and impaired glucose tolerance are correlated they are not synonymous and represent different underlying pathologies. On the other hand, Lamport et al (2012) reported that neither diabetics, nor older adults with better GT, benefited from a low (toast and yogurt) rather than high GL meal (glucose drink) after thirty or one hundred and twenty minutes. However, given the small number of control participants (n = 10) this study may have lacked the power to detect differences between meals.

Although in the present study a low GL meal did not generally influence cognition or mood in older adults with poorer GT, it is interesting that on a number of occasions they selectively benefitted from a higher GL meal during the immediate postprandial period. Specifically, those with poorer GT had better memory and more positive moods thirty minutes after eating if they had consumed glucose, rather than sucrose or isomaltulose based meals. Similar short term effects have been reported previously. Table 18 shows the studies investigating the short term effects of glucose on cognition in older adults with better and poorer glucose tolerance.

Reference.	N (average age).	Glucoregulatory Status.	Effect of glucose on episodic memory.
Messier et al. (2003).	11M 46F (72 years)	Better	ns
		Poorer	+
Kaplan (2000)	10M 10F (72 years)	Better	ns
		Poorer	+
Messier et al. (2010)	9 M, 6 F (70 years)	Better	ns
		Poorer	+
Messier et al. (1997)	19 M 74F (62 years)	Better	+ (males)
		Poorer	- (males)
Riby et al. (2008)	33 M 34 F (45 years)	Better	+
		Poorer	ns
Craft et al. (1994)	27 M Younger (23.5 years) 32 M Older (67.5 years)	Better	+ (older) - (younger)
		Poorer	+ (younger) ns (older)

Table 18. Studies investigating the short term effects of glucose on cognition in older adults with better and poorer glucose tolerance. +, significant beneficial effect. ns, not significant. -, significant negative effect.

Messier et al. (2003) found that drinking 50g of glucose, rather than a placebo, attenuated the deficits observed in older adults with poor GT, and reduced the difference between better and poorer gluco-regulators in memory in the period up to forty-five minutes after consumption. In a subsequent study Messier et al. (2010) confirmed these findings; 50g of glucose reduced the association between glucose regulation and cognition by improving performance in those with the poorest GT. Similarly Kaplan et al (2000) gave older adults meals that provided 50g of carbohydrate that differed in the speed of absorption (glucose drink, potato and barley). Although those with poorer glucoregulation had greater improvements in memory after all three meals (tested up to one hundred and five minutes) the nature of the carbohydrate was not important. However, these effects have not always been confirmed; Riby et al (2008) found that 50g, but not 25g, improved performance of better but not poorer glucoregulators. Interestingly, a study by Craft et al (2004) found that drinking 50g of glucose, rather than a placebo,

improved memory in older men with better but not poorer GT. However, glucose also improved the memory of younger men with poorer GT but in contrast decreased the performance of younger men with better GT. It is interesting that older men with better GT were comparable to younger men with poorer GT, in terms of GT, thus there could be an optimal range of GT within which glucose facilitates memory. However, in this range of studies there was substantial variation in the definition used to classify those having better or poorer GT, such that a range of mechanisms may be influential that could differentially interact with a meal.

Although the mechanisms mediating the effect of GT on cognitive decline are yet to be elucidated Convit (2005) proposed that individuals with glucose intolerance have poorer cognitive performance because they have endothelial dysfunction and are less able to transport glucose across the BBB into the brain. It is interesting that our sample of older adults with poorer GT benefitted in the short-term from a higher rather than a lower GL. It is possible that those with impaired GT may require higher blood glucose and insulin responses to drive glucose utilization by the brain. Although the transport of glucose into the brain is not thought to be insulin dependent, insulin may act as a vasodilator (Muniyappa and Sowers, 2013) and thus increased insulin (as a result of a glucose load) may improve endothelial function and indirectly aid transport of glucose across the BBB. Consistent with this, Hirvonen et al. (2011) found that a higher concentration of insulin was required to stimulate cerebral glucose metabolism in those with poorer GT compared to healthy controls. Clearly, more research is required.

A further finding from the present study was that the tendency to develop lower levels of blood glucose did not moderate the effect of glycaemic load on cognitive performance. If anything the tendency to develop a LBG below baseline values seemed to reduce the benefits observed in those with better GT. For example, those with better GT, and LBG above baseline values, had better memory throughout the entire morning after eating isomaltulose. However, those with better GT and the tendency for a lower LBG only benefitted from a lower GL during the late postprandial period (180 minutes) (Figure 16a).

In chapter 3 it was reported that a tendency to develop low blood glucose levels benefitted those with poor glucose tolerance. Similarly, it is well documented that some insulin treated diabetics, after re-current hypoglycaemic episodes, develop 'hypoglycemia unawareness' (Bakatselos, 2011). This not only involves a reduction in sympathetic nervous system symptoms (Cryer, 2005) but also a reduction in cognitive dysfunction during subsequent hypoglycaemic episodes (Mellman et al, 1994; Fruenwald-Schultes et al, 2000). The mechanisms involved in this process are not yet determined, however, there is evidence that brain adaptation can result from exposure to hypoglycaemia. For example, in animals that previously had experienced hypoglycaemia hippocampal glucose concentrations were subsequently higher under normal glycaemic conditions (McNay et al. 2006). In healthy humans' experimental exposure to recurrent hypoglycaemia resulted in the preservation of cognitive performance and the uptake of glucose by the brain when on later occasion's low glucose levels were again experienced (Boyle et al., 1994). It is possible that the older adults in our sample that had a tendency for LBG to fall below baseline did not benefit from the

lower GL meal and sustained supply of glucose from the periphery because they had already developed compensatory mechanisms (i.e. increased brain interstitial glucose levels) to deal with a transient shortage in peripheral energy supply.

Chronic hyperglycaemia, that is characteristic of glucose intolerance, is thought to contribute to cognitive decline (Ravona-Springer et al, 2012), however, to our knowledge this is the first study to consider the acute effect of the GL of a meal on the cognitive performance of older adults with cognitive decline. Small et al (2006) found improvements in memory and cerebral metabolism after a fourteen day healthy lifestyle program that incorporated a low GL diet. However, as this program also involved relaxation, physical and mental exercise the role of diet was unclear. The present study tested whether older adults with early signs of cognitive decline may benefit from a lower rather than a higher GL meal. Reductions in cerebral glucose metabolism are evident even at the early stages of cognitive decline (Landau et al, 2012) and may precede the onset of cognitive symptoms (Mosconi et al, 2007). Given our participants displayed only a very mild degree of impairment it was possible that providing a steady supply of glucose to the brain may have aided their performance. The evidence from the present study was, however, very clear. Although there was evidence that the measure of cognitive decline used was associated with both poorer episodic and working memory, on no occasion did the nature of the meal consumed benefit either the mood or cognition of those whose memory was poorer than predicted by NART.

Alterations in brain glucose metabolism that correlate with cognitive decline (Landau et al, 2012), may potentially reflect a range of underlying pathologies:

defects in brain glucose transport; a disruption of glycolysis; impaired insulin signalling; mitochondrial dysfunction; neuronal atrophy (Cunnane et al, 2011; Correia et al, 2011). It appears, however, that even at this early stage of cognitive decline changes in the nature of glucose provision is no longer sufficient to enhance cognition, presumably a reflection of the malfunctioning of one of the above, or similar, mechanisms. It is possible that alternative approaches that aim to either correct the disruption in cerebral glucose metabolism or bypass deteriorating brain glucose metabolism altogether (e.g Ketonemia) (Cunnane et al, 2011) may prove more beneficial.

The aim of this chapter was to examine the effect of modulating the GL of breakfast on cognition and mood of older adults and determine whether GL interacts with glucoregulatory status and cognitive decline. A lower rather than a higher GL meal improved cognition and mood in healthy older adults with better GT. These effects were strongest during the late postprandial period (105 - 180min post meal). A lower GL meal did not benefit older adults with poorer GT or older adults with cognitive decline; although a higher GL meal may improve memory and mood in those with poorer GT after 30 minutes. It is possible that defects in the brain glucose metabolism/transport may help explain why older adults with poorer GT or cognitive decline are unable to benefit from a lower GL meal in the same way as healthy older adults. More research is required to determine the pathologies that underlie glucose intolerance related cognitive decline which in turn may help in the development of alternative interventions.

CHAPTER 5

The glycaemic load of Energy Drinks interacts with caffeine to influence young adult's glucose tolerance, cognition and mood.

5.1 Introduction

Although most dietary caffeine is consumed in the form of tea and coffee, recently the market for caffeinated beverages such as energy drinks has grown substantially (Heckman et al, 2010). Manufacturers claim that energy drinks improve physical endurance, reaction times and concentration. Supposedly, the interaction between the various ingredients is critical; however, as reviewed in chapter 1 there is insufficient evidence to support this claim. Indeed as previously discussed there is reason to believe that the consumption of caffeine and a highly glycaemic carbohydrate simultaneously may be undesirable. This study investigates the psychological effects of the interaction between the caffeine and GL of its vehicle in a sample of young adults.

5.2 Method

5.2.1 Participants

Three hundred and forty-five undergraduates were recruited when they responded to a circular email and were randomly and blindly allocated to one of the six conditions. 175 were male and 162 female with a mean age 21.78 years (SD 3.508). Participants were excluded if they were diabetic, suffered from any food allergies/intolerances or if their BMI was outside the healthy range (18.5-25.0).

Table 19 shows the baseline data for each group prior to receiving one of the six drinks.

		Milk	Glucose	Water	Milk Caffeine	Glucose Caffeine	Water Caffeine	P
Gender	M F	32 28	30 27	27 28	28 28	31 30	29 27	ns
Age		22.2(3.4)	21.5(3.4)	21.5(3.4)	22.5(3.4)	21.2(3.4)	21.1(3.4)	ns
Habitual caffeine intake		108 mg (17.5)	134 mg (24.7)	155 mg (29.0)	179 mg (35.0)	126 mg (24.5)	132 mg (20.0)	ns
Depressed/Elated before testing		45.8(26.3)	45.3(25.5)	45.9(27.6)	44.4(27.2)	44.3(26.4)	48.1(27.3)	ns
Depressed/Elated After testing		44.5(24.3)	47.1(24.1)	47.6(25.0)	46.2(25.8)	44.7(24.4)	46.9(25.5)	ns
Anxious/Composed Before testing		45.5(39.6)	44.4(41.4)	42.8(41.1)	48.4(40.9)	44.1(39.2)	50.6(41.2)	ns
Anxious/Composed After testing		45.4(34.1)	44.4(35.5)	45.5(35.5)	43.1(35.6)	42.7(34.1)	49.7(35.8)	ns
Agreeable/Hostile Before testing		42.7(41.4)	44.0(43.1)	40.8(44.1)	42.4(43.0)	44.6(41.5)	49.6(43.9)	ns
Agreeable/Hostile After testing		42.5(36.1)	43.9(33.3)	41.4(38)	43.8(37.1)	43.9(35.0)	46.9(37.1)	ns
Confident/ Unsure Before testing		45.5(37.6)	44.4(37.5)	42.8(41)	48.2(40.5)	44.1(41.1)	40.6(41.4)	ns
Confident/ Unsure After testing		43.5(35.8)	42.5(32.9)	39.9(40)	46.1(38.9)	39.1(39.7)	40.1(38)	ns
Energetic/ tired Before testing		41.4(28.9)	48.5(29.4)	41.6(30)	45.7(29.1)	44.3(28.9)	40.2(30)	ns
Energetic/ tired After testing		43.5(27.1)	44.9(28.2)	47.3(29)	48.0(28.7)	45.6(28.4)	41.0(28)	ns
Clearheaded/ Confused before testing		40.9(37.2)	46.6(35.8)	42.6(39)	44.1(38.4)	44.2(29.1)	45.8(31)	ns
Clearheaded/ Confused after testing		45.5(29.0)	42.9(35.6)	47.3(29)	49.6(38.7)	44.6(29.0)	43.0(28)	ns

Immediate memory	8.4(4.2)	8.0(4.3)	8.0(4.2)	8.7(4.3)	8.7(4.1)	8.3(4.1)	ns
Delayed memory	6.0(3.1)	5.7(3.2)	5.7(3.0)	6.2(3.9)	6.1(3.7)	6.2(3.7)	ns
Vigilance RT	637.9(197.7)	613.0(210.8)	630.7(186.1)	573.2(197.1)	612.1(187.4)	686.4(236.4)	ns
1 st min	605.9(156.8)	635.7(167.5)	626.3(153.4)	631.7(186.3)	646.4(154.6)	637.1(161.3)	ns
2 nd min	569.9(185.9)	566.0(241.5)	562.5(189.9)	537.3(163.4)	528.1(186.6)	537.9(144.4)	ns
3 rd min	647.6(248.9)	591.7(298.8)	166.3(178.9)	616.4(186.6)	609.6(204.6)	580.6(202.7)	ns
4 th min	640.2(183.9)	611.2(254.0)	605.6(232.5)	590.3(216.0)	653.6(180.7)	601.8(259.0)	ns
5 min	4.5(1.8)	4.1(2.0)	4.9(1.7)	4.2(1.9)	4.4(2.0)	4.6(1.8)	ns
Vigilance accuracy	4.3(1.8)	4.0(1.9)	4.8(1.9)	4.5(1.6)	4.3(2.0)	4.7(1.9)	ns
1 st min	3.4(1.6)	3.2(1.7)	3.8(1.7)	3.5(1.8)	3.4(1.8)	3.6(1.8)	ns
2 nd min	4.1(1.7)	3.6(1.8)	4.0(1.8)	4.5(1.7)	4.2(1.9)	4.1(1.9)	ns
3 rd min	3.9(1.8)	3.5(1.6)	3.6(1.7)	4.0(1.8)	3.9(1.6)	3.9(1.9)	ns
4 th min	300.7(31.7)	318.1(36.8)	310.2(33.1)	3.8.8(34.5)	307.4(36.9)	303.3(35.4)	ns
5 th min	320.3(44.2)	327.2(41.6)	324.6(44.9)	329.0(40.1)	325.1(43.5)	320.0(39.5)	ns
Decision times	330.6(41.2)	345.5(47.8)	353.3(49.3)	341.5(41.5)	340.3(46.1)	330.7(40.9)	ns
1 lamp	347.5(51.3)	360.6(47.8)	368.4(63.1)	355.1(42.5)	364.3(68.1)	347.8(58.9)	ns
2 lamp	108.1(64.5)	205.7(74.1)	198.0(78.1)	193.2(58.1)	187.1(58.4)	188.4(62.7)	ns
4 lamp							
8 lamp							
Movement times							
1 lamp							

2 lamp	117.8 (60.1)	188.9 (58.6)	209.9 (60.1)	187.5 (58.9)	187.7 (56.5)	197.9 (59.6)	ns
4 lamp	182.0 (61.6)	193.0 (54.8)	205.9 (80.5)	195.5 (55.9)	196.7 (65.4)	187.6 (60.0)	ns
8 lamp	188.9 (59.9)	200.5 (52.2)	203.4 (67.7)	202.1 (53.6)	202.4 (56.6)	190.4 (52.8)	ns
Serial sevens RT	1808.0(1424.4)	2197.5(1515.6)	1917.3(1356.3)	1951.7(1465.4)	1941.8(1532.1)	1946.2 (1489.7)	ns
Serial sevens errors	3.1 (5.3)	4.1 (5.6)	3.6 (5.5)	3.8 (5.5)	3.5 (5.2)	3.7 (5.3)	ns
Focused attention RT.	607.3 (153.2)	604.6 (167.9)	619.7 (161.5)	600.7 (164.6)	603.2 (155.0)	588.1 (155.8)	ns
Focused attention errors.	2.7 (3.2)	2.6 (3.6)	2.2 (3.5)	2.7 (3.7)	2.5 (3.1)	2.5 (3.3)	ns

Table 19. Baseline data for each group prior to receiving one of the six drinks. There were no significant differences in performance or mood at baseline.

5.2.2 Procedure

The procedure was followed with the approval of the Swansea University ethics committee (reference number: 0825/2010/1) and only after the participants had given written informed consent (Appendix 12). At 0800 after fasting overnight participants came to the laboratory and had their fasting blood glucose levels measured. They then consumed a standard breakfast. After ninety minutes they consumed an experimental drink but no other food throughout the morning. The test battery was administered on four occasions, once before the drink, and after breakfast, when all participants were nutritionally equal (which gave participants an opportunity to practise). Then testing took place three times after participants had consumed the drink. Figure 17 outlines the experimental procedure.

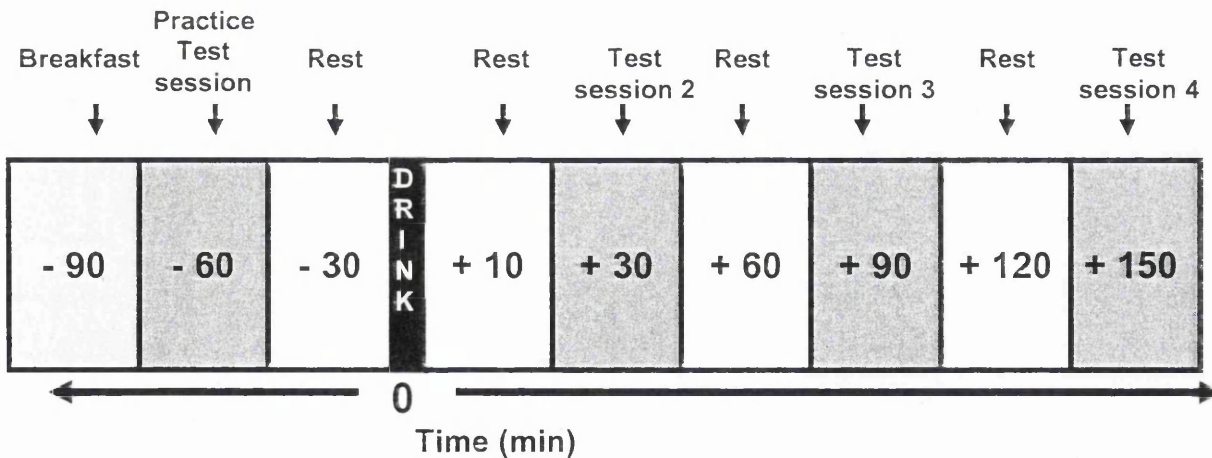


Figure 17. Schematic representation of the time in minutes that each testing session took place relative to when the drink was consumed.

5.2.3 Glucose measurements

Most studies investigating the effect of differences in postprandial glucose on cognition utilise intermittent finger prick blood glucose measurements (e.g Benton et al, 1998; 2003). However, this approach has the potential to miss a large amount of information regarding fluctuations in glycaemia. Therefore a sub portion of the participants (N=38) attended the laboratory the day prior to the experiment and were fitted with an interstitial glucose monitor (Medtronic I pro continuous glucose monitoring system; Medtronic Ltd, Watford, UK) which they wore throughout the test procedure the following day. A small glucose sensor was inserted under the subject's skin and this enabled glucose levels to be recorded every 5 minutes for the duration of the study. This approach has been recently validated against arterialised venous blood, the best available proxy for blood glucose delivery to the brain, and capillary samples, measured by finger prick (Dye et al. 2010). In addition, the entire sample had their capillary blood glucose monitored from finger pricks using an ExacTech sensor (Medisense Britain Limited) that uses an enzymic method, coupled with microelectronic measurement, and has been shown to be accurate (Matthews et al. 1987).

5.2.4 Breakfast / Drinks

All participants consumed a breakfast of Cornflakes (30g) with 120ml of skimmed milk. In total this amounted to 151 kcal and offered 32g of carbohydrate. In addition a decaffeinated drink of either tea or coffee was consumed.

The sample was randomly divided into six groups (n) that mid-morning consumed one of the following 250ml drinks.

- 1) Milk-based product (GL 3.6) with no added caffeine (60)
- 2) Milk-based product (GL 3.6) with 80mg caffeine (56)
- 3) 30g glucose (GL 30) with no added caffeine (57)
- 4) 30g glucose (GL 30) with 80mg caffeine (61)
- 5) Flavoured water with no caffeine (55)
- 6) Flavoured water with 80mg caffeine (56)

Variants 1-4 contained 155 kcal and all drinks were flavoured orange. The water based drinks were artificially sweetened using sucralose so that they matched the sweetness of the glucose based drinks. All the drinks were provided in opaque plastic bottles labelled A – F. Participants were blind as to the nature of the drink that they were consuming and were unaware that their drinks may have differed from others. The researcher was blind as to the nature of the drinks. The participants were given a maximum of ten minutes to consume the drink after which they sat quietly reading or watching television.

5.2.5 Test battery

The test battery was completed on four occasions always in the following order. 1) Mood, 2) Memory – immediate recall of word list, 3) Serial sevens – working memory, 4) Arrow Flankers – selective attention, 5) Simple and Choice reaction times, 6) Vigilance – sustained attention, 7) Memory – delayed recall of word list, 8) Mood.

The tests of Memory, Working memory, Simple and Choice reaction times, and Vigilance described in section 3.2.3. Mood was described in section 4.2.4.2.

5.2.5.1 Arrow Flankers Test

A modified version of the Eriksen and Eriksen (1974) flanker task was used to measure selective attention as it has been reported to be susceptible to caffeine administration (Addicott & Laurienti. 2009). Each trial presents five arrows that are either congruent (>>>>), incongruent (>><>>), neutral (□□<□□). The task is to indicate whether the middle arrow is pointing to the right or left and the reaction times and accuracy are recorded. On occasion crosses (xx<xx) are also presented that indicate to participants that they should not respond at all. The Arrow Flankers test measures the ability to direct attention and ignore peripheral information. There is a tendency to respond to the distracting flankers meaning the subject had to inhibit this inclination. There was a randomly varying inter-stimulus interval of between 1 and 3 s. Outcomes were accuracy (% incorrect) and reaction time in milliseconds.

5.3 Statistical analysis

Preliminary tests found that there were no significant differences at baseline suggesting that randomisation had been successful. Baseline data are shown in table 19. Initial analysis considered whether gender influenced participants' response to the drinks. There was a main effect of gender on movement times ($F(2,318) = 17.785, p < 0.0001$); males were faster than females. Although there were occasional higher order interactions (see appendix 13) these represented no consistent pattern and therefore were considered to be chance variations. Gender

was therefore not further reported. The habitual level of caffeine consumption was estimated from reports of the level of daily intake of caffeine-containing beverages such as tea, coffee, cola and EDs. The daily intake was on average 139 (147) mg and the habitual caffeine intake did not differ between groups (Table 19). The data were analysed using appropriate Analysis of Variance designs. The basic approach involved the Vehicle (Milk, Glucose, Water) X Caffeine (0 or 80mg) X Test session (30min, 90min, 150min). Only outcomes that involved the vehicle (i.e. milk, glucose, water) or caffeine are reported and where higher order interactions are not mentioned it can be assumed that they were non-significant.

5.4 Results

5.4.1 The glycaemic response to the test drinks

Data are presented from thirty-eight young adults who wore an interstitial glucose monitor (Medtronic I pro continuous glucose monitoring system; Medtronic Ltd, Watford, UK) for the duration of the experiment. Data are reported in successive five minute periods. For clarity of presentation the data are separated into three time periods that are of interest. The response to breakfast (-90 min to 0 min, with 0 min being the point where the drink was consumed); the short term response to drink (0 min to +90 min) and the longer term response to the drink (+90 min to +165 min). Analysis of variance was calculated for each of these time periods, with the vehicle and whether caffeine was consumed as the between subject factor and time as a within subject factor. Where Mauchly's Test of Sphericity was significant Greenhouse-Geisser correction was reported.

The response to breakfast

When considering the effect of breakfast (-90 min to 0 min); there was a significant effect of time ($F(2.5, 5.024) = 91.731, p < 0.0001$), interstitial glucose rose quickly immediately following consumption until -60min when it began to fall, reaching baseline scores by 0 min (time of drink). There were no significant effects of whether caffeine was subsequently consumed ($F(1,32) = 2.749, p = .104$) or the Vehicle that was subsequently drunk ($F(2,32) = .143, p = .867$). It was clear that prior to a drink the six experimental groups responded in a similar manner to their breakfast, that is in terms of their glycaemic response they were well matched. Glucose response to breakfast can be seen in appendix 14.

The response to drink

When considering the immediate effect of the drinks (0 min to +90 min) the effect of caffeine and vehicle were considered using a three way analysis Vehicle X Caffeine X Time. The interactions Vehicle X Time ($F(5.7, 92.2) = 9.94, p < 0.001$; Figures 18) and Caffeine X Time ($F(2.8, 92.2) = 8.43, p < 0.001$; Figures 19) reached statistical significance. The consumption of glucose as expected resulted in a marked increase in blood glucose values. Similarly caffeine consumption resulted in higher blood glucose values towards the end of the time period.

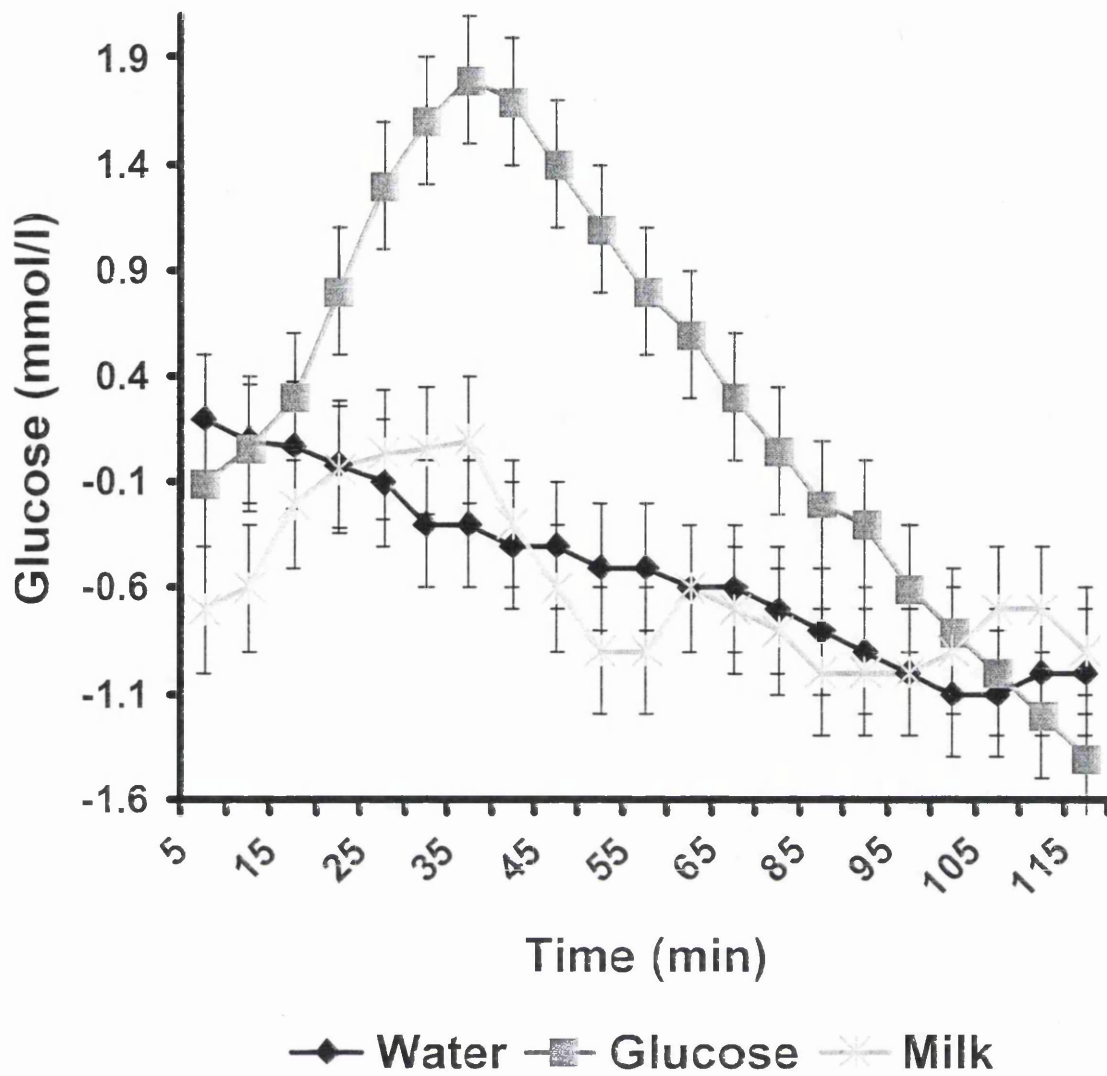


Figure 18. The effect of the vehicle on the immediate glycaemic response. The data presented are average changes every five minutes in interstitial glucose (mmol/L), from the value immediately prior to consuming the drink. Those who drank glucose experienced a greater glycaemic response than those who drank milk or water.

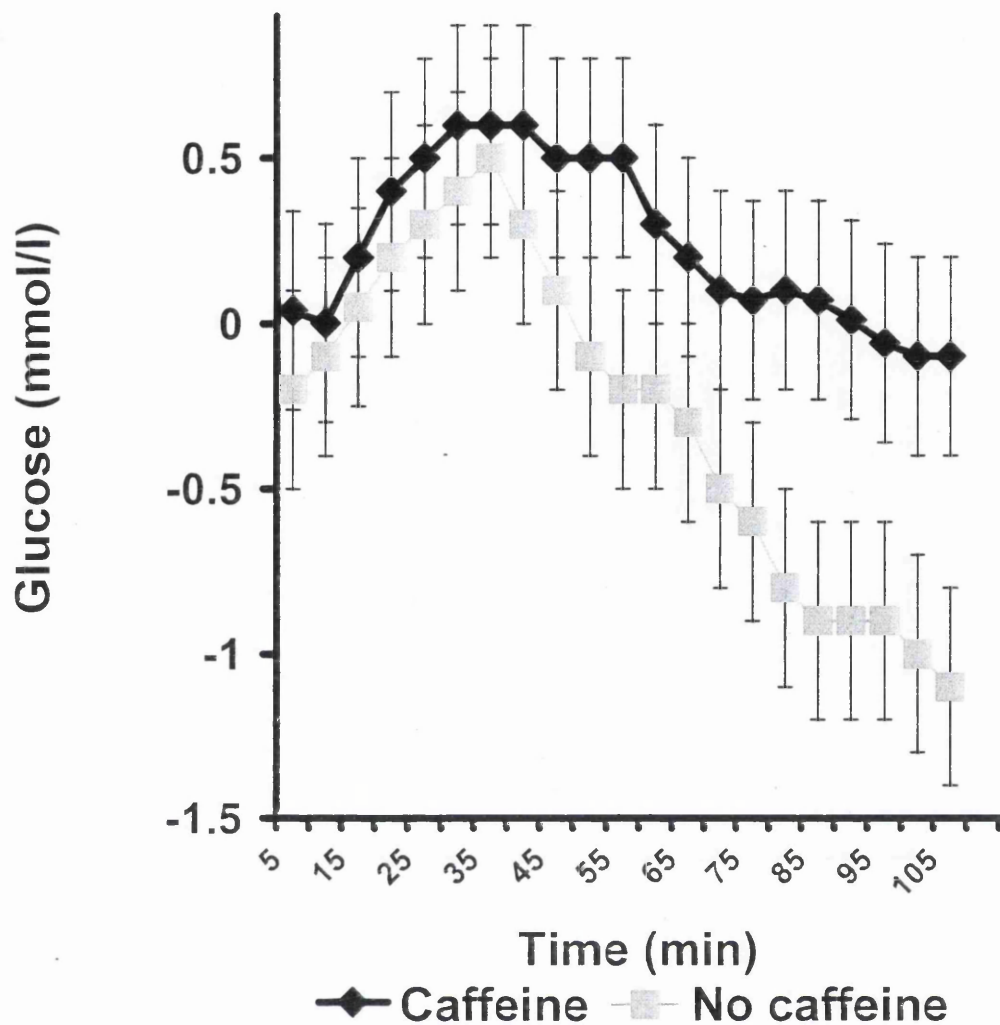


Figure 19. The effect of the caffeine on the immediate glycaemic response. The data presented are average changes in interstitial glucose, as mmol/L, from the value immediately prior to consuming the drink. Those who drank caffeine had higher interstitial glucose levels after 35 minutes than those who did not consume caffeine. The effect occurred irrespective of the vehicle with which it was consumed.

When in the longer term (+90 min to + 165 min) interstitial glucose levels were considered the main effect of caffeine was again significant ($F(1,32) = 19.30$, $p < 0.001$; Figure 20) whereas the Vehicle was not ($F(2,32) = 1.03$, n.s; Figure 21). Thus the addition of caffeine, irrespective of the nature of the drink, was associated with higher levels of interstitial glucose from 30 minutes until the end of the experiment (150 minutes post consumption).

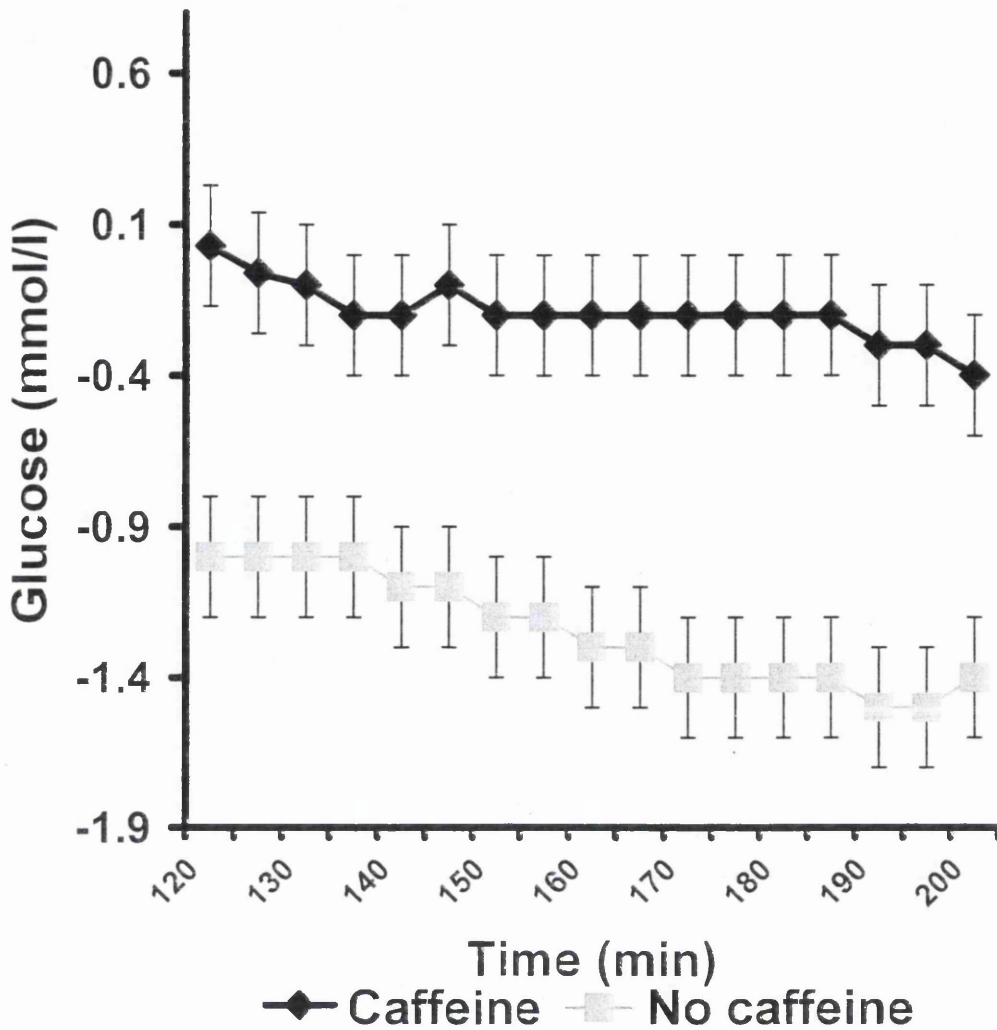


Figure 20. The effect of caffeine on the longer term glycaemic response. The data presented are average changes in interstitial glucose, as mmol/L, from the value immediately prior to consuming the drink. Those that drank caffeine had higher interstitial glucose levels at the end of the morning than those that did not consume caffeine.

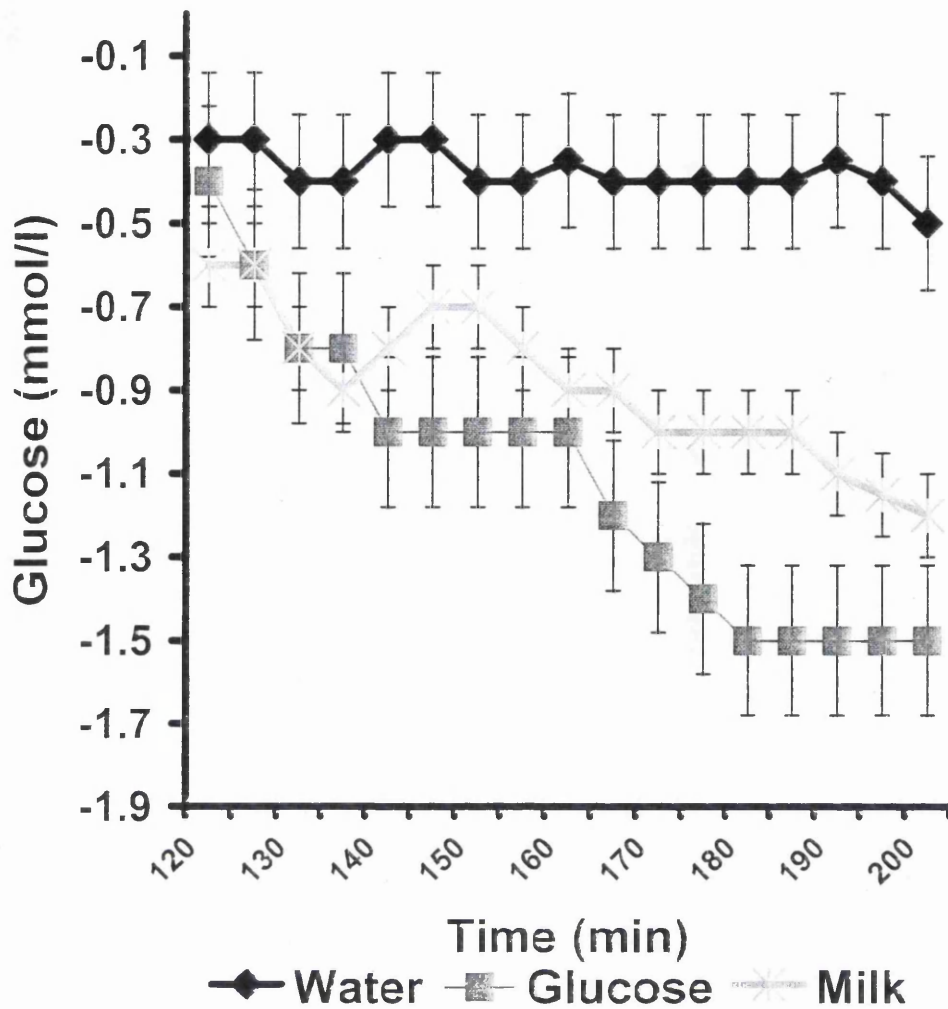


Figure 21. The effect of vehicle on the longer term glycaemic response. The data presented are average changes in interstitial glucose, as mmol/L, from the value immediately prior to consuming the drink. Those who drank milk maintained a higher interstitial glucose level at the end of the morning than those who drank glucose or water.

Finger prick measures of glucose

The remainder of the participants (as well as those fitted with an interstitial glucose monitor) had their blood glucose measured from finger pricks at -90 min, -30 min, 0 min, +30 min, +90 min and +150min. The patterns of results were the same as the interstitial glucose measurements and therefore not reported.

5.4.2 Mood

All dimensions of mood were analysed using a four way analysis of variance: Vehicle (Milk, Glucose, Water) X Caffeine (0 or 80mg) X Before / after cognitive testing X Test session (30, 90 and 150 min post drink). In all cases a higher rating was more positive. Where Mauchly's Test of Sphericity was significant Greenhouse-Geisser correction was reported.

Energetic / Tired

The interaction Vehicle X Caffeine X Test session reached statistical significance ($F(3.9, 640.2) = 2.65, p < 0.04$) and is reported in Figure 22. Caffeine had a different influence depending on the nature of the drink. With water based drinks those who consumed caffeine felt more tired in the late morning, whereas with the milk-based drink caffeine made participants significantly more energetic. In contrast caffeine had little influence when a glucose containing drink was consumed. Post hoc tests failed to find any difference when caffeine had not been consumed. However, when taking the milk-based drink those who received caffeine were more energetic after 90min ($p < 0.02$) and there was a similar trend after 150 minutes ($p < 0.06$) compared to those who did not receive caffeine. With the water based drinks, at both 90 minutes ($p < 0.008$) and 150 minutes ($p < 0.002$),

those receiving caffeine reported being more tired than those without. When participants drank glucose there were no differences depending on whether or not they received caffeine.

Milk-based rather than glucose-based drinks resulted in feeling more energetic after 90 minutes ($p < 0.03$) but only in those who received caffeine. Similarly a milk-based rather than water-based drink resulted in greater energy after 90 minutes ($p < 0.0001$) and 150 minutes ($p < 0.0001$) but again only in those who received caffeine. Also at both 90 ($P < 0.005$) and 150 minutes ($p < 0.002$) glucose rather than water resulted in feeling more energetic but again only in those who received caffeine. There were no differences at 30 minutes post consumption. All effects occurred later in the morning suggesting that the time scale over which effects are examined is important.

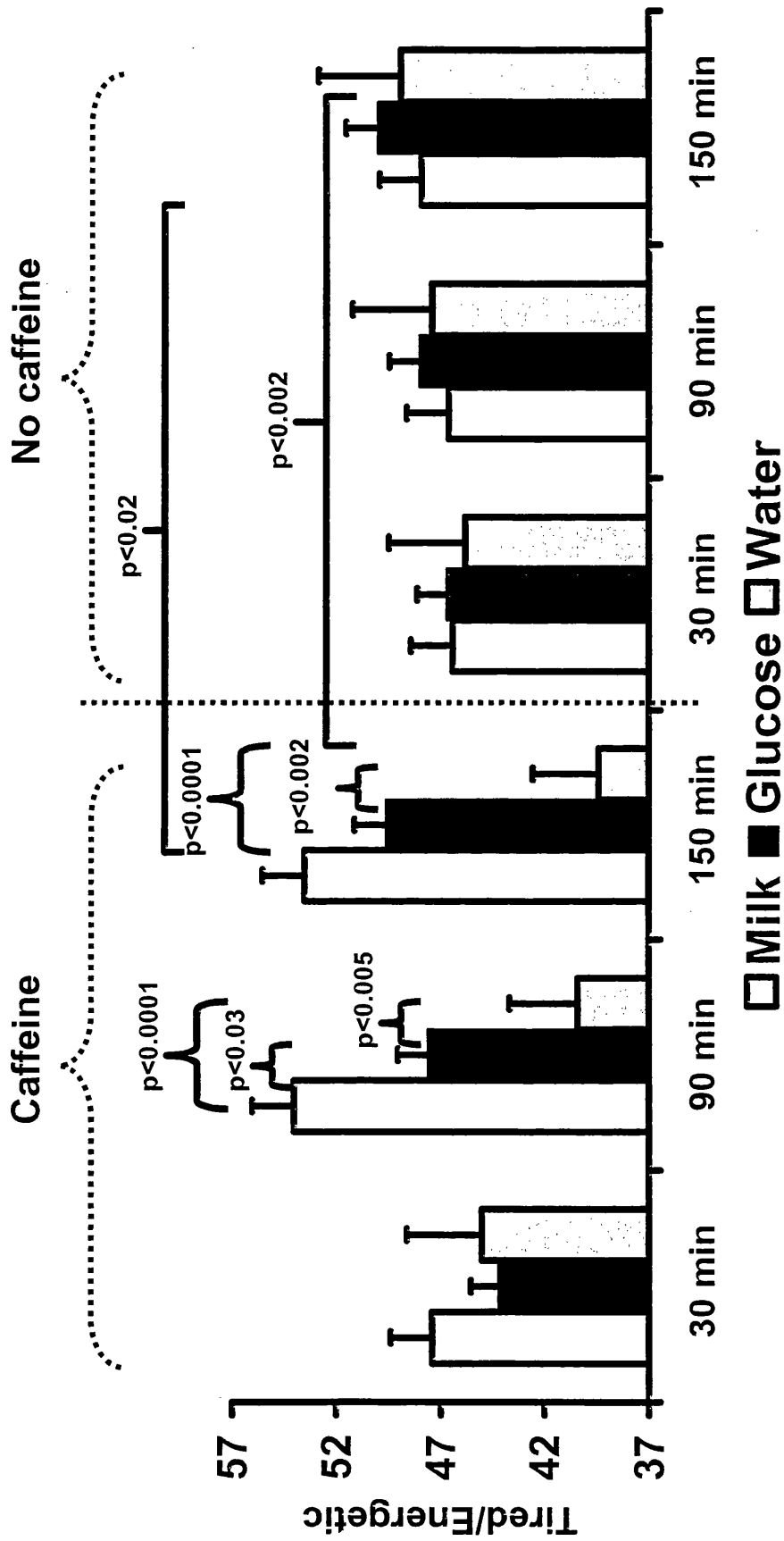


Figure 22. The influence of vehicle, with and without caffeine, on feeling energetic over the morning. Data are mean rating for VAS energetic / tired at each testing session ($n = 345$). The left side of the graph represents scores for each vehicle when caffeine was consumed and the right side represents scores when caffeine was not consumed. There were no differences between vehicles when caffeine was not consumed. At 90 minutes when caffeine was consumed those that drank milk were more energetic than those that drank glucose or water. Also after 90 and 150 minutes when caffeine had been consumed those that drank glucose were more energetic than those that drank water. When water was consumed those that had caffeine were significantly more tired at 90 and 150 minutes than those that did not have caffeine. When milk was drunk those that had caffeine were more energetic than those without.

There was also a Vehicle X Caffeine X Before / after test session interaction ($F(2, 318) = 3.33, p < 0.04$). Figure 23 illustrates the effect. Again the combination of a water-based drink and caffeine resulted in reports of feeling tired after, rather than before, the testing session ($p < 0.02$). When glucose was consumed there were no differences between those who consumed caffeine and those who did not, however, when milk was consumed participants who had caffeine reported feeling more energetic after a test session ($p < 0.02$) compared to those who consumed milk with no caffeine. Irrespective of the testing session those who drank glucose reported feeling less tired after testing than those consuming water ($p < 0.03$) but only if they consumed caffeine. Similarly those who drank milk reported feeling less tired after testing than those consuming water ($p < 0.0001$) but only if they consumed caffeine. There was a trend ($p < 0.07$) for those consuming milk and caffeine to be more energetic following a test session than those consuming glucose and caffeine (milk + caffeine 18.1 (33.4) glucose + caffeine 7.0 (35.2)). There were no differences in the response to the vehicles in those who did not consume caffeine.

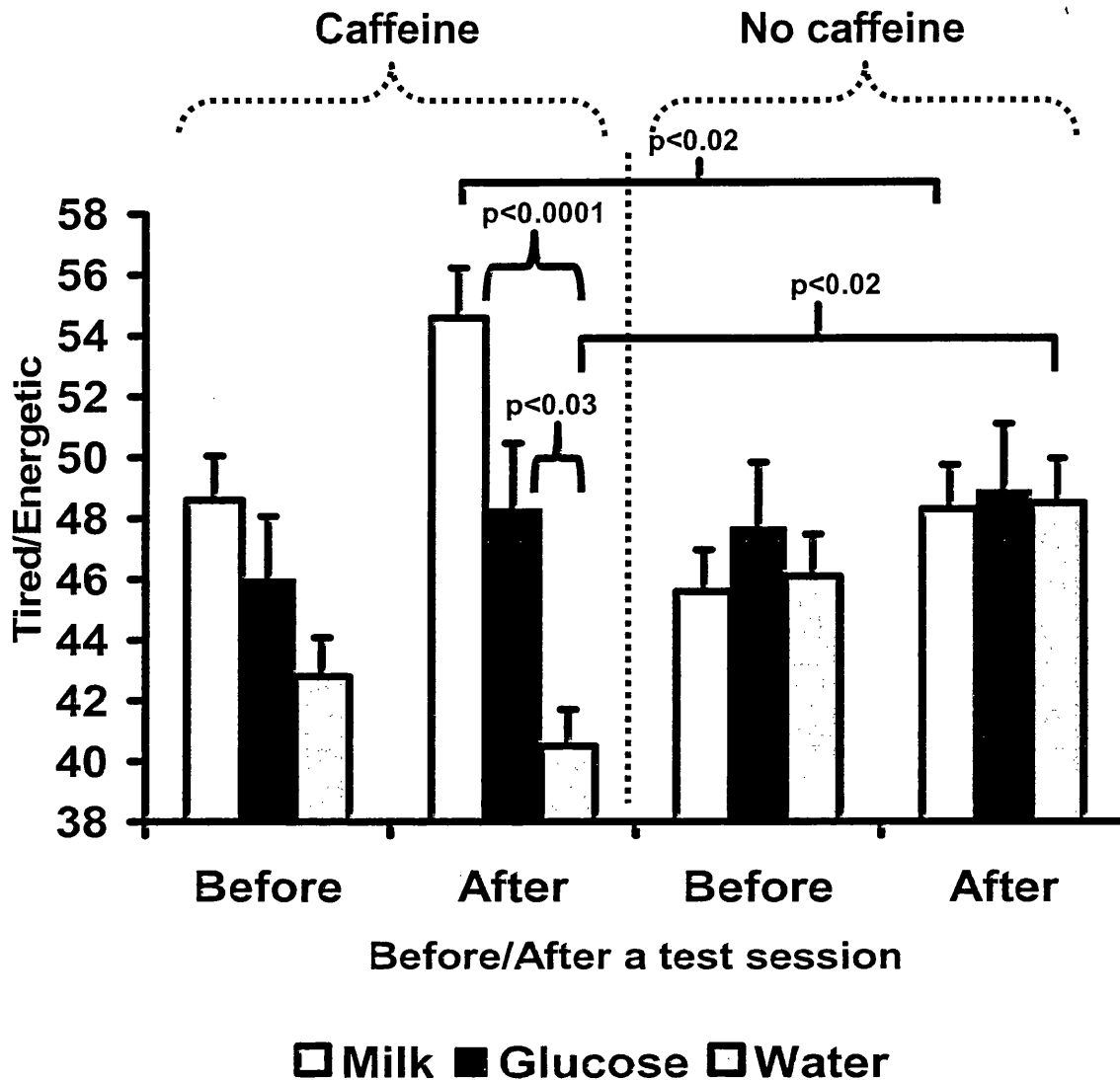


Figure 23. The influence of vehicle, with and without caffeine, on feeling energetic before and after a test session. Data are mean rating for energetic / tired before and after each testing session (n = 345). The left side of the graph represents scores for each vehicle when caffeine was consumed and the right side when it was not consumed. There were no differences between vehicles when caffeine was not consumed. When caffeine was consumed those that drank milk rather than water were more energetic after a testing session. When caffeine was consumed those that drank glucose rather than water were more energetic. When water was consumed those that had caffeine were significantly more tired after a test session than those that did not. When milk was consumed those that had caffeine were more energetic than those who had not.

Given the unexpected response to caffeine in water-based drinks the immediate influence of caffeine 30 minutes post consumption, before taking any tests, was considered. Ratings of being energetic / tired were examined by subtracting ratings before a drink was consumed from the ratings half an hour later, but before a test battery was performed. The main effect of caffeine was not significant ($F(1,331) = 0.002$, ns), however, the interaction Vehicle X Caffeine reached statistical significance ($F(2,331) = 3.21$, $p < 0.04$). Table 20 reports the interaction with a positive score indicating feeling more energetic and a negative score feeling more tired. Those who drank caffeine in combination with water reported being significantly more energetic than those who drank caffeine with glucose ($p < 0.03$) or milk ($p < 0.03$). There were no differences between the glucose and milk conditions when caffeine was taken. There were also no significant differences between the three drinks in those consuming no caffeine ($F(2,165) = 0.52$, n.s.). That is there was a short term benefit of consuming caffeine when it was taken with water.

	No caffeine	Caffeine
Milk-based	+2.26 +/- 2.59	- 2.30 +/- 2.68
Glucose-based	+1.13 +/- 2.64	- 2.25 +/- 2.55
Water based	-1.19 +/- 2.68	+6.46 +/- 2.66*

Table 20. The immediate influence of drinks with and without caffeine on feeling energetic. Data are mean changes from ratings before a drink was consumed to the ratings half an hour later, but before a test battery was performed. When water was consumed those that had caffeine were significantly more energetic 30 minutes later than those that did not have caffeine. There were no differences when milk or glucose had been drunk. * Differs from the no caffeine condition $p < 0.04$.

Elated/ Depressed

Ratings of feeling elated rather than depressed were not influenced by the nature of the drink. Neither the Vehicle ($F(2, 315) = 0.64, n.s$), whether caffeine was consumed ($F(1, 315) = 1.03, n.s$), nor did the interaction between these factors achieved statistical significance ($F(2, 315) = 0.95, n.s$).

Anxious/ Composed

Similarly when ratings of being composed / anxious were examined the Vehicle ($F(2, 316) = 1.15, n.s$) and Caffeine main effects ($F(1, 316) = 1.19, n.s$) were not significant, and neither were the interaction between these factors ($F(2, 316) = 2.20, n.s$).

Confident/ Unsure

With ratings of being Confident / unsure there was a four way interaction ($F(3.9, 629.9) = 2.51, p < 0.04$), but as these higher order interactions appeared to reflect chance differences in one cell, it was not further considered.

Agreeable/ Hostile

With ratings of being Agreeable / hostile the interaction Vehicle X Caffeine X Test session reached statistical significance ($F(3.0, 507.2) = 4.07, p < 0.004$). Post hoc tests, however, failed to find any significant differences between conditions. However, caffeine appeared to have a different influence depending on the nature of the drink. In particular there was a trend for there to be a negative influence of caffeine when taken with a water based drink later in the morning (Water + caffeine 39.1(2.3); glucose + caffeine 47.4(2.2); milk + caffeine 49.2(2.3).

There was also a Vehicle X Caffeine X Before / after test session interaction ($F(2, 321) = 7.32, p < 0.02$). When water was consumed those who received caffeine rated themselves as more hostile following a test session than those who did not receive caffeine ($p < 0.007$). Caffeine had no effect in those who drank glucose or milk. Those who consumed water rated themselves as less agreeable than those who consumed glucose ($p < 0.02$) and those who consumed milk ($p < 0.008$), but only if they had also consumed caffeine. As the vehicle did not influence ratings of agreeableness when caffeine was not drunk, this effect therefore appeared to reflect a negative reaction to caffeine in water based drinks.

Clearheaded / Confused

When reports of feeling Clearheaded / confused were examined the interaction Vehicle X Caffeine X Test session reached statistical significance ($F(3.1, 507.8) = 3.47, p < 0.007$). Again there was a trend ($p < 0.07$) for caffeine to increase feelings of being confused as opposed to clearheaded, when it was consumed with a water based drink 150 minutes after consumption (water + caffeine 43.2(3.4); glucose + caffeine 50.6(2.2); milk + caffeine 50.6(2.3). Post hoc tests, however, failed to find any statistically significant differences.

There was again a Vehicle X Caffeine X Before / after test session interaction ($F(2, 317) = 3.17, p < 0.04$). When water was drunk caffeine increased ratings of being confused as opposed to clearheaded ($p < 0.02$), however, when milk or glucose were drunk caffeine had no significant effect. When caffeine was not consumed there were no effects of the vehicle on ratings of being clearheaded. When caffeine was consumed a milk based drink rather than a water based drink

increased clear-headedness ($p < 0.02$). Again this effect reflected a negative reaction to caffeine in water based drinks.

5.4.3 Episodic memory

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(2) = 9.897$, $p < 0.007$ (Time), therefore, epsilon was estimated and the degrees of freedom were adjusted using the Greenhouse-Geisser correction. The data were considered in a four-way analysis of variance; Vehicle X Caffeine X Short term/ Long term Memory X Test Session. There was a main effect of Caffeine. Those who consumed caffeine recalled significantly more words than those who did not ($F(1, 341) = 5.02$, $p < 0.03$: 6.3 words \pm 0.91 c.f. 6.94 \pm 0.19). The interaction Vehicle X Test session was also significant ($F(3.8, 664.9) = 2.61$, $p < 0.03$; Figure 24) The Vehicle did not influence memory 30 or 90 minutes after a drink, but after 150 minutes those consuming milk-based products tended to perform better than those consuming either glucose or water based drinks (6.5 \pm 0.26 c.f. 5.8 \pm 0.26 glucose and 5.9 \pm 0.26 water). Although the difference between milk and water based drinks was not significant the difference between the milk and glucose based drinks approached statistical significance ($F(1, 226) = 3.27$, $p < 0.07$).

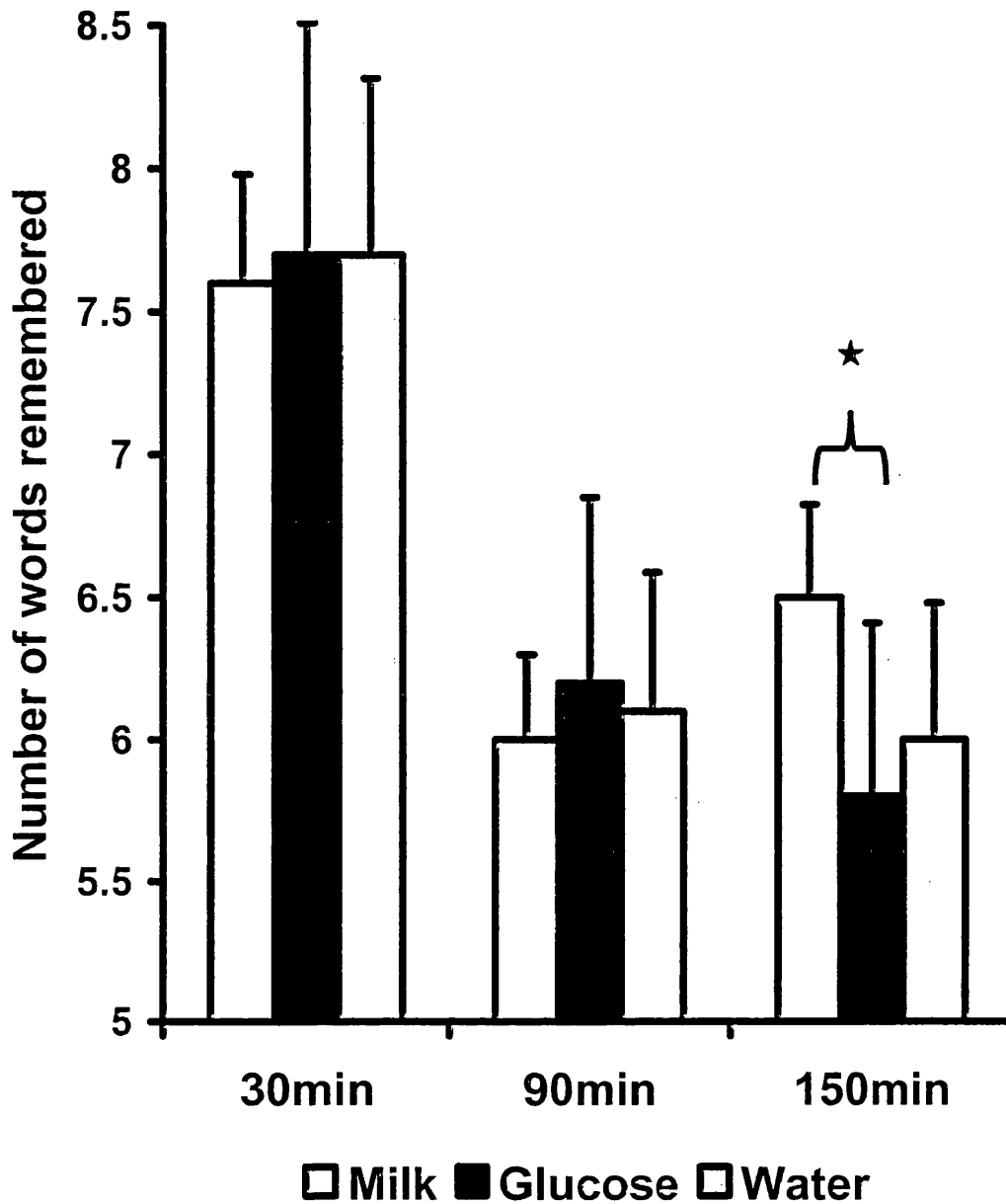


Figure 24 The influence of the type of drink on the number of words recalled over test sessions. Data are mean (standard error) for the number of words recalled after each vehicle regardless of caffeine. After 150 minutes those consuming milk-based products tended to perform better than those consuming either glucose or water based drinks ★ $p < 0.07$.

5.4.4 Working memory

Accuracy

The data were considered using a three way analysis of variance: Vehicle X Caffeine X Test session. Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(2) = 312.697$, $p < 0.001$ (Time). therefore epsilon was estimated and the degrees of freedom were adjusted using the Greenhouse-Geisser correction. When the number of incorrect responses were examined Caffeine ($F(1,329) = 0.24$, n.s), the Vehicle ($F(2,329) = 0.57$, n.s) and the interaction between these two variables ($F(2,329) = 1.50$, n.s) were all non-significant.

Reaction times

Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(2) = 2.690$, $p = .261$ (Time). Although accuracy was not influenced the speed of doing the test was affected. Those who consumed caffeine performed the task faster than those that did not ($F(1,329) = 3.88$, $p < 0.001$; 1319 seconds +/- 27.0 c.f. 1244 +/- 26.6). The Vehicle just missed statistical significance ($F(1,329) = 2.79$, $p < 0.06$). However, post hoc tests found that the consumption of glucose based drinks at 90 minutes ($p < 0.03$) and 150 minutes ($p < 0.04$), but not the 30 minutes, were associated with taking longer to perform mental arithmetic than after a milk-based product. Similarly water based rather than glucose based drinks were associated with quicker responding in the second ($p < 0.05$) and third ($p < 0.05$) testing sessions. Those consuming milk tended to be faster than those who drank water based drinks although this was not statistically different (Figure 25).

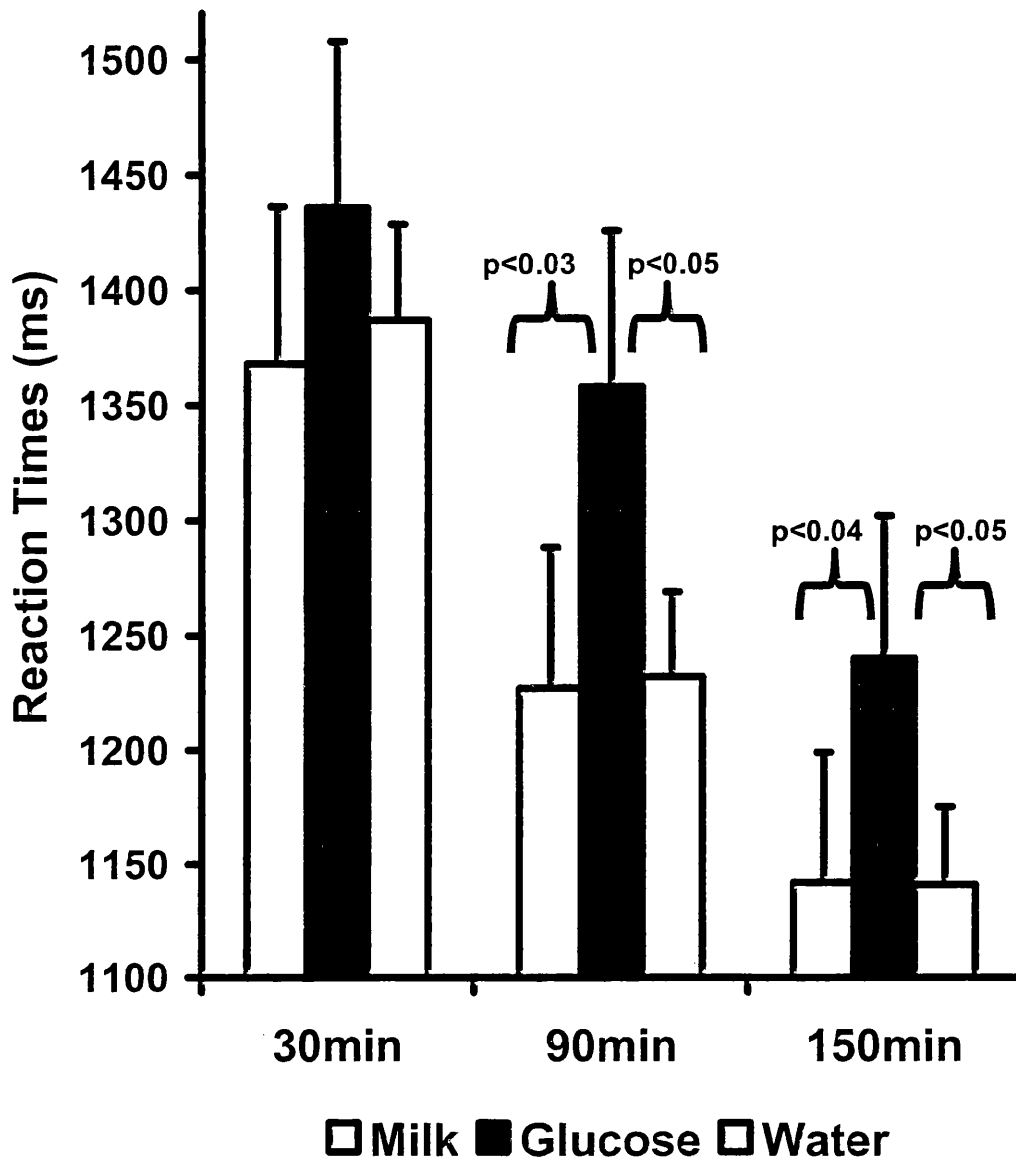


Figure 25. The influence of the type of drink on the time in milli-seconds to perform the serial sevens test. Data are mean (standard error) for reaction times on the serial sevens task. Those that the consumed glucose based drinks at 90 minutes ($p<0.03$) and 150 minutes ($p<0.04$) were slower than those that consumed milk. Similarly those that drank water rather than glucose were faster after 90 ($p<0.05$) and 150min ($p<0.05$).

5.4.5 Focused attention

Reaction times

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated $\chi^2(2) = 14.559, p < .001$ (Time) therefore epsilon was estimated and the degrees of freedom were adjusted using the Greenhouse-Geisser correction. Focused attention was considered using the Arrow flankers task in a three way analysis of variance: Vehicle X Caffeine X Test session. As expected with the congruent stimuli there was no effect of either Caffeine ($F(1,324) = 0.85, n.s$), Vehicle ($F(2,324) = 0.51, n.s$) or the interaction between the two ($F(2,234) = 0.157, ns$). Similarly there was no effect of either Caffeine ($F(1,324) = 2.503, ns$), Vehicle ($F(2,324) = 1.109, ns$) or the interaction between the two ($F(2,324) = 0.149, ns$) for neutral stimuli. With the incongruent stimuli there was, however, an interaction between Caffeine and the Vehicle ($F(2,324) = 3.51, p < 0.03$). There were no differences between vehicles when caffeine was not drunk however when caffeine was consumed there was a trend for the speed of responding to be faster with a glucose rather than milk based drink ($p < 0.07$). When milk or water were drunk the addition of caffeine made no difference but when participants consumed the glucose based drink the addition of caffeine tended to decrease the time taken for the task; this effect just missed statistical significance ($p < 0.07$).

Accuracy

Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(2) = 5.306, p = .70$ (Time). Accuracy (number of incorrect responses to incongruent stimuli) was considered using the analysis Vehicle X Caffeine X Test Session. There were no significant effects of either Caffeine

(($F(1,324) = 1.23$, n.s), Vehicle ($F(2,324) = 1.05$, n.s) or the interaction between the two ($F(2,234) = 0.99$, ns).

5.4.6 Reaction times

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated $\chi^2(2) = 366.965$, $p < .0001$ (Lamps) and $\chi^2(5) = 160.453$, $p < .0001$ (Time) and $\chi^2(20) = 1308.357$, $p < .0001$ (Time X Lamps) therefore epsilon was estimated and the degrees of freedom were adjusted using the Greenhouse-Geisser correction. Decision times and movement times were considered separately. The decision times were examined with Vehicle X Caffeine X Test session X Number of lamps (1, 2, 4 or 8 lamps) design. The interaction Vehicle X Caffeine X Test session reached statistical significance ($F(2.5,402.8) = 2.46$, $p < 0.05$). Caffeine quickened decision times after 30 ($p < 0.008$), 90 ($p < 0.001$) and 150 minutes ($p < 0.0001$) in water based drinks. Although adding caffeine to glucose based drinks produced slightly quicker decision times, the effects were non-significant and caffeine made little difference when added to milk. When participants consumed caffeine their decision times were significantly faster with water rather than milk at 30 ($p < 0.05$), 90 ($p < 0.03$) and 150 ($p < 0.02$) minutes and significantly faster with water rather than glucose 90 ($p < 0.009$) and 150 ($p < 0.007$) minutes post ingestion. There were no significant differences between milk and glucose when caffeine was consumed. Although there was a trend for decision times to be faster after drinking milk when compared to either glucose or water, when caffeine was not consumed these effects were not significant. The general pattern of results showed that decision times became slower over the morning when water or glucose had been drunk and that caffeine prevented this decline, more so when

taken with water. Reactions did not slow over the morning when milk was consumed and the addition of caffeine did not affect this. The effects are illustrated in Figure 26.

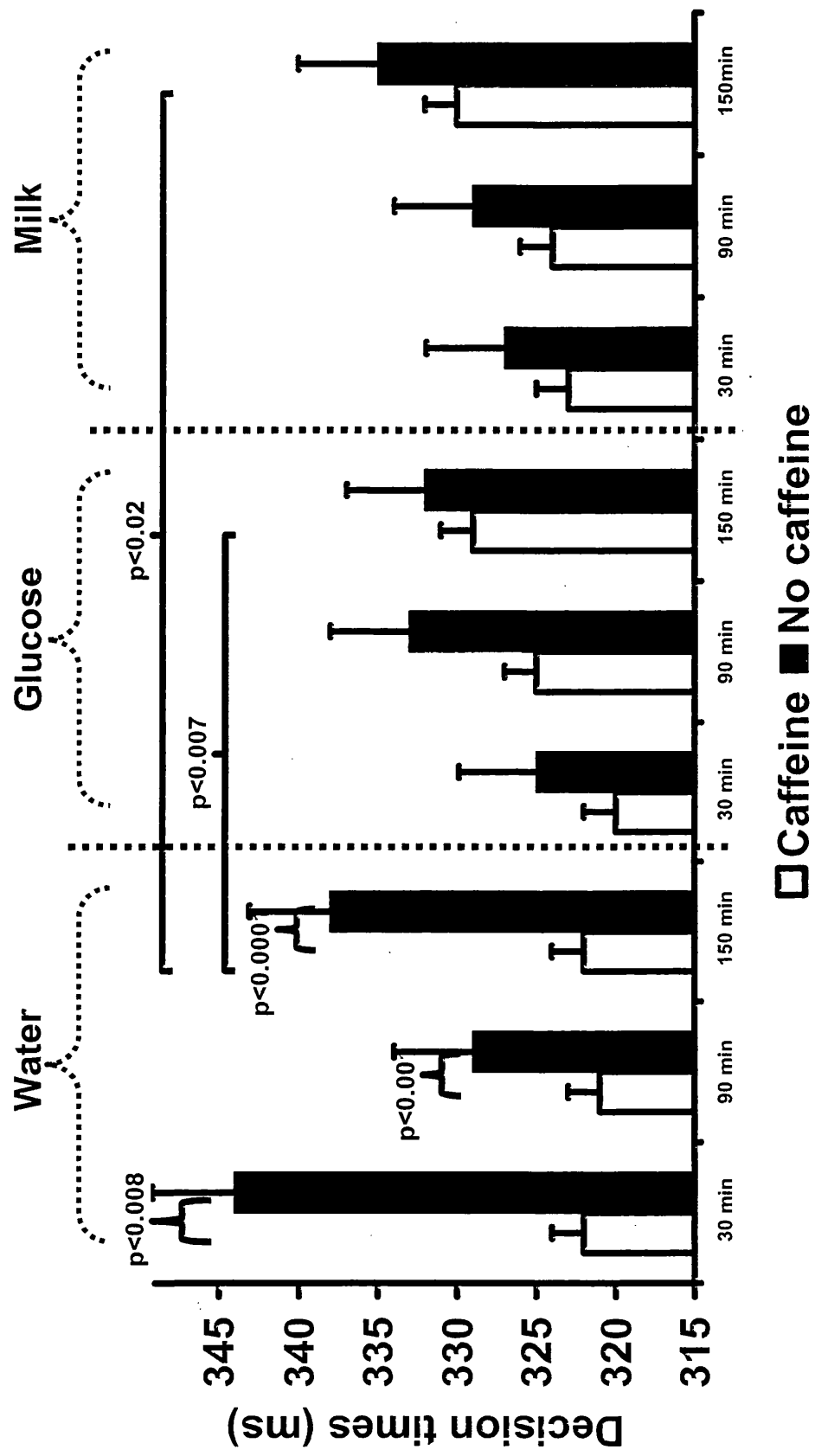


Figure 26. The influence of the type of drink on decision times over the morning. Data are mean (standard error) for decision times in ms. When subjects consumed caffeine their decision times were faster with water compared to milk at 30 ($p < 0.05$), 90 ($p < 0.03$) and 150 ($p < 0.02$) minutes and significantly faster with glucose 90 ($p < 0.009$) and 150 ($p < 0.007$) minutes post ingestion. Caffeine quickened decision times after 30 ($p < 0.008$), 90 ($p < 0.001$) and 150 minutes ($p < 0.0001$) in water based drinks.

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated $\chi^2(2) = 17.537, p < .0001$ (Lamps) and $\chi^2(5) = 19.646, p < .0001$ (Time) and $\chi^2(20) = 155.443, p < .0001$ (Time X Lamps) therefore epsilon was estimated and the degrees of freedom were adjusted using the Greenhouse-Geisser correction. When movement times were examined the interaction Vehicle X Caffeine X Test session reached statistical significance ($F(3.7,593.9) = 3.018, p < 0.02$). In general movement times were faster with caffeine although the only significant difference occurred in the second test session in water based drinks ($p < 0.02$).

5.4.6 Vigilance

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated $\chi^2(2) = 8.362, p < .02$ (Min) and $\chi^2(9) = 20.948, p < .02$ (Time) therefore epsilon was estimated and the degrees of freedom were adjusted using the Greenhouse-Geisser correction. The number of sequences correctly identified was considered with a Vehicle X Caffeine X Test Session X Minute of test design. There was a significant interaction Caffeine X Minute of test ($F(3.8,1251.5) = 4.73, p < 0.001$) as well as a main effect of caffeine ($F(1,323) = 5.777, p < 0.02$) Post hoc test revealed that participants who consumed caffeine correctly identified more sequences during the first ($p < 0.03$), second ($p < 0.005$), forth ($p < 0.01$) and fifth ($p < 0.002$) minute of the test. The third minute of testing just missed statistical significance ($p < 0.06$), but as it was considered to be the result of a chance variation in one cell, the main effect of caffeine is reported ($p < 0.004$). Participants who consumed a caffeinated drink identified more sequences correctly. There were no effects involving Vehicle.

Next the reaction times when correct sequences had been identified were examined. This analysis required the removal of participants who had failed to identify any of the correct sequences in any testing period. There was no evidence that the number of such incidences differed according to which drink participants had consumed. Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated $\chi^2(9) = 38.501, p < .0001$ (Time) and $\chi^2(35) = 80.119, p < .0001$ (Time X Min) therefore epsilon was estimated and the degrees of freedom were adjusted using the Greenhouse-Geisser correction. There was a significant main effect of caffeine ($F(1,288) = 7.62, p < 0.006$), the responses were quicker when caffeine had been consumed (583.59 ± 6.01 c.f. 560.73 ± 5.69). The interaction Vehicle X Minute of test was also significant ($F(7.5,1217.6) = 3.24, p < 0.04$). Post hoc tests revealed that when glucose was consumed reaction times were significantly slower towards the end of the testing period. Participants who consumed water had faster reactions than those who consumed glucose during the third ($p < 0.008$), fourth ($p < 0.001$) and fifth ($p < 0.005$) minute of the test. Similarly, participants who consumed milk had faster reactions than those who consumed glucose during the fourth ($p < 0.005$) and fifth ($p < 0.05$) minute of the test. There were no significant differences between those who consumed milk and those who consumed water. Thus the consumption of glucose slowed reaction times towards the end of the task.

5.5 Summary

Blood glucose

- Young adults', who consumed glucose, rather than milk or water, had higher interstitial glucose levels during the early postprandial period. Similarly, those that drank caffeine had higher glucose levels during the early postprandial period.
- Young adults' that drank caffeine had higher interstitial glucose levels during the later postprandial period.

Mood

- 30 min after consumption caffeine, when taken with water but not milk or glucose, increased ratings of feeling energetic.
- With the water-based drinks, at both 90 min and 150 min, those receiving caffeine reported being more tired.
- However, in those consuming the milk-based drink, the addition of caffeine increased reports of energy at 90 min and 150 min.
- Caffeine, when added to the glucose-based drink, did not influence ratings of energy at any stage throughout the morning.
- A similar pattern of results were observed for ratings of clearheaded-confused and agreeable-hostile.

Cognition

- Young adults', who drank milk, rather than glucose or water, had quicker working memory responses after 90 and 150 minutes and there was a similar trend for episodic memory.

- Reaction times on the vigilance task were faster during the fourth, and fifth, minute of the test if participants drank milk rather than glucose.
- Young adults', who consumed caffeine recalled more words, had quicker choice reaction times, faster working memory, and better and quicker responses on the vigilance task.

5.6 Discussion

The Effect of Vehicle

As predicted the present study found that the participants who consumed milk rather than glucose based drinks had a more gradual increase in blood glucose and tended to experience less of a decline towards the end of the morning (Figures 18 and 21). Presumably participants were provided with a steadier and more consistent supply of energy if they had drunk milk than if they had drunk glucose. The consumption of water did not affect participants interstitial or blood glucose levels as it did not provide participants with a source of energy.

Since low GL meals have been associated with better cognitive functioning and mood in the late morning (Benton et al. 2003, 2007), it was predicted that a low GL milk based drink would improve both mood and cognitive functioning two to three hours after consumption compared to the high GL glucose or water. There were in fact several findings that the response to glucose and milk differed. In the second and third testing session the consumption of milk with caffeine, rather than glucose or water with caffeine, resulted in reports of increased energy (Figure 22). The time required to do mental arithmetic was longer in the serial sevens test 90 and 150 minutes after a drink when glucose or water, rather than milk, was consumed

(Figure 25). Reaction times on the vigilance task were faster during the fourth and fifth minute of the test if participants drank milk rather than glucose. There was also a trend for more words to be recalled after 150 minutes when milk rather than glucose had been consumed (Figure 24).

Many studies have reported similar decrements when examining the effects of a glucose drink on mood and cognitive performance. In general previous findings suggests that even if the consumption of glucose is initially beneficial, after two or three hours the subsequent fall in blood glucose may have negative consequences for both mood (Donohoe and Benton, 1999a) and memory (Donohoe and Benton 1999b, 2000; Benton et al., 2003). Thus if consuming glucose has longer term negative consequences the possibility arises that other types of nutrition that sustain blood glucose homeostasis may have a longer benefit. This expectation was confirmed by the present study; energy provided in the form of milk rather than glucose benefited in aspects of mood and cognition in the late morning (Figures 21 to 25).

Although the benefits of a low compared with high GL meals or snacks are often interpreted as a consequence of changes in postprandial blood glucose levels actual blood glucose measurements have not been consistently found to correlate with performance (Fischer et al, 2001; Benton et al, 2003; Benton and Nabb, 2004). A change in blood glucose level has various metabolic consequences, both centrally and peripherally: for example, changes in levels of acetylcholine, serotonin, glutamate, insulin, glucagon and cortisol (Hoyland et al, 2008). The

mechanism by which low GL benefits cognition and mood thus may involve one or many correlates of blood glucose.

The effect of caffeine

It is now commonly accepted that caffeine can improve certain aspects of cognition and mood, however, negative effects of caffeine on glucose homeostasis have been previously reported. In the present study the addition of caffeine, irrespective of vehicle, was associated with higher levels of glucose. This effect of caffeine has been documented previously. For example Pizziol et al (1998) in a placebo-controlled study found that 200mg caffeine increased blood glucose levels during the third and fourth hour of an oral glucose tolerance test (OGTT) that involved the consumption of 75g of glucose. In addition Graham et al, 2001 reported that after the consumption of 5mg/kg caffeine (average of 325mg) participants had a 24% greater area under the curve (AUC) for blood glucose and a 60% greater AUC for serum insulin during the last 90 minutes of an OGTT. In a similar fashion, Moisey et al (2007) investigated the effect of caffeinated coffee (5mg/kg caffeine), compared to decaffeinated coffee, given with either a high (GL 65.75) or a low glyceamic load (GL 30.75) meal. They found that caffeinated coffee was associated with a larger area under the curve (AUC) for both blood glucose and insulin, suggesting significantly impaired glucose management after the consumption of caffeine. Insulin sensitivity was also significantly reduced when caffeine was taken with the high GL meal but not when it was taken with the low GL meal. Although these findings are relevant, the studies used fairly high doses of caffeine (200-350mg), higher than what would be found in a standard cup of coffee or most energy drinks. However the present study demonstrates that the ingestion

of a smaller dose (80mg) of caffeine similarly impairs glucose tolerance and results in a disruption of blood glucose homeostasis.

In regards to cognition, when participants consumed caffeine it produced a series of changes in performance of the type that would be expected from previous research. Its consumption resulted in recalling more words, quicker reaction times in the choice reaction time test and the working memory task, and better and quicker responses on the vigilance task. Subjective energy levels were also increased 30 minutes after consumption when taken with water (Table 20). These effects of caffeine have been frequently reported and reviewed (Smith, 2002; Glade, 2010; Nehlig, 2010) and are probably the result of caffeine's antagonistic actions at the A₁ and A_{2A} subtypes of the adenosine receptors (Lorist and Tops, 2003).

Although there was consistent evidence that caffeine speeded reaction times there was an interesting failure to find a consistent change in subjective mood over the morning: rather the response depended on the vehicle to which it was added (Figures 22 and 23). Later in the morning the addition of caffeine to milk-based drinks increased subjective energy. In contrast, caffeine made little difference to ratings of energy when combined with glucose. Later in the morning caffeine resulted in feeling more tired if it was combined with water, a novel finding as reviews of the topic have suggested that in "low to moderate doses, caffeine have been quite consistently shown to increase positive affect" (Smith et al., 2004). Similarly, Rogers et al. (2011) concluded that "a clear result is that caffeine compared with placebo increases alertness". Although such comments are

received wisdom they reflect mostly studies of the short-term response to consumption rather than the effect after two to three hours. To date the longer term effects of caffeine consumption have been considered less frequently.

Furthermore, there has been only limited attempts to consider the nature of the drink in which caffeine was administered. To our knowledge only six studies have systematically examined the effect of different vehicles in combination with caffeine and their effects on mood or cognition. Such vehicles have included tea (Smith, Sturgess, et al, 1999; Quinlan, Lane, et al, 1997;1999), coffee (Liguori, Hughes, et al, 1997; Smith, Sturgess, et al, 1999; Quinlan, Lane, et al, 1997;1999; Hindmarch, Rigney, et al, 2000), diet cola (Liguori, Hughes, et al, 1997; Smith, Sturgess, et al, 1999), cola (Smith, Sturgess, et al, 1999), water (Quinlan et al, 1999) and the effect of adding milk to tea or coffee (Quinlan, Lane et al, 1997). All reported a main effect of caffeine on measures of mood regardless of the vehicle. Although drinks such as tea and coffee contain different phytonutrients they are similar in their macronutrient content to the extent that these studies essentially examined the effect of caffeine when taken with a water based drink. In addition, most of these studies only examined effects up to 90 minutes after consumption. With this in mind previous reports are not inconsistent with the present finding that after half an hour the consumption of caffeine was associated with reports that participants felt more energetic, but only if it was provided in a water-based drink (Table 20).

The only other context that has examined caffeine in combination with other ingredients is 'energy drinks'(ED). There are reports that EDs (glucose, caffeine and a range of other ingredients) increase alertness (Alford et al., 2001; Reyner

and Horne, 2002; Kennedy and Scholey, 2004; Smit et al., 2004), attention (Alford et al., 2001; Kennedy and Scholey 2004; Reyner and Horne, 2002; Warburton et al. 2001) and reaction times (Alford et al., 2001; Horne and Reyner, 2001). It has been concluded that caffeine is the component that is responsible for the beneficial effects of EDs (Van den Eynde et al, 2008). However, most often studies such as these have examined a 'whole' ED containing both glucose and caffeine and compared it to a placebo (usually water), making it impossible to determine the relative contribution of each ingredient. In addition, participants typically were tested 30 – 60 minutes after consumption providing only limited information on the time scale of such results. Interestingly, the present study found that those who drank a combination of caffeine and glucose rated themselves similar in energy to those that consumed only water at 30 minutes after consumption. It was only when considering the components individually that significant differences emerged. As expected caffeine when taken with water, when compared with water alone, increased reported energy after 30 minutes. However when caffeine was combined with glucose no such benefit was observed. This suggested that the consumption of glucose may have antagonised the effect of caffeine.

To our knowledge few studies have tried to distinguish the relative influence of caffeine and glucose. Scholey and Kennedy (2004) gave participants glucose, caffeine, a combination of both, or a placebo and found that compared to the placebo only the combination of glucose and caffeine improved memory and speed of attention. There were no effects on mood. They concluded that the pattern of results would not be predicted from the effects of glucose and caffeine in isolation and that there was some degree of synergy between the two. However, only the combination of glucose and caffeine differed significantly from the placebo and as

comparisons were not made with caffeine or glucose alone, the interaction between the two ingredients can not be determined. Adan and Serra-Grabulosa (2010) found that a combination of caffeine and glucose improved reaction times but only when compared with water, however both caffeine and glucose when taken alone also speeded reaction times compared with water. Adan and Serra-Grabulosa (2010) also reported that memory was improved after a combination of glucose and caffeine rather to water, caffeine alone or glucose alone, suggesting that there may be a synergistic interaction between the two ingredients. Adan and Serra-Grabulosa (2010) findings contrast with the present finding that only caffeine increased the number of words that could be recalled; there was no interaction with glucose, and glucose did not differ significantly from water. Similarly to Scholey and Kennedy (2004), Adan and Serra-Grabulosa (2010) found that there was no effect of either glucose or caffeine on mood. In both studies participants were tested 30 minutes after the drink and as the present study demonstrated that effects on mood may occur at a later stage this needs to be further considered.

Smit, et al, (2004) systematically studied the effects of glucose and caffeine taken alone, or together, and a placebo up to 90 minutes after consumption. Similar to the present results they found that caffeine speeded reaction times and that in the absence of caffeine perceived energy decreased over time. The decrease in energy over time meant that significant benefits of caffeine were seen after 60 minutes; glucose had no effect. Although this supports the notion that effects on mood may appear at a later stage Smit et al. (2004) found that caffeine improved energy irrespective of glucose. In contrast, the present study suggested that the effect of caffeine may be dependant on the vehicle with which it is consumed. Ninety minutes following the drink caffeine only increased energy in

those that consumed it with milk (Figures 22 and 23). Although those that drank glucose and caffeine rated themselves as more energetic than those that consumed water and caffeine, this reflected a negative response: participants became more tired if they drank caffeine with water.

To our knowledge only one study has so far examined the effect of ED components longer than 90 minutes post consumption. Giles et al (2012) studied the effects of 200mg caffeine, with or without taurine, in participants who did or did not consume glucose (50g) up to 120 min post consumption. Similar to the present findings caffeine improved attention and speeded up reaction times. In addition glucose (regardless of caffeine) slowed down reaction times. Furthermore, in line with the present finding, that caffeine when taken with water reduced ratings of energy 90 and 150 minutes after a drink, Giles et al. (2012) reported that between 0 (time of drink) and 60 minutes caffeine reduced fatigue, however, when caffeine was taken with taurine fatigue increased between 0 and 120 minutes. These findings support the notion that over time, under certain circumstances, caffeine may reduce reported energy levels. Also consistent with the present finding Giles et al. (2012) found no effect of glucose on any measure of mood.

Given the present observation that caffeine impaired glucose tolerance, and that participants who consumed milk had a steadier interstitial glucose response, it is possible that alterations in blood glucose homeostasis may mediate interactions between caffeine and vehicle. Indeed it was clear that of the participants who had drunk caffeine and water, those who rated themselves the most tired, had the highest glucose levels at the end of the morning (Appendix 15 and 16). Impaired

glucose tolerance has been related to poorer cognition (Donohoe and Benton, 2000) and mood (Nabb and Benton, 2006a,b) As potentially a similar decrement may occur when an artificial state of impaired glucose tolerance is produced by the consumption of caffeine this possibility should be further examined.

The present results should not, however, be over-interpreted or over-generalized. The results of any study potentially reflect specific aspects of the design. The generality of the findings need to be established and critical parameters established. It is possible that the negative response to caffeine and water, observed in this study, reflected the consumption of the high GI breakfast that was followed by no additional food consumption. 'Participants' blood glucose were already declining at the time of the drinks. It is possible that the consumption of caffeine without additional energy further compromised blood glucose homeostasis resulting in the decline in subjective energy. It is possible that a low GL breakfast might have produced a different pattern of results. Many previous reports of a positive response to energy drinks and caffeine used participants who had fasted overnight, a condition that is not likely to occur typically in everyday life. Fasted participants are also caffeine deprived. To what extent have the phenomena presently reported been a reflection of the reversal of withdrawal effects? The highly demanding, long and monotonous testing procedure may also have contributed to an increased level of tiredness, after drinking caffeine and water. It is unclear whether similar levels of tiredness would have been reported in the absence of these conditions. These methodological differences need to be further considered.

The aim of this chapter was to determine whether caffeine, which causes glucose intolerance, interacts with the GL that it is consumed with, to affect cognition and mood. Caffeine when taken with water reduced energy levels 90 and 150 minutes later. In contrast, taking caffeine with milk (low GL) increased energy levels during this time. Although taking caffeine with glucose (high GL) prevented the decline in energy seen when caffeine was taken with water, an increase in energy was not observed following this combination (Figures 22 and 23). Interesting questions emerge from the present study. If an adverse response to adding caffeine to water based drinks is confirmed there are obvious implications. Would comparable findings result from the consumption of coffee, tea, cola-type or ED? This is an important issue as coffee, for example, is widely consumed with the anticipation that it will decrease tiredness or to maintain alertness when driving. The current data suggest that this effect may be short lived and in the longer-term even detrimental. So far the questions have been mostly ignored as to whether the effect of caffeine depends on the macronutrients with which it is consumed and the time scale over which it is measured.

CHAPTER 6

The role of endothelial dysfunction in glucose tolerance related cognitive decline – a pathway for nutritional interventions?

6.1 Introduction

In summary, the data presented found that older adults with poorer GT had a greater degree of amnesic cognitive decline and rated themselves more anxious and depressed than those with better GT (Figures 12 and 13). It is note worthy that these effects were stronger in those aged 61 or above. Interestingly, a tendency for LBG to fall below baseline was not related to poorer cognition, in fact older adults with poorer GT and LBG below baseline had less cognitive decline and depression than those with poorer GT and no LBG (Figures 12 and 13). In both children and healthy older adults a lower rather than a higher GL meal improved cognition and mood, most notably during the late postprandial period (Figures 4-9 and 14-16). This, however, was not the case for older adults with poorer GT, or those with early signs of cognitive decline. Although older adults with poorer GT had a transient improvement in memory and mood following a high GL meal, this was limited to the first 30 minutes after consumption (Figures 14 - 16). In young adults caffeine induced a mild degree of glucose intolerance (Figures 19 and 20). Although caffeine (taken with water) increased subjective energy after 30 minutes, it resulted in decreased energy levels later in the morning (Figure 22). However, caffeine taken with milk was associated with an increase in subjective energy after 90 and 150 minutes (Figure 22). There were no effects of caffeine on ratings of energy when it was taken with glucose. Drinking a lower GL drink (milk

or water rather than glucose), regardless of caffeine, improved young adults episodic and working memory 150 minutes later.

This chapter presents a theoretical overview that attempts to account for these findings and highlights possible areas for future research. First glucose uptake and metabolism in the brain are reviewed in greater detail than in chapter 1; a particular emphasis is placed on the role of the epithelial cells of the BBB. This is followed by a detailed account of the evidence that suggests nitric oxide plays a critical role not only in epithelial – mediated vasodilatation, and hence cerebral blood flow, but also in glucose uptake and utilisation by the brain. Next it is argued that these vital mechanisms are impaired in both aging, and those with glucose intolerance, and may help account for the cognitive deficits that occur when these conditions combine. With this backdrop it is explained how this perspective can account for many of the findings of this thesis. Finally, areas of future research based on this theoretical perspective are suggested.

6.2 The supply of energy to the brain.

Due to the relative lack of stored brain carbohydrate (except a small amount of glycogen, mostly in astrocytes) the brain relies upon continuous cerebral blood flow and the passage of glucose across the BBB to meet its energy needs. GLUT1, the main glucose transporter, is found at the luminal and abluminal membranes of the BBB endothelial cells and plays an essential role in the facilitated diffusion of glucose across the membrane (Farrell et al, 1991). Additional glucose transporters have also been identified at BBB endothelial cells; GLUT3 (Gerhart et al, 1992), GLUT4 (Ngarmukos et al, 2001) SGLT1 (sodium-dependent

glucose transporters) (Matsuoka et al, 1998) and SGLT2 (Martin et al, 2006) but at much lower levels than the GLUT1 protein (Shah et al, 2012). Thus any changes affecting GLUT 1 function and expression will dramatically affect brain glucose homeostasis and brain functioning. An excellent example of this is a rare disorder De Vivo disease; an autosomal dominant developmental disorder associated with a deficiency of GLUT 1 which is characterized by a low glucose cerebrospinal fluid concentration (hypoglycorrachia), a type of neuroglycopenia, that results from impaired glucose transport across the BBB (Lankford et al. 2012).

As would be expected regional glucose use and consumption in the brain is strongly related to local blood flow (Clarke and Sokoloff, 1999), thereby linking neuronal activity, metabolic rate, and blood flow. Upon the activation of local cortical and subcortical networks, during cognitive demand, there is an increase in energy consumption, followed by a drop in local interstitial glucose, resulting in an increased need for energy substrate delivery and transport (McNay et al, 2000; 2001). This localized drop in glucose levels within the brain increases the transbarrier concentration difference and thus, via GLUT1, drives glucose entry into the brain interstitium (Leybaert et al, 2007). The supply of blood and nutrients to meet the local needs of the brain cells also relies upon the process of neurovascular coupling, that is, the coupling of vessel diameter and thus blood flow, to neuronal activity (Harder et al, 1998; Kuschinsky, 1997). This process is highly, although not exclusively, dependent on the ability of brain endothelium to induce vasodilatation (Girovard and Ladecola, 2006; Toda et al, 2010).

6.3 The role of nitric oxide

Nitric oxide (NO) is a small inorganic, labile, gaseous molecule, which is produced by the endothelium and mediates the relaxation of blood vessels (Furchgott and Zawadzki, 1980). NO and L-citrulline are synthesized by the enzyme nitric oxide synthase (NOS) through a two-step conversion process from L-arginine and the intermediate N-hydroxy-L-arginine. This enzymatic reaction requires L-arginine, oxygen, and NADPH as co-substrates (equation 1). The guanidino nitrogen in L-arginine and oxygen provide the nitrogen and oxygen atoms in NO, respectively. The co-factors involved in the reaction are flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and tetrahydrobiopterin (BH₄). Calmodulin and heme are the prosthetical groups involved in the reaction (Knowles, 1997). In vivo NO has a half-life of only 1-2ms, however, it can be oxidised to nitrate and nitrite that have half-lives of 5-8hrs and 1-20min respectively (Lidder and Webb 2013). These molecules are transported in the blood, accumulate in tissue and have the potential to be converted back to NO under particular physiological and pathological conditions, for example, during hypoxia when the oxygen dependent NOS is compromised (Lundberg et al, 2008).

Equation 1



There are three isoforms of NOS, the genetic sequence of each residing on three distinct chromosomes: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS), however, it is now known that nNOS and eNOS are also expressed in

other tissues, for example, the mitochondria (also known as mtNOS) (Law et al, 2001). ENOS is responsible for NO-endothelial mediated vasodilatation.

6.3.1 Nitric oxide may mediate neurovascular coupling.

NO formed via endothelial NO synthase (eNOS) induces vasodilatation, blood flow increase, and reduces blood pressure; effects that are mediated by the binding of NO to soluble guanylyl cyclase (cGC) and the subsequent production of cyclic guanosine monophosphate (cGMP) (Toda et al, 2010). In the brain, relationships have been observed between neuronal activation, NO production and blood flow (Okamura et al, 2002). Indeed, although the quest for mediators is ongoing, it has been argued that NO itself might directly mediate the neurone to vessels communication and thus has an important role to play in the coupling of neuronal activity and increased blood supply aka neurovascular coupling (Girovard and Iadecola, 2006; Laranjinha et al, 2012). There is evidence that the vasoactive actions of glutamate were reduced by nitric oxide synthase (NOS) inhibitors (e.g. N-nitro-L-arginine) (Lovick et al, 1999; Meng et al, 1995; Dirnagl et al, 1993) and that local application of NOS inhibitors suppressed the activity-dependent vasodilatation by about 50% (Duchemin et al, 2012). Interestingly both eNOS and nNOS derived NO appear to be important (Toda et al, 2009). For example, it has been argued that the release of nNO from interneurons upon activation initiates the vascular response to neuronal stimulation (Duchemin et al, 2012), although more research is needed to confirm if this is the case. Whether NO mediates neurone to vessel communication, or not, there is no doubt that NO plays crucial roles in the regulation of blood flow in various organs including the brain (Toda et al, 2010).

The relationship between NO and neurovascular coupling is illustrated in Figure 29.

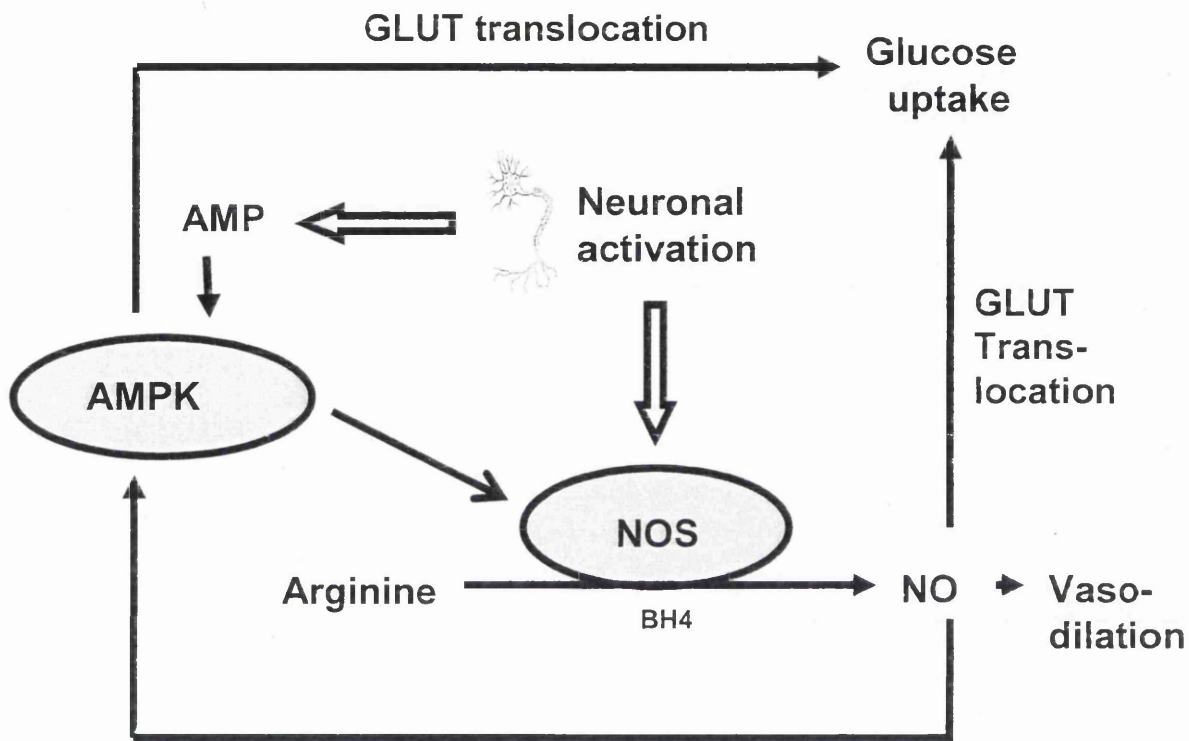


Figure 27. A schematic representation of the roles of AMPK and NOS in neurovascular, neurometabolic and neurobarrier coupling. Upon neuronal stimulation energy is consumed and hence AMP is increased. The increase in AMP increases AMPK activity which enhances GLUT translocation and increases glucose uptake. Simultaneously, neuronal activation, both directly and indirectly via AMPK, activates NOS, increasing NO, leading to vasodilation.

6.3.2 Nitric oxide may regulate neurometabolic and neurobarrier coupling.

Interestingly, eNO activity also appears to be intimately related to that of adenosine-3',5'-monophosphate-activated protein kinase (AMPK) (Chen et al, 2000; Shearer et al, 2004; Zou et al, 2004) which suggests that eNO may play a vital role in the regulation of the metabolism of energy substrates. The overall effect of AMPK, via a number of mechanisms, is to switch off the ATP-consuming pathways such as lipogenesis or gluconeogenesis while switching on the ATP-producing pathways such as fatty acid and glucose oxidation (Jobgen et al, 2006). Previous studies have revealed that AMPK promotes the production of endothelium-derived NO (Chen et al, 2009; Schulz, 2009). For example, experiments with aortic rings in mice lacking AMPK showed reduced eNOS phosphorylation and AMPK knockout mice displayed decreased eNOS-mediated NO production (Chen et al, 2009). Interestingly, NO itself can activate AMPK (Zhang et al, 2008); AMPK-mediated eNOS activation might lead to a sustained NO release owing to a positive feedback loop (Figure 29). It appears that eNO may mediate AMPK stimulated glucose uptake. For example, NOS inhibitors diminished AMPK-stimulated glucose uptake (Fryer et al, 2000; Li et al, 2004).

Stimulation of AMPK activity increased glucose uptake by GLUT 4 in skeletal and cardiac muscle during exercise (McConnell and Kingwell, 2006; Hayashi et al, 1998; Li et al, 2004) via an insulin independent mechanism. In the brain AMPK activation caused an increase in the surface expression of the neuronal glucose transporter GLUT3 (Weisova et al, 2009) and similar observations have been made regarding GLUT1 transporters in the plasma membrane (Abbud et al, 2000; Barnes et al, 2002). As mentioned previously, GLUT 1 is the glucose transporter that is found in

the epithelial cells of the BBB. Thus NO could modulate the transport of glucose by GLUT 1 and hence brain interstitial glucose levels. It has been argued that in the brain AMPK is a crucial mediator that couples increased energy demands with neuronal activity and glucose uptake (Amato and Man, 2011), and there is evidence that eNO is at least partially responsible for this effect (McConnell and Kingwell, 2006; Ciudad et al, 2004). Taken together these findings suggest that eNO may play a vital role in the uptake of glucose in the brain. It is possible that AMPK-eNOS-mediated vasodilatation and GLUT expression provides a means to increase blood, and hence glucose supply, to neurons during metabolic demand, providing a link between metabolism and blood perfusion (Schulz, 2009).

In the periphery NO may also play a vital role in insulin stimulated glucose transport in skeletal muscle and adipose tissue and hence affects gluco-regulatory ability. Inhibition of NOS impairs glucose tolerance in rats (Roy et al, 1998) and humans (Baron et al, 1995). Dietary supplementation of arginine (NOS substrate) has been shown to reduce plasma glucose levels in streptozotocin-induced diabetic rats (Kohli et al, 2004) and Zucker diabetic fatty rats (Fu et al, 2005). These latter effects may be mediated by an increase in blood flow and hence glucose delivery to muscle and adipose tissue (Baron et al, 1995), an increase in insulin receptor sensitivity, or an increase in the surface expression of GLUT 4. Therefore any factor that affects the bioavailability or production of NO may simultaneously impair endothelial functioning, glucose tolerance, the supply of energy to the brain, glucose metabolism within the brain and cognitive function. Indeed a growing body of research implicates both eNOS and nNOS derived NO in the modulation of learning and memory in experimental animals (Bohme et al,

1991; Haley et al. 1992; Chapman et al, 1992; Rickard et al, 1988; 1999; Bernabeu et al, 1995).

6.3.3 Aging reduces NO availability and impairs endothelial and cognitive function.

Aging is associated with changes in cerebrovascular structure and functioning which may contribute to cognitive decline (Sonnteg et al, 2007). For example, fMRI studies have demonstrated a reduction of cerebral activation in older participants, which may be associated with age-related changes in the mechanism that links neuronal activity to vascular changes (Nielson et al, 2004; Taoka et al, 1998). Similarly, a recent review of transcranial doppler ultrasonography studies concluded that aging was associated with a slowing of blood flow velocity (Keage et al. 2012). These changes may, at least in part, be attributed to age related endothelial dysfunction (Figure 30).

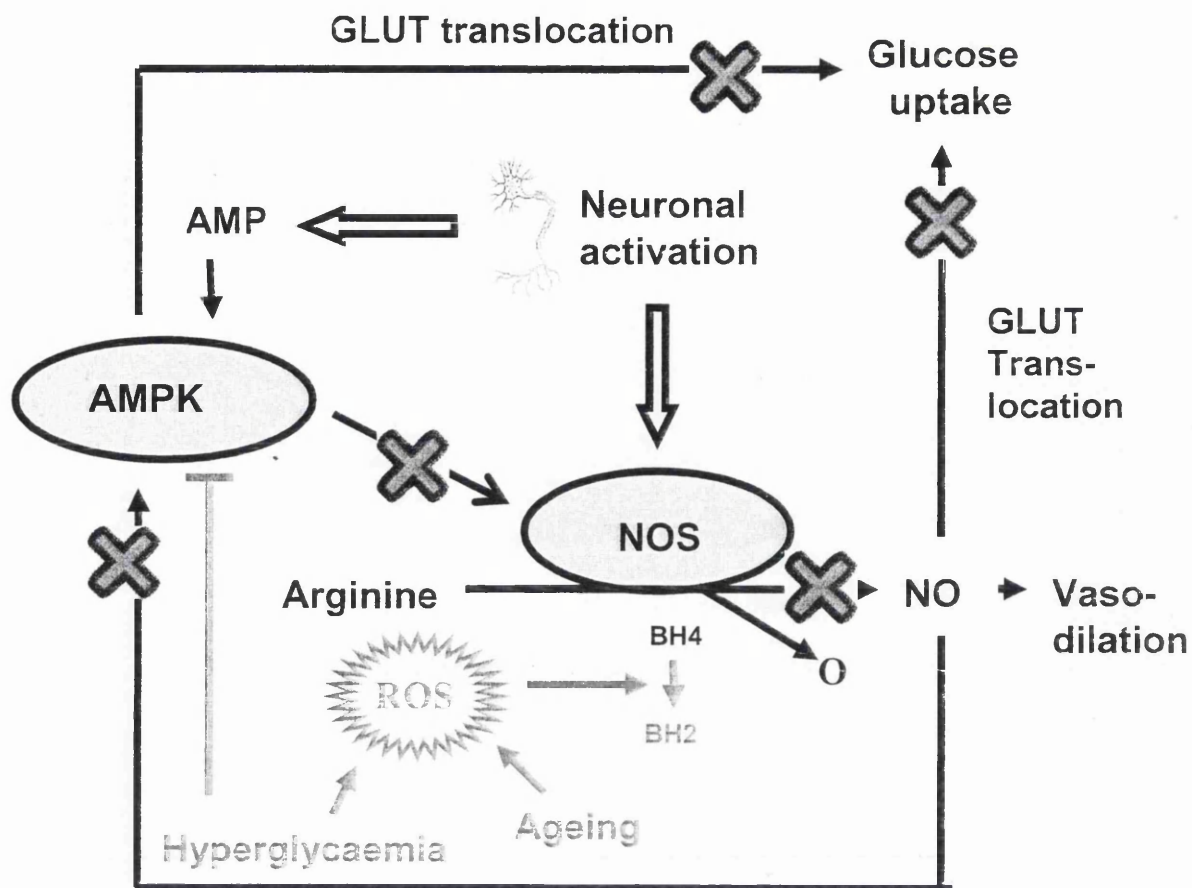


Figure 28. A schematic representation of the effect of hyperglycaemia and aging on AMPK and NOS activity and neurovascular, neurometabolic and neurobarrier coupling. In both aging and hyperglycaemia there is an increase in reactive oxygen species (ROS) that leads to NOS uncoupling due to the oxidation of BH4 to BH2. As a result less NO is available thus vasodilation, AMPK activity, GLUT translocation and glucose uptake are reduced. Hyperglycaemia also directly inhibits AMPK activity further reducing neuronal glucose availability.

When we are young and healthy, the endothelial production of NO is effective and sufficient, however, as we age we lose our ability to synthesize endothelial derived NO (Torregrossa et al, 2011). For example, Taddei et al (2001) studied endothelium dependent forearm blood flow and concluded that dysfunction of the NO system contributed towards reductions in endothelium functioning in older adults. Similarly, Egashira et al (1993) reported that there may be up to a 75% loss of endothelium-derived nitric oxide in 70-80 year olds compared to 20 year olds. In regards to cerebral blood flow Okamoto et al (2001) reported that the administration of L-arginine (NO substrate) increased cerebral blood flow velocity to a lesser extent in older participants (average 70 years of age) than younger participants (average 29 years of age), suggesting a diminished NOS activity, or NO availability, and hence reduced NO-mediated cerebral vasodilatation in aging participants. Similar effects have also been observed in aged rats, for example Mayhan et al, (2008) found that NO dependant dilatation of cerebral arterioles was impaired in aged compared to younger adult rats. Similarly, Geary and Buchholz (2003) noted that aging increased cerebral artery tone and reduced NO dependant dilatation in male rats. Taken together these findings suggest that with aging cerebral blood flow reactivity is reduced, possibly the result of diminished NO – mediated vasodilatation.

Age related reductions in NO could also alter BBB transport of glucose via GLUT 1 (de al Torre and Stefano, 2000). Emerging studies indicate that the responsiveness of AMPK signalling declines with aging, impairing metabolic regulation. For example, Reznick et al (2007) found that acute stimulation of AMPK activity (by 5'-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside) was reduced

in aged rats. Similarly, Ljubicic and Hood (2009) demonstrated that the activation of AMPK, induced by muscle contractions, was reduced in the muscles of older individuals. Given that NO is thought to mediate AMPK stimulated glucose uptake (Fryer et al, 2000; Li et al, 2004), reduced NO availability may account for these findings. In line with this Deshmukh et al (2010) found that treatment of skeletal muscle with a NO donor increased AMPK mediated glucose uptake. As previously mentioned AMPK activation causes an increase in the surface expression of the neuronal glucose transporter GLUT3 (Weisova et al, 2009) and GLUT 1 in the plasma membrane (Abbud et al, 2000; Barnes et al, 2002). Thus it is possible that a decline in AMPK signalling may inhibit glucose transport across the BBB and into neurons when needed. Consistent with this, is evidence in rodents that suggests that the drop in interstitial glucose levels, associated with neuron activity, is deeper and lasts longer in older animals suggesting a reduced ability to adapt to the depletion of interstitial glucose (McNay and Gold, 2001). This diminished supply of fuel produces a less than perfect environment for neuronal functioning which may contribute to age-related deficits in learning and memory. These findings may also help explain the lower brain glucose metabolism observed with PET scans in older participants (Bentourkia et al, 2000; Kalpouzios et al 2009), MCI (Mosconi et al, 2005; 2009) and those with dementia (Drzezga et al, 2003; Mosconi et al, 2005; 2009). Given the hippocampus's vulnerability to damage (Mattson et al, 1989; McEwen, 1997) it is possible that over time a combination of reduced blood flow reactivity, and diminished glucose transport, could lead to neuronal death and a loss of hippocampal volume.

There are probably many mechanisms underlying age related reductions in NO availability. It has been suggested that age associated superoxide production may be responsible for reducing available NO (Mayhan et al, 2008; van der Loo et al, 2000). Superoxide can react with NO to produce peroxynitrite (van de loo et al, 2000), which, in turn, can oxidize tetrahydrobiopterin (BH₄) (the co-factor of eNOS) to dihydrobiopterin (BH₂), thus reducing its availability to eNOS (Pitocco et al, 2010). In the presence of reduced concentrations of BH₄, eNOS becomes uncoupled and transfers electrons to molecular oxygen instead of L-arginine to produce superoxide, rather than NO, thus promoting a perpetuating decline in NO availability and endothelial dysfunction. The relationships between aging, superoxide formation, NO, AMPK, vasodilatation and glucose uptake can be seen in Figure 30.

6.3.4 Hyperglycaemia reduces NO availability and impairs endothelial and cognitive function.

Type two Diabetes mellitus (T2DM) has been associated with both vascular and Alzheimer's dementia (Craft, 2009). Furthermore, in those without diabetes a reduced ability to regulate blood glucose is associated with poorer attention (Donohoe et al, 2000), slower reaction times (Yaffe et al, 2012; Donohoe et al, 2000) and poorer memory (Yaffe et al, 2012; Awad et al, 2002), changes in functioning that are more pronounced in older age (Ryan and Geckle, 2000). Currently one in every 10–15 cases of dementia can be attributed to T2DM (Kloppenborg et al, 2008). There is evidence that the age-related reduction in cerebral blood flow may be accelerated in T2DM (Wakisaka et al, 1990). Similarly, cerebral artery reactivity has been reported to be reduced in T2DM compared to

controls (Moghaddasi et al, 2010). Therefore it appears that the natural changes that occur with age may be exacerbated further by conditions such as T2DM. It has been documented in human studies that endothelial cells in T2DM fail to produce sufficient amount of NO, thus smooth muscle cells fail to relax in response to endothelium-dependent vasorelaxants (e.g. acetylcholine, bradykinin, shear stress, etc.) (Avogaro et al, 2006 for a review). The development of diabetes mellitus is characterized by high serum glucose levels. Glucose is a prooxidant molecule that can lead to the overproduction of reactive oxygen species (ROS) such as superoxide and hydrogen peroxide. The mitochondrion represents an important source of reactive oxygen species in the diabetic endothelium. During hyperglycaemia there is an overproduction of electron donors from the tricarboxylic acid cycle and this causes the mitochondrial proton gradient to increase. It is suggested that there is a threshold over which the life of superoxide-generating electron transport intermediates is prolonged (Nishikawa et al, 2000), once this threshold is exceeded superoxide production is markedly increased (Korshunov et al, 1997). Thus an overproduction of oxidant molecules in T2DM may reduce the bioavailability of NO, cause endothelial dysfunction and hence reduce cerebral blood flow.

AMPK signalling is also negatively regulated by chronic exposure to high levels of glucose. For example, incubation of epithelial cells with high glucose concentrations reduces AMPK activity (Ido et al, 2002) and therefore reduces glucose uptake by all cells (Viollet et al, 2010), including neurons (Weisova et al, 2009) and the plasma membrane (Abbud et al, 2000; Barnes et al, 2002). In addition, studies show that GLUT 1 is down regulated in animals with diabetes,

possibly as a result of chronically elevated blood glucose levels (Pardridge et al, 1990). This suggests that glucose transport across the BBB is likely to be reduced in those with poorer GT. Figure 30 illustrates the relationships between hyperglycemia, superoxide formation, NO, AMPK, vasodilatation and glucose uptake.

6.4 Relevance to the present findings.

This thesis presented a number of findings that may be explained by the theory presented above.

6.4.1 The combined effects of aging and hyperglycaemia.

In chapter 3 it was reported that adults 61 years and over with poorer GT had poorer memory than younger participants with poorer GT or older participants with better GT. In fact it is commonly observed that glucose intolerance associated cognitive decline is more pronounced after age 60 (Convit et al, 2003; Ryan and Geckle, 2000; Lamport et al, 2009). Both aging and hyperglycaemia lead to an increased production of oxidative species and hence a reduction in the bioavailability of NO and endothelial dysfunction. It is possible that this combined deleterious effect of hyperglycaemia and aging is enough to result in a marked reduction in cerebral blood flow and glucose transport across the BBB, which in turn deprives neurons of their fuel supply and consequently results in cognitive decline (Figure 30).

6.4.2 Low blood glucose, AMPK and cognitive decline.

The novel finding that older adults with poorer GT and LBG had less cognitive decline and depression, than those with poorer GT and LBG above baseline, was presented in chapter 3. To our knowledge the effects of low levels of blood glucose on brain adaptation and cognitive decline in humans has not been considered. However, similar effects have been observed in diabetics (Lobmann et al, 2000) and animals (McNay et al, 2006; Boyle et al, 1994; Jacob et al, 1999; McNay and Sherwin, 2004). For example, animals exposed to low blood sugar levels (2.5-3.0mmol/l) have increased hippocampal interstitial glucose concentrations (McNay et al, 2006; McNay and Sherwin, 2004); an effect that subsequently enhances cognition and learning (McNay et al, 2006; McNay and Sherwin, 2004). Similarly, in humans Boyle et al. (1994) found that cognition was preserved during episode low blood glucose, if previous exposure had occurred. It is possible that this increase in brain glucose levels is enough to compensate for reduced glucose transport across the BBB in those with poor GT.

Although the mechanisms underlying these findings remain unclear falling glucose levels result in an increase in the AMP:ATP ratio and thus activate AMPK and eNOS (McCrimmon, 2008). As mentioned eNOS derived NO increases cerebral blood flow and AMPK results in increased glucose uptake via GLUT transporters. In line with this GLUT 1 at the endothelium (Boado et al, 1993; Kumagai et al, 1995), the neuronal glucose transporter GLUT 3 (Uehara et al, 1997) are both up regulated following hypoglycaemia, and these adaptations lead to an increased level of brain glucose (Lei et al, 2006). It is possible that our sample of older adults with poor GT and the tendency for LBG may have developed adaptations to

previously experienced LBG and that these adaptations were able to compensate for the deleterious effect of poor GT on cerebral blood flow and glucose transport (Figure 31).

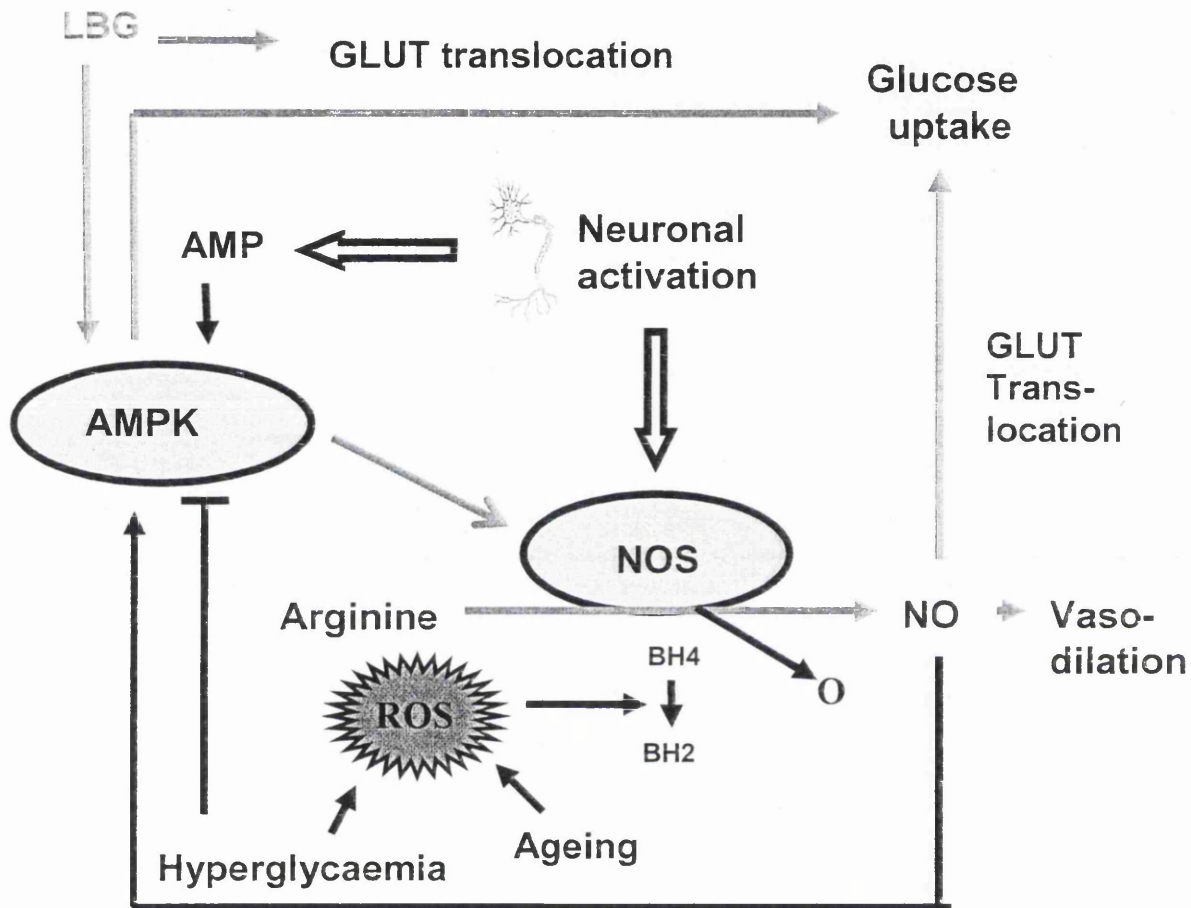


Figure 29. A schematic representation of the effect of LBG on AMPK activity and neurometabolic and neurobarrier coupling. LBG stimulates AMPK activity and increases GLUT translocation and glucose uptake. In turn the increase in AMPK activity stimulates NOS, increasing NO availability and vasodilation.

6.4.3. Endothelial function, glycaemic load and cognition.

In both children (chapter 2) and healthy older adults (chapter 4) a lower rather than a higher GL meal improved cognition and mood, most notably during the late postprandial period. As previously noted, chronic hyperglycaemia impairs endothelial function and there is also plenty of evidence suggesting that this is also the case during acute hyperglycaemia (Mah and Bruno, 2012 for a review). For example, it was found that blood flow to the forearm following compression is reduced by 20-42%, 30 – 90 minutes after an OGTT (Mah et al, 2011), and this effect remained for a period of time after blood glucose had returned to normal (Suzuki et al, 2012). Interestingly, the consumption of a lower rather than higher GL meals ameliorate these reductions in blood flow observed following a higher GL meal (Buscemi et al, 2012; Lavi et al, 2009). Brain imaging studies involving GL are few and to our knowledge there are none that have considered areas involving memory. A logical next step would be to examine the effect of GL on cerebral blood flow and glucose uptake/utilisation, and relate these to changes in endothelial functioning that occur in the periphery. There is also evidence that AMPK activity is increased after foods with a lower GL and decreased during hyperglycemia (Jun et al. 2011). These changes in AMPK activity may have implications for brain glucose uptake and metabolism. It is possible that changes in endothelial function and/or AMPK activity may mediate the effect of GL on cognitive performance. Figure 32 illustrates the relationship between GL, AMPK activity, endothelial function and glucose uptake in children and older adults with better glucose control.

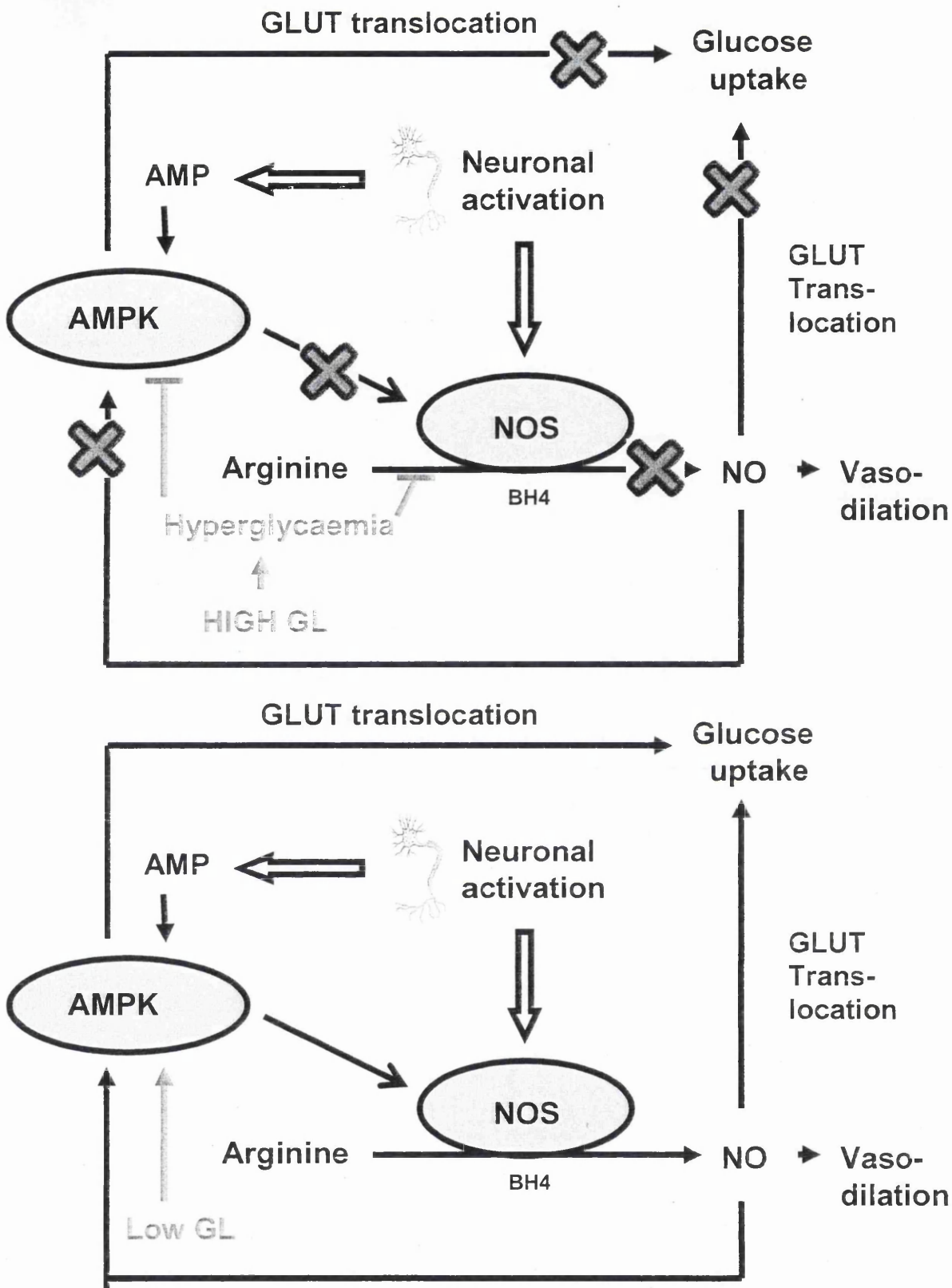


Figure 30. A schematic representation of the effect of GL on AMPK and NOS activity and neurovascular, neurometabolic and neurobarrier coupling. Meals that have a high GL result in hyperglycaemia which inhibits both NOS and AMPK and in turn reduces vasodilation, GLUT translocation and glucose uptake. However, meals that have lower GL's avoid hyperglycaemia allowing AMPK and NOS to function optimally. In addition meals with lower GL directly stimulate AMPK enhancing glucose uptake.

It was interesting that older adults with poorer GT did not benefit from a low rather than a high GL (chapter 4). One might predict that given their poor GT they may have some degree of endothelial dysfunction (Figure 30). Thus moderating the GL may prove beneficial for endothelial functioning and hence improve cognitive performance. However, endothelial dysfunction that occurs in such populations is a cumulative outcome that has occurred over an extended period of time. Thus it is unlikely that a single low GL meal will be enough to alter endothelial function significantly. It is possible that longer term low GL diet may prove beneficial, for example, Buscemi et al (2012) allocated obese participants to either a high or a low GL diet for three months. The low GL diet was associated with improvements in endothelial function and glycaemic variability. However, these participants were not diabetic and whether these vascular improvements translate into cognitive gains needs to be explored.

Of note was that older adults with poorer GT had a transient improvement in memory and mood 30 minutes after a high GL meal (chapter 4). Glucose intolerance is either the result of impaired insulin release from the pancreatic B cells, or a resistance to the effect of insulin by tissue receptors. The usual response to a rise in insulin resistance is compensatory hyperinsulinemia (Polonsky et al, 2000). Although we did not measure insulin it is possible that an increase in insulin levels following the high GL meal may have in some way been beneficial. Although the transport of glucose into the brain is not thought to be insulin dependent, insulin does modulate cognition (Shemesh et al. 2012) and acts as a vasodilator by increasing NOS activity (Muniyappa and Sowers, 2013). It is possible that an increase in insulin may in some way improve cognition in those

with poorer GT, perhaps though improved endothelial function (Figure 33). In support of this, Shimabukuro et al. (2004) found that although an OGTT impaired endothelial function in T2DM patients, this effect was ameliorated by a prior use of nateglinide (an insulin secretagogue). In addition, Hirvonen et al. (2011) found that a higher concentration of insulin was required to stimulate cerebral glucose metabolism in those with poorer GT, compared to healthy controls. Thus it is possible that the benefit of a high GL meal, to older adults with poorer GT, may not be attributed to an increase in plasma glucose per se but a simultaneous increase in insulin levels. Whether a beneficial effect of insulin is great enough to offset the negative effects of hyperglycaemia needs to be explored.

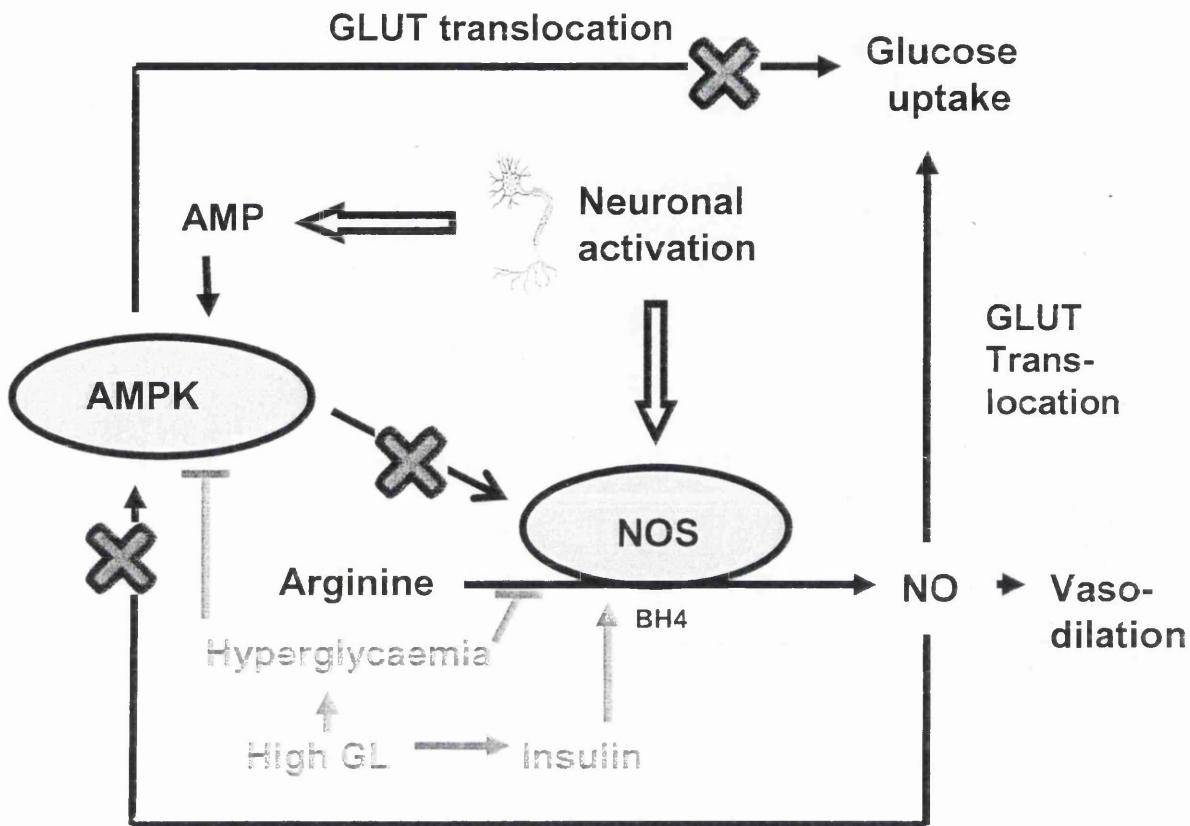


Figure 31. A schematic representation of the effect of a high GL and insulin on AMPK and NOS activity. Meals that have a high GL result in hyperglycaemia which inhibits both NOS and AMPK and in turn reduces vasodilation, GLUT translocation and glucose uptake. However, high GL's also increase insulin levels which increases NO production and vasodilation via the stimulation of NOS.

Glucose has also been shown to improve memory in older adults with cognitive decline (Riby et al, 2008), however, we did not observe this to be the case (chapter 4). Alterations in brain glucose metabolism are apparent in those with cognitive deterioration (Mosconi, 2007) and correlate with cognitive decline (Landau et al. 2012). These changes may potentially reflect a range of underlying pathologies: defects in brain glucose transport across the BBB; reduced cerebral blood flow, a disruption of glycolysis; impaired insulin signalling; mitochondrial dysfunction; neuronal atrophy (Cunnane et al, 2011; Correia et al, 2011). It appears, however, that even at this early stage of cognitive decline changes in the nature of glucose provision are no longer sufficient to enhance cognition, presumably a reflection of the malfunctioning of one of the above, or similar, mechanisms.

If indeed endothelial dysfunction has a role to play in glucose intolerance related cognitive decline it may not necessarily have a role to play in cognitive decline per se. There are many pathways to cognitive decline and only when a major cause specific to that individual is identified can there be hope of treatment. Unfortunately, much current research focuses on 'dementia', 'AD' or 'MCI' as if they are homogeneous groups; they are not. More research is needed that focuses on particular groups of individuals who are identified as 'at risk' due to a specific pathology such as glucose intolerance. In this way an intervention can become more individualised and potentially more effective.

6.4.4 Caffeine, hyperglycaemia, cognition and mood.

The final significant finding was that caffeine, when taken with water, increased subjective energy after 30 minutes but decreased energy levels later in the morning. However, when caffeine was taken with milk it was associated with an increase in energy after 90 and 150 minutes (chapter 5). When taken acutely caffeine induces glucose intolerance (Pizziol et al. 1998; Graham et al. 2001). Indeed we also reported that young adults who consumed caffeine had significantly higher blood glucose levels and that this persisted until the end of the study period (~ 180minutes) (Figure 21). How caffeine produces this effect is not clear, however, it may be mediated by an increase in the release of epinephrine (Thong and Graham, 2002). Epinephrine is known to both increase hepatic glycogenolysis and reduce glucose uptake by tissues (Vicini et al. 2002). In addition, because caffeine blocks the adenosine receptors it stimulates a reflex activation of the sympathetic system which leads to an increased heart rate and possibly increased arterial pressure (Marlies et al. 2005). These vasoconstrictor properties of caffeine also lead to reduced cerebral blood flow (Chen et al. 2009). However, at least in healthy participants, there appears to be a compensatory increase in oxygen extraction (Chen et al. 2009; Perthen et al. 2008) which represents the uncoupling of blood flow and oxygen metabolism in order to maintain cerebral metabolism. There is also evidence that cerebral glucose metabolism is increased despite reduced cerebral blood flow (Grome and Stefanovich, 1986). Interestingly evidence suggests that caffeine consumption also increases the production of NO (Echeverri et al, 2010) and AMPK activity (Egawa et al. 2009), which as previously mentioned have a powerful influence on glucose uptake and metabolism although caffeine induced hyperglycaemia is likely

to ameliorate this effect. It appears that the net effect of these mechanisms may be positive, or negative, depending on the circumstances (Figure 34).

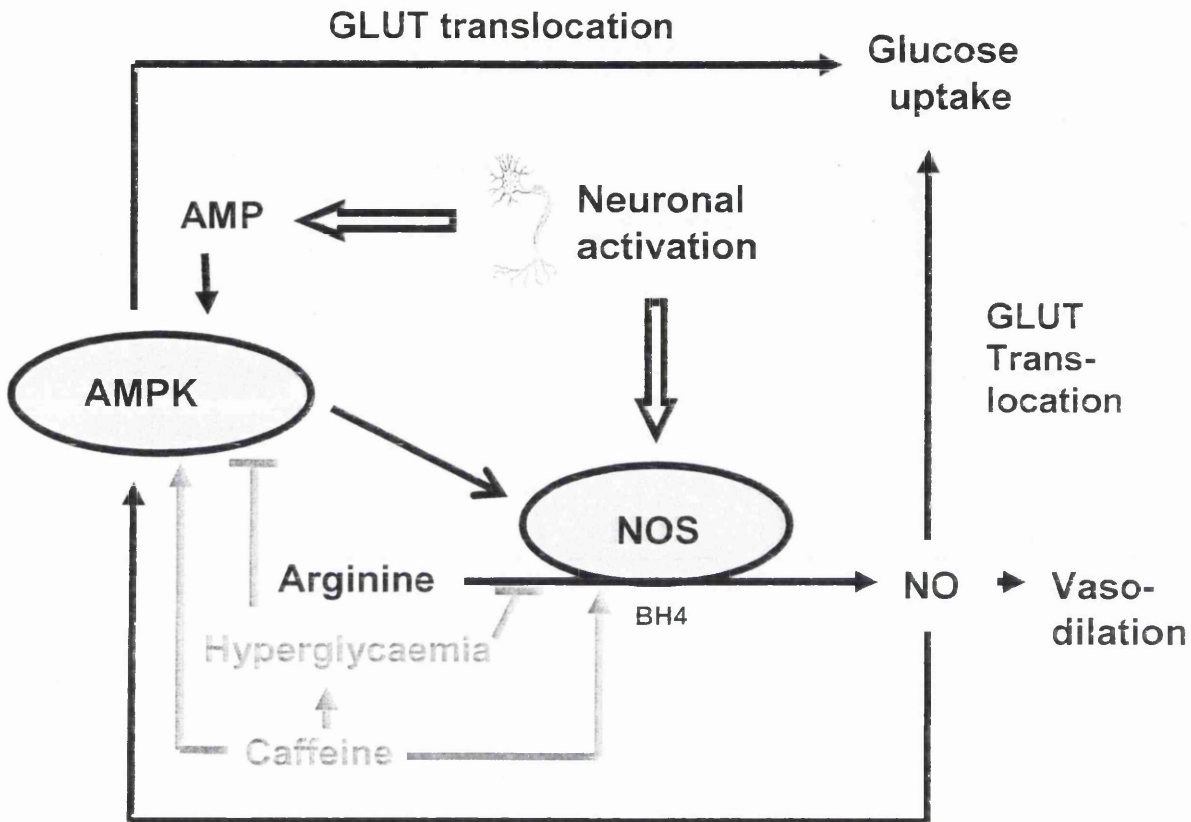


Figure 32. The effect of caffeine and hyperglycaemia on AMPK and NOS activity. Caffeine causes hyperglycaemia which inhibits both NOS and AMPK and in turn reduces vasodilation, GLUT translocation and glucose uptake. However, caffeine also activates NOS and AMPK which increases NO production, vasodilation GLUT translocation and glucose uptake.

The above mechanisms may help explain the findings observed in chapter 5. Our sample of young adults consumed a relatively small but high GL breakfast at 8.30am (cornflakes) and by the time they consumed their drink at 10am their blood glucose levels had already begun to drop. Those that consumed caffeine experienced a mild degree of glucose intolerance, suggesting a reduced uptake of glucose by the tissues. Caffeine may also have caused minor vasoconstriction and reduced cerebral perfusion. However, caffeine also increases glucose metabolism in the brain which may improve mood and cognition in the short term (as observed after 30min) (Table 12) but also heightens the need for delivery, especially when faced with ongoing demanding cognitive testing. Given that during neuronal activation there are localised drops in brain interstitial glucose levels, caffeine induced glucose intolerance and cerebral hypoperfusion may have prevented the timely delivery of energy, resulting in increasing fatigue as the morning progressed.

Importantly, when young adults consumed caffeine in a milk based drink they reported higher energy levels: participants were more energetic after 90 and 150 minutes. It is known that the consumption of milk produces a disproportionately high insulin response (Hoyt et al. 2005). As previously mentioned insulin may act as a vasodilator (Muniyappa and Sowers, 2013) and therefore may improve cerebral blood flow (Figure 35). In fact there was a much smaller difference in blood glucose levels towards the end of the morning in those that drank milk, and those who had drunk milk plus caffeine ($\sim 0.05\text{mmol/l}$), than there was between those that drank water and those that drank water plus caffeine ($\sim 1.3\text{mmol/l}$), suggesting that the consumption of milk (and possibly raised insulin levels) may have been

able to somewhat overcome the impaired glucose tolerance. The low GI of milk would also have provided a steady and consistent supply of energy over the morning, helping to prevent declines in mood and performance. Further work is needed to replicate this study and also test the effect of GL and caffeine over time under a range of circumstances, for example, after a low rather than a high GL breakfast.

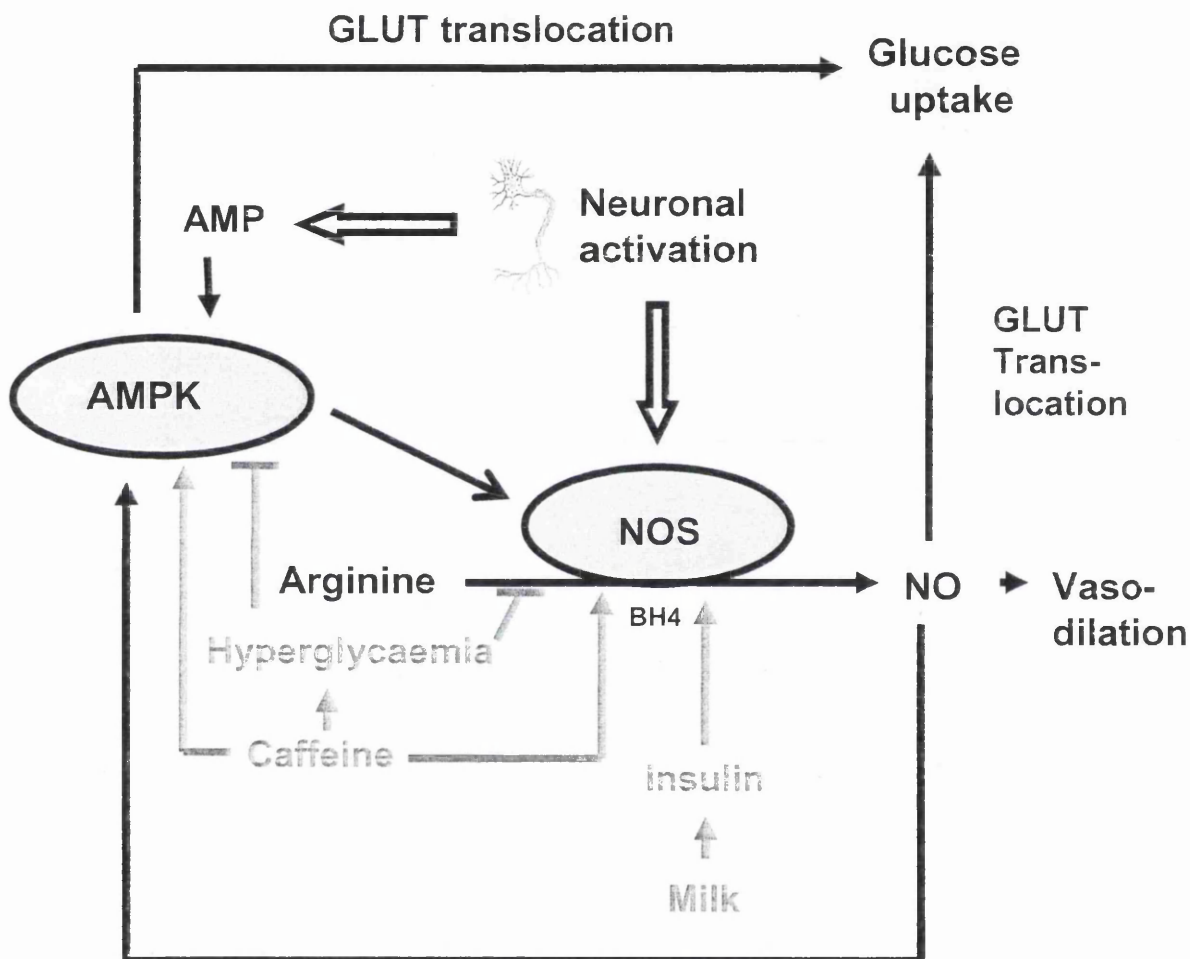


Figure 33. The effect of caffeine and milk on AMPK and NOS activity. Caffeine causes hyperglycaemia which inhibits both NOS and AMPK and in turn reduces vasodilation, GLUT translocation and glucose uptake. However, caffeine also activates NOS and AMPK which increases NO production, vasodilation GLUT translocation and glucose uptake. Milk causes an increase in insulin levels which increases NO production and vasodilation via the stimulation of NOS.

6.5 Concluding remarks

In summary, this thesis supports the conception that glucose tolerance is related to cognitive performance and mood, however, the relationship is affected by a number of factors including the age of the subject and the tendency to develop LBG. This thesis also provides evidence that under particular circumstances, such as prolonged periods of cognitive testing, caffeine-induced glucose intolerance can also lead to a decline in energy levels. Although caffeine induced glucose intolerance and fatigue may be ameliorated by the co-consumption of energy from foods; most notably in the form of low GI milk, cognitive decline caused by pathological reductions in glucose tolerance, cannot be altered by a low GL breakfast. However, both children and healthy older adults had better memory and mood after eating a low rather than a high GL meal, opening the possibility that modulation of GL maybe a useful preventative strategy in the longer term. Further research is planned to test the postulations put forward in this final chapter.

APPENDICES

Appendix 1. Parental consent form.

Breakfast study

Dear Parent,

We are writing because your child attends the breakfast club of _____ School. Swansea University is interested in finding out if what your child eats helps his or her school work. As you know if you miss breakfast you do not work as well during the morning. However, it is not known is whether some breakfasts are better than others. We are trying to find the best breakfast for children. To do this we are asking if your child can take part in a simple study. They will need to come to the club on two days, a week apart, without first eating at home. If you are happy for them to take part then please sign the next sheet.

Your son/daughter will attend the breakfast club as normal but on two days they will receive a breakfast of cornflakes with milk and sugar, a yogurt and a drink. Although they will look and taste the same, one will release energy quickly and the other more slowly. They contain different naturally occurring sugars. If your child has a mid-morning snack, this will be given towards the end of the morning.

They will then attend class as normal but twice they will be asked to take some simple tests that will be presented as games. For example a test of memory by trying to remember some objects and how quickly they can push a button when a light comes on.

Naturally if your child is unhappy they will be free to drop out at any point. Nobody is going to try to make them eat something they do not like. They will only be asked to do what they are happy to do.

The information gathered is not of a sensitive nature but the results will not be made available to the school.

Should you wish further information we would be pleased to arrange a meeting or alternatively you could call ----- or email -----

It is very important that if you believe that your child reacts badly to the foods listed above, for example they suffer with a food allergy, they should not take part.

If it is appropriate we hope that you will allow your son or daughter to take part. Only studies such as this one can help us find out what is best for a child. If you would like to help please fill in the next page and return it to the school.

Yours faithfully

David Benton

Hayley Young

PERMISSION FOR MY CHILD TO TAKE PART IN A BREAKFAST STUDY

YES NO

- 1) I have read about the study and any questions have been answered

- 2) I have enough information to allow me to say whether my child should take part

- 3) I understand that my child does not need to take part and can withdraw at any time and this will not affect their education

- 4) I understand that my child's results will not be given to the school

- 5) It is safe for my child to eat all the foods listed on the information sheet (that there is no medical reason to avoid these foods)

- 5) My child has never had an allergic or other reaction to food

IF YOU HAVE ANSWERED YES TO ALL THE ABOVE AND ARE HAPPY FOR
YOUR CHILD TO TAKE PART PLEASE FILL IN THE DETAILS BELOW

Name of child

Signed **Parent / Guardian**

Address **Phone**

.....

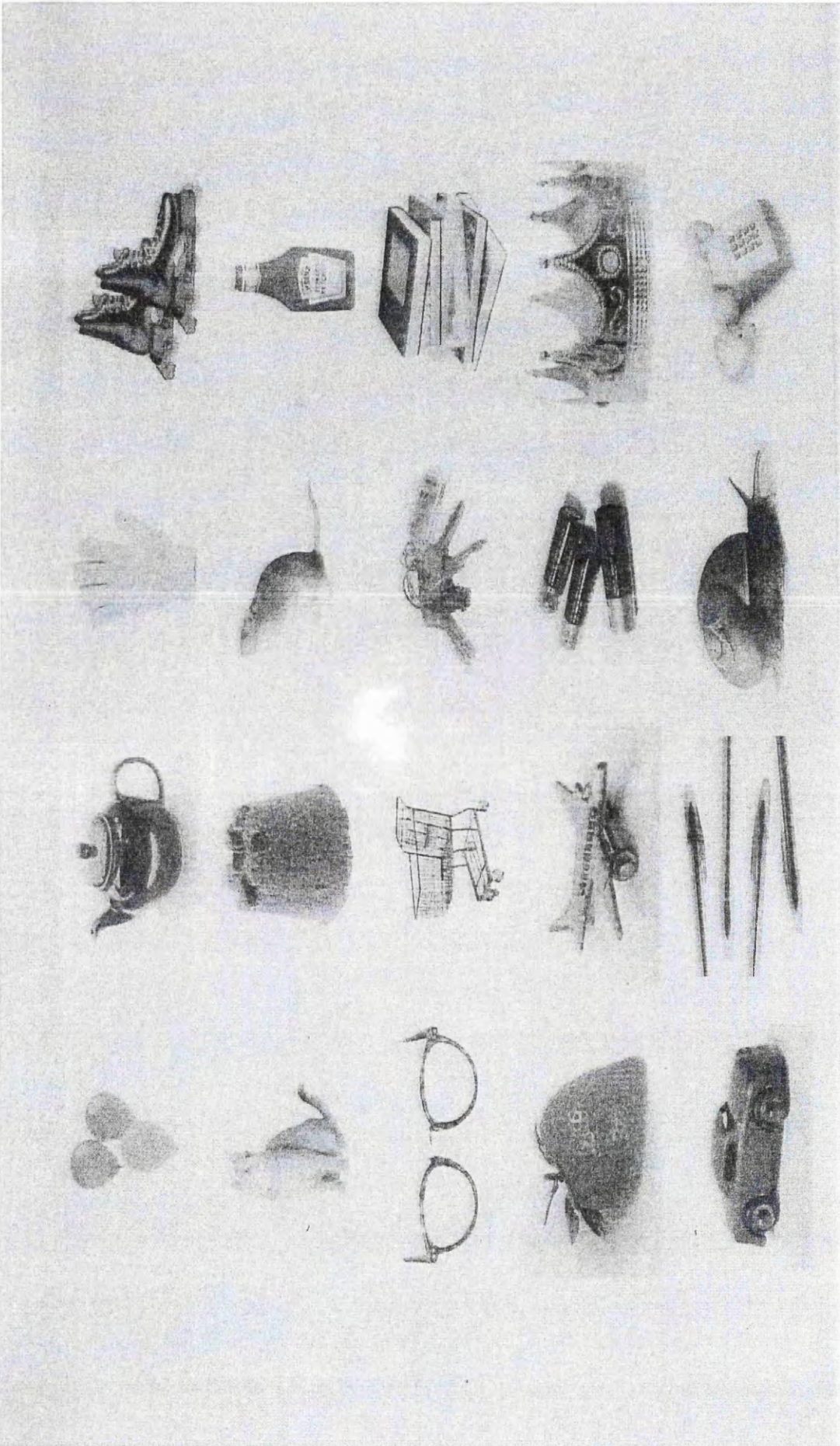
.....

On the days of the testing breakfast will be eaten from 8 o'clock to half past 8.

Please list dates when it will be convenient for your child to arrive early

.....

Appendix 2. The recall of objects task. Children viewed the card for 40 seconds and when the card was removed had 60 seconds to remember as many objects as possible.



Appendix 3 – Older adults’ participant information sheet.

Breakfast Study

Dear Sir / Madam

We are very grateful that you have decided to take part in our study about the benefits of eating breakfast. This letter is just to give you a little more information about what you will be asked to do during your time at the University.

As we age we may start to notice subtle flaws in our memory. Eating breakfast has been shown to help mental performance; however, some breakfasts may be more helpful than others. The broad aim of this study is to determine if by changing what people eat for breakfast we can help improve memory.

The study will take place on two mornings between 8.45am and 1.00pm. You will need to arrive at the University having not eaten or drink anything other than water for the preceding 12 hours, that is from 8.45 the evening before. This includes tea and coffee.

DAY 1

On arrival you will be asked to give a blood glucose reading that involves a small finger prick. This should cause only minor discomfort. This procedure will take place across the morning at 30 min intervals to track changes in your blood glucose.

You will be asked to consume a lemon flavoured drink containing glucose. This procedure is called a glucose tolerance test and will allow us to determine how well your body controls its glucose levels, an important predictor of future health. In case of any problem we will inform you so that you can discuss the matter with you doctor.

Three times throughout the morning you will also take a test battery that includes measures of memory, attention, reaction times and mood. You will also be asked to fill out some questionnaires to do with personality and lifestyle.

At the end of the morning you will be provided with a light lunch.

Day 2

On a second day you will consume a breakfast of toast with jam, a low fat yogurt and an orange drink. Again you will complete the test battery on three occasions throughout the morning and will be offered lunch at the end of the study. Measures of height, weight and other general health checks will also take place on this day.

You may also bring something to read whilst you are in the lab as you will be sitting and waiting in between testing. You will be reimbursed for any expenses occurred during your travel/stay at the University. If you have any packing receipts then please keep them.

If you require any additional information regarding the study please contact Hayley Young at -
----- or phone ----- . In addition if you know anyone else who may be interested in taking part in this study please tell them to contact us.

Your help in these matters is greatly appreciated. It is only with the help of people like you that research can proceed, hopefully in the long term to the greater good.

Yours faithfully

David Benton

Hayley Young

David Benton

Appendix 4. Older adults informed consent form.

Informed consent – breakfast study

Swansea University requires that all persons who participate in research studies give their written consent to do so. Please read the following and answer the questions below then sign it if you agree with what it says.

I freely and voluntarily consent to be a participant in the research project about the benefits of breakfast to be conducted by Hayley Young as principal investigator, who is a postgraduate student in the School of Human Sciences at Swansea University under the supervision of Professor David Benton. The broad goal of this research study is to explore the effect of different breakfasts on memory across the morning. Specifically, I have been asked to attend Swansea university on two occasions, consume breakfast that will be provided for me, provide finger prick samples of blood and complete some memory tasks and some questionnaires. I understand that I will be required on both occasions for the full duration of the morning.

I have been told that my responses will be kept strictly confidential. I also understand that my participation in this study is completely voluntary and that if at any time during the study I feel unable or unwilling to continue, I am free to leave. In addition, should I not wish to answer any particular question or questions, I am free to decline. My name will not be linked with the research materials, and I will not be identified or identifiable in any report subsequently produced by the researcher.

I have been informed that if I have any general questions about this project, I should feel free to contact Hayley Young at ----- or -----.

Please turn over and answer the following questions.

- | | | |
|---|-----|----|
| 1) I have read about the study and any questions have been answered | YES | NO |
| 2) I have enough information to decide whether to take part | YES | NO |
| 3) I understand that my participation is voluntary and that I am free to withdraw at any time | YES | NO |
| 4) There is no medical reason for me to avoid any foods | YES | NO |
| 5) I have never had an allergic or other adverse reaction to food | YES | NO |
| 6) I have normal or corrected to normal vision and hearing | YES | NO |
| 7) I do not have any digestive difficulties such as Crohn's disease | YES | NO |
| 8) Are you currently taking any medications? | YES | NO |

IF YES PLEASE SPECIFY _____

If you agree to take part in this study please provide the information below.

Participant's Signature _____

PRINT NAME _____

Address _____

POST CODE _____

Date _____

Phone _____

I have explained and defined in detail the research procedure in which the respondent has consented to participate. Furthermore, I will retain one copy of the informed consent form for my records.

Researcher signature

Date

Appendix 5. Words lists that were used for the episodic memory task. A. List used in chapter 3. B. list used in chapter 4 and 5 (1st test battery). C. list used in chapter 4 and 5 (2nd test battery). D. list used in chapter 4 and 5 (3rd test battery).

A.	Pedal	Globe	Daisy
	Brick	Flute	Linen
	Spike	Paste	Beard
	Shark	Prune	Cedar
	Tooth	Ankle	Queen
	Chart	Plane	Belly
	Organ	Arrow	Crypt
	Mixer	Honey	River
	Trunk	Couch	Ruler
	Ivory	Spade	Elbow
B.	Beach	Thick	Table
	Meant	Rifle	Force
	Earth	Brief	Horse
	Clear	Chain	Reach
	Paper	Least	Woman
	Order	Shore	Guess
	Drink	Eight	Glass
	Issue	Coast	Think
	Truck	Worse	Child
	Quick	Staff	Value
C.	Chest	Whole	River
	Moral	Light	Quiet
	Blood	Claim	Metal
	Under	Smile	Dozen
	Phone	Right	Frame
	Extra	Uncle	South
	Wheel	Allow	Knife
	Learn	Crowd	Rural
	Heart	Truth	Teeth
	Apart	Court	Proud
D.	Spoke	Power	Cover
	Phase	Radio	Alone
	Judge	Short	Flesh
	Minor	Bible	Ideal
	Money	Happy	Stone
	Wrote	Hotel	Carry
	Water	Might	Novel
	Empty	China	Event
	Plant	Break	Dress
	Theme	Board	Style

(Lists were recorded as follows: Pedal, Globe, Daisy, Brick, Flute, Linen.....)

GENERAL HEALTH QUESTIONNAIRE



Please read this carefully:

We should like to know if you have had any medical complaints, and how your health has been in general, over the past ~~four~~ weeks. Please answer ALL the questions on the following pages simply by underlining the answer which you think most nearly applies to you. Remember that we want to know about present and recent complaints, not those you had in the past. It is important that you try to answer ALL the questions.

Thank you very much for your co-operation.

HAVE YOU RECENTLY:

- | | | | | | |
|----|---|----------------------|---------------------|---------------------------|----------------------|
| 1 | – been able to concentrate on whatever you're doing? | Better than usual | Same as usual | Less than usual | Much less than usual |
| 2 | – lost much sleep over worry? | Not at all | No more than usual | Rather more than usual | Much more than usual |
| 3 | – been having restless, disturbed nights? | Not at all | No more than usual | Rather more than usual | Much more than usual |
| 4 | – been managing to keep yourself busy and occupied? | More so than usual | Same as usual | Rather less than usual | Much less than usual |
| 5 | – been getting out of the house as much as usual? | More so than usual | Same as usual | Less than usual | Much less than usual |
| 6 | – been managing as well as most people would in your shoes? | Better than most | About the same | Rather less well | Much less well |
| 7 | – felt on the whole you were doing things well? | Better than usual | About the same | Less well than usual | Much less well |
| 8 | – been satisfied with the way you've carried out your task? | More satisfied | About same as usual | Less satisfied than usual | Much less satisfied |
| 9 | – been able to feel warmth and affection for those near to you? | Better than usual | About same as usual | Less well than usual | Much less well |
| 10 | – been finding it easy to get on with other people? | Better than usual | About same as usual | Less well than usual | Much less well |
| 11 | – spent much time chatting with people? | More time than usual | About same as usual | Less time than usual | Much less than usual |
| 12 | – felt that you are playing a useful part in things? | More so than usual | Same as usual | Less useful than usual | Much less useful |
| 13 | – felt capable of making decisions about things? | More so than usual | Same as usual | Less so than usual | Much less capable |

PLEASE TURN OVER

HAVE YOU RECENTLY:

14 — felt constantly under strain?	Not at all	No more than usual	Rather more than usual	Much more than usual
15 — felt you couldn't overcome your difficulties?	Not at all	No more than usual	Rather more than usual	Much more than usual
16 — been finding life a struggle all the time?	Not at all	No more than usual	Rather more than usual	Much more than usual
17 — been able to enjoy your normal day-to-day activities?	More so than usual	Same as usual	Less so than usual	Much less than usual
18 — been taking things hard?	Not at all	No more than usual	Rather more than usual	Much more than usual
19 — been getting scared or panicky for no good reason?	Not at all	No more than usual	Rather more than usual	Much more than usual
20 — been able to face up to your problems?	More so than usual	Same as usual	Less able than usual	Much less able
21 — found everything getting on top of you?	Not at all	No more than usual	Rather more than usual	Much more than usual
22 — been feeling unhappy and depressed?	Not at all	No more than usual	Rather more than usual	Much more than usual
23 — been losing confidence in yourself?	Not at all	No more than usual	Rather more than usual	Much more than usual
24 — been thinking of yourself as a worthless person?	Not at all	No more than usual	Rather more than usual	Much more than usual
25 — felt that life is entirely hopeless?	Not at all	No more than usual	Rather more than usual	Much more than usual
26 — been feeling hopeful about your own future?	More so than usual	About same as usual	Less so than usual	Much less hopeful
27 — been feeling reasonably happy, all things considered?	More so than usual	About same as usual	Less so than usual	Much less than usual
28 — been feeling nervous and strung-up all the time?	Not at all	No more than usual	Rather more than usual	Much more than usual
29 — felt that life isn't worth living?	Not at all	No more than usual	Rather more than usual	Much more than usual
30 — found at times you couldn't do anything because your nerves were too bad?	Not at all	No more than usual	Rather more than usual	Much more than usual

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Appendix 7. Hypoglycaemia symptoms questionnaire.

Instructions: Place one cross on each line to indicate how much each statement applies to you during the last week. Please use the entire line to indicate the degree to which each statement is true.

I eat when nervous
Exactly like me _____ Not at all like me

My tiredness is relieved by eating
Exactly like me _____ Not at all like me

I get shaky when hungry
Exactly like me _____ Not at all like me

I am often eating something
Exactly like me _____ Not at all like me

I tend to snack late morning
Exactly like me _____ Not at all like me

I often choose not to eat breakfast
Exactly like me _____ Not at all like me

I feel faint if I haven't eaten in a while
Exactly like me _____ Not at all like me

I prefer to eat little and often
Exactly like me _____ Not at all like me

I often experience hunger pangs
Exactly like me _____ Not at all like me

I tend to snack mid afternoon
Exactly like me _____ Not at all like me

I put on weight easily
Exactly like me _____ Not at all like me

I often experience food cravings
Exactly like me _____ Not at all like me

I can be irritable if I do not eat
Exactly like me _____ Not at all like me

In the late afternoon I lack energy
Exactly like me _____ Not at all like me

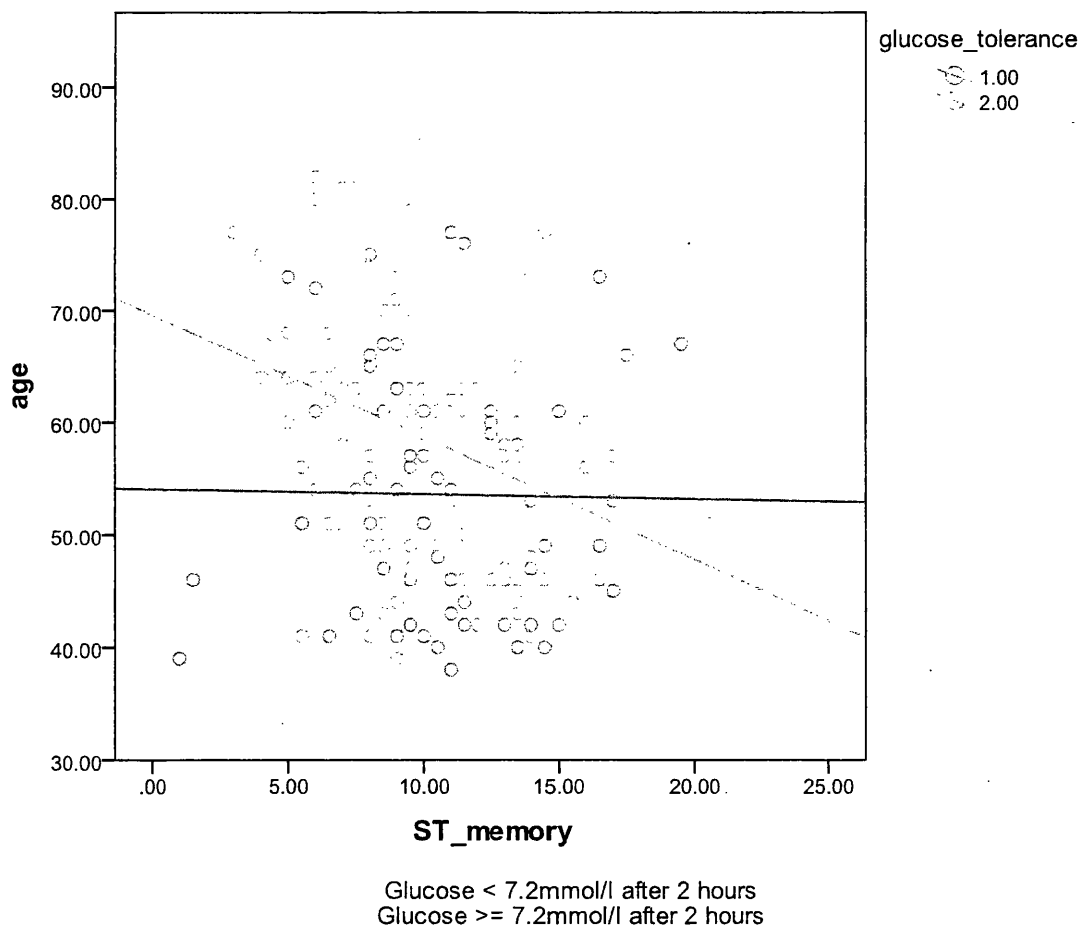
I need to eat regularly
Exactly like me _____ Not at all like me

- I tend to snack in the evening
Exactly like me _____ Not at all like me
- I snack to reduce tiredness
Exactly like me _____ Not at all like me
- Sometimes my heart pounds for no reason
Exactly like me _____ Not at all like me
- I often find it hard to concentrate
Exactly like me _____ Not at all like me
- Sometimes my thinking is confused
Exactly like me _____ Not at all like me
- Sometimes my vision is blurred
Exactly like me _____ Not at all like me
- I tend to become anxious
Exactly like me _____ Not at all like me
- My memory is poor
Exactly like me _____ Not at all like me
- I have difficulty making decisions
Exactly like me _____ Not at all like me
- Sometimes I feel dizzy or light headed
Exactly like me _____ Not at all like me
- There are times when I feel too warm for no apparent reason
Exactly like me _____ Not at all like me
- Sometimes I run on 'autopilot'
Exactly like me _____ Not at all like me
- I often blush
Exactly like me _____ Not at all like me
- I tend to have cold hands and feet
Exactly like me _____ Not at all like me
- I often sweat
Exactly like me _____ Not at all like me
- Sometimes my legs feel wobbly and I have to sit down
Exactly like me _____ Not at all like me
- Sometimes my hands tremble
Exactly like me _____ Not at all like me

Appendix 8. Slope analysis for the effects of glucose tolerance and age on memory.

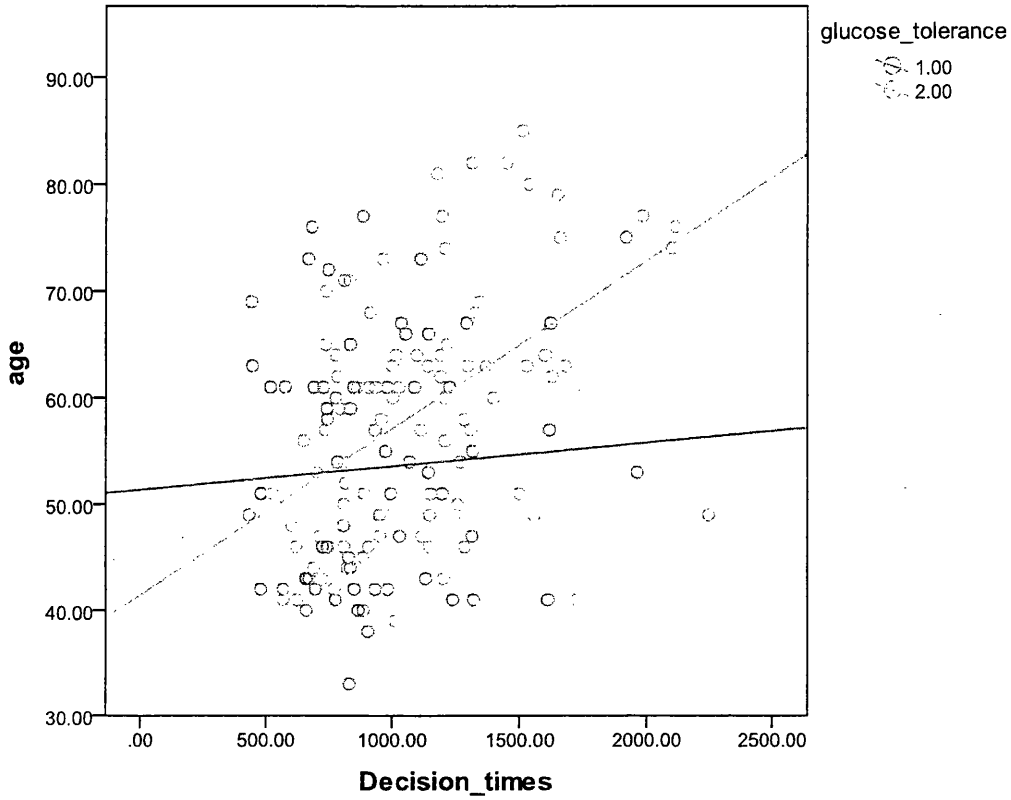
	Coefficient	Standard error	t	p
Glucose Tolerance. *	2.0397	0.6521	2.9767	0.003
Age (years)	0.2009	0.0791	2.5389	0.01
Age*Glucose Tolerance.	-.0347	0.0115	-3.0239	0.002

The effect of Glucose tolerance, Age and their interaction on memory. *Glucose tolerance is mmol/l 2 hours after OGTT.



The effect of age on memory at glucose levels above and below 7.2mmol/l.

Appendix 9. Scatter plot showing the effect of age on decision times in those with better or poorer glucose tolerance.



1 = glucose < 7.2 after 2 hours
2 = glucose > or the same as 7.2 after 2 hours

The effect of age on decision times at glucose levels above and below 7.2mmol/l.

Appendix 10. Mood VAS that was used for measuring mood in chapter 4 and 5.

Please place one cross on each line to indicate the way you feel *at the moment*. For example if neither adjective describes you then put a mark in the middle; if you tend towards one end of the line then place the cross towards that end to the extent that the adjective describes you.

EXAMPLE

Happy	_____	Sad
	<i>I am neither happy nor sad</i>	
Happy	_____	Sad
	<i>I am very happy</i>	
Happy	_____	Sad
	<i>I am a little sad</i>	

Now please complete the following:

Agreeable	_____	Hostile
Clearheaded	_____	Confused
Composed	_____	Anxious
Elated	_____	Depressed
Confident	_____	Unsure
Energetic	_____	Tired

		Agreeable/ Hostile	Clearheaded/ Confused	Composed/ Anxious	Elated/ Depressed	Confident/ Unsure	Energetic/ Tired	
Better GT LBG	GLU	-2.7(19.6)	-1.7(8.7)	1.8(10.6)	6.2(8.5)	4.5(7.9)	7.5(11.1)	
		-4.9(13.7)	-2.2(10.7)	-3.6(9.3)	1.9(8.2)	-3.2(14.2)	-1.2(8.4)	
		-1.5(15.9)	-3.0(15.0)	-4.5(12.3)	-1.4(9.8)	1.7(11.3)	-7.4(12.9)	
	SUC		-23.2(31.6)	-16.0(22.9)	-14.6(20.6)	-19.5(20.8)	-24.9(20.3)	-30.2(20.7)
			-18.0(19.2)	-15.2(20.1)	-15.5(20.3)	-14.2(18.7)	-15.5(23.1)	-12.4(20.2)
			-9.7(10.2)	-5.9(9.9)	-7.8(9.2)	4.5(15.7)	-4.9(12.0)	-3.4(16.7)
			3.2(17.8)	21.1(20.9)	20.0(15.9)	9.0(20.2)	12.2(20.4)	17.9(20.1)
			6.1(9.3)	7.7(22.0)	12.0(12.1)	5.6(16.2)	5.2(11.2)	13.3(19.1)
			2.9(8.9)	7.5(12.4)	7.0(10.1)	8.4(22.3)	2.0(9.4)	1.7(13.2)
			-5.9(16.8)	-1.7(19.1)	4.2(15.1)	6.8(17.5)	-2.2(15.7)	3.5(13.2)
	ISO		-1.5(12.9)	2.3(19.7)	3.6(12.2)	-1.2(20.1)	1.6(20.5)	3.1(20.5)
			91.5(8.8)	-0.7(13.0)	7.0(13.1)	2.6(16.4)	2.6(17.5)	-2.9(10.4)
		3.1(5.1)	9.9(18.5)	5.5(14.1)	4.5(12.0)	10.0(16.0)	9.1(17.6)	
0.3(8.3)		-1.4(13.9)	-4.1(5.5)	-6.5(18.6)	-4.4(17.5)	-0.6(10.5)		
-0.8(10.4)		5.7(9.5)	0.7(3.2)	-0.1(11.0)	7.7(10.4)	-4.0(20.2)		
0.8(5.9)		1.2(10.9)	3.2(6.5)	-9.0(14.6)	-0.7(11.4)	-13.3(20.5)		
Poorer GT LBG	GLU	-10.6(16.1)	0.9(9.5)	-0.1(9.8)	-5.8(9.1)	3.0(12.6)	-9.6(17.6)	
		-7.8(17.7)	-0.2(8.3)	-6.5(10.3)	-9.1(10.5)	3.2(12.6)	-13.3(16.7)	
		-3.2(17.8)	-1.0(20.2)	-4.0(10.2)	4.9(22.4)	1.1(18.7)	-1.6(20.5)	
	SUC		-8.0(19.9)	-10.0(19.7)	-6.0(19.7)	-7.3(20.5)	-9.4(19.6)	-9.1(20.6)
			-3.2(12.1)	-4.6(19.3)	-3.0(18.7)	0.4(20.2)	-1.6(19.4)	-5.1(20.3)
			-8.2(18.9)	-10.1(20.9)	-9.2(20.5)	-6.5(20.1)	-12.5(17.0)	-12.5(20.3)
			-6.9(18.3)	-4.9(22.5)	-4.3(18.5)	-1.9(20.5)	-11.6(20.1)	-8.6(20.3)
			-5.7(18.6)	-6.0(22.2)	-7.2(20.3)	0.8(21.0)	-8.4(20.5)	-5.8(20.1)
			1.5(10.9)	-0.4(17.6)	-0.9(20.7)	-5.7(20.2)	-0.2(20.8)	0.3(21.3)
			-2.0(13.5)	-2.0(17.2)	-2.7(20.2)	-4.7(19.5)	-10.7(20.6)	-7.5(23.3)
		1.5(13.6)	-8.8(17.3)	-4.2(16.8)	-3.9(18.6)	-7.0(23.5)		

		-8.5(13.5)	-9.8(12.3)	-11.8(19.5)	-15.3(20.3)	-12.3(17.0)	-20.9(24.9)
		-3.0(15.0)	-2.3(15.3)	-9.4(18.8)	-10.7(20.7)	-11.6(20.1)	-20.7(27.4)
		-4.9(17.2)	-5.9(18.4)	-12.7(20.3)	-4.8(20.7)	-8.1(20.5)	-17.4(29.6)
	ISO	4.6(9.5)	9.5(14.2)	3.5(11.6)	1.4(10.6)	4.5(14.1)	13.0(20.5)
		-4.0(11.3)	-5.7(20.8)	-2.1(21.3)	-2.5(20.2)	-0.9(20.9)	0.4(28.4)
		-0.2(10.3)	3.1(13.0)	0.5(16.4)	-1.9(18.0)	2.4(15.8)	-0.7(21.2)
		-3.4(10.2)	-7.8(15.5)	6.5(15.1)	-3.1(19.5)	-4.3(16.5)	-9.4(24.7)
		-4.3(12.0)	-4.6(13.3)	-5.5(16.3)	-6.4(19.5)	-0.5(18.7)	-3.5(21.5)
		-7.8(19.9)	-9.2(22.5)	-8.6(15.6)	-6.3(20.0)	-9.8(19.3)	-11.8(28.3)
	GLU	1.2(11.2)	11.2(17.3)	2.5(9.0)	0.3(15.2)	3.0(13.0)	6.5(11.7)
	Poorer GT No LBG	-8.2(15.1)	-2.8(21.2)	-0.3(19.9)	-6.4(16.8)	-6.2(14.5)	2.6(14.8)
		-6.8(19.6)	-1.0(20.6)	-3.0(18.5)	-9.0(21.1)	-5.6(19.6)	-6.1(13.5)
		-7.7(14.0)	-7.0(20.2)	-2.3(22.5)	-9.5(16.3)	-13.1(14.0)	-18.1(19.5)
		-17.6(22.2)	-8.0(18.8)	-8.9(20.9)	-13.2(20.7)	-9.8(20.8)	-16.9(29.4)
		-8.1(16.8)	-23.6(20.2)	-9.4(22.1)	-12.4(20.6)	-9.8(20.4)	-24.5(25.1)
	SUC	-5.2(17.6)	6.4(20.2)	-1.0(11.6)	-0.5(11.0)	-1.8(16.9)	-4.1(18.3)
		-3.2(9.8)	1.9(20.3)	-10.5(20.2)	-3.2(10.0)	-8.2(18.2)	-3.6(20.9)
		-13.5(25.3)	-4.3(19.5)	-7.0(34.4)	-2.6(20.8)	-8.8(20.8)	-12.5(16.2)
		-9.5(16.6)	-16.8(20.7)	-9.5(20.4)	-4.8(20.2)	-15.5(21.7)	-15.6(20.5)
		-6.3(16.2)	-4.2(22.3)	-1.4(26.1)	-2.8(19.8)	-3.2(20.4)	-10.9(20.9)
		-11.3(19.1)	-9.1(20.3)	-10.9(34.3)	-3.0(20.4)	-10.2(30.5)	-13.8(28.3)
	ISO	-2.4(12.2)	1.4(17.4)	0.4(11.6)	0.6(16.2)	1.0(14.5)	2.1(24.0)
		-6.7(17.0)	-12.6(16.5)	-7.9(18.2)	-2.7(15.4)	-6.5(20.2)	-2.8(20.9)
		-1.0(15.0)	-4.5(26.3)	-4.3(20.4)	0.6(17.1)	0.2(19.2)	-7.7(24.4)
		-4.6(16.2)	-13.7(21.0)	-8.7(19.5)	-3.7(19.0)	-11.3(20.3)	-17.9(28.2)
		-9.0(20.0)	-11.7(22.3)	-11.6(13.6)	-7.6(18.1)	-7.4(20.3)	-17.5(31.9)
		-5.7(18.2)	-8.1(22.5)	-5.8(17.5)	-1.5(17.6)	-9.7(20.3)	-9.3(21.3)
	GLU	-8.8(14.1)	-3.3(15.8)	-6.4(122.9)	-7.8(12.1)	-3.8(13.0)	-3.3(23.8)
	Better GT	-5.5(9.0)	-6.6(16.3)	-8.8(7.8)	-7.5(12.4)	-10.2(11.0)	-8.3(18.7)

No LBG		-9.3(8.2)	-7.3(9.9)	-6.2(7.5)	-8.7(9.7)	-5.1(12.1)	-9.5(16.4)	
		-15.0(13.6)	-14.5(18.1)	-14.0(14.4)	-10.7(11.4)	-20.0(18.9)	-20.1(18.1)	
		-13.0(9.6)	-7.3(12.0)	-12.5(12.8)	-13.0(10.8)	-16.8(10.9)	-14.6(19.2)	
		-14.2(10.5)	-20.0(17.5)	-18.5(14.8)	-20.3(12.5)	-20.6(17.1)	-25.8(22.8)	
	SUC		-7.2(15.2)	-0.7(12.8)	-2.3(16.7)	-3.0(10.9)	-2.8(21.9)	4.2(24.2)
			-14.8(22.9)	-10.2(20.8)	-12.5(20.6)	-7.7(17.5)	-8.8(20.5)	3.3(24.4)
			2.2(15.7)	4.0(14.2)	2.6(16.1)	4.1(10.2)	5.9(13.3)	5.6(22.2)
			3.0(14.6)	2.7(15.0)	-0.5(20.9)	5.7(10.9)	5.0(15.3)	6.6(19.5)
			10.9(16.7)	7.7(17.8)	10.8(19.7)	4.0(9.0)	6.2(18.1)	10.0(20.5)
	ISO		3.0(17.8)	-2.1(15.3)	1.9(13.7)	9.4(14.6)	4.5(15.1)	6.2(22.5)
			-2.8(13.3)	2.0(19.5)	-1.9(16.0)	1.2(18.3)	-3.7(16.1)	-1.3(21.4)
			-9.0(15.7)	-13.4(20.5)	-9.3(14.6)	-3.5(19.0)	-6.9(18.6)	-9.3(19.1)
			-6.8(18.2)	-9.2(20.3)	-6.0(14.3)	-4.2(20.5)	-6.5(24.2)	-9.5(20.4)
		5.2(19.2)	-13.2(20.4)	-13.2(17.5)	7.9(20.3)	-2.9(24.0)	2.5(21.7)	
		3.8(14.3)	11.1(20.3)	-3.5(20.1)	3.7(20.6)	11.7(20.5)	10.2(20.3)	
		2.5(20.5)	21.7(19.3)	-3.0(22.2)	8.2(20.5)	3.3(17.4)	25.0(19.8)	
Significant effects	Main effect meal. (F(2,140)=2.88, p<0.05). ISO > SUC > GLU	Meal X Group X Time interaction. (F(11.4,266.3) = 2.33, p<0.009). ISO > SUC > GLU @ 195 min for Better GT no LBG group.	No significant effects.	Meal X Group X Time interaction. (F(11.4,266.2)= 2.65, p<0.05). ISO and SUC > GLU @ 195 min for Better GT no LBG group.	Meal X Group X Time interaction. (F(11.1,260.9)= 2.07, p<0.05). ISO and SUC > GLU @ 195 min for Better GT no LBG group.	Meal X Group X Time interaction. (F(10.6,249.4)= 2.57, p<0.003). ISO and SUC > GLU @ 195 min for Better GT no LBG group.		

Data are mean (SD) for change in mood from baseline. GT – Glucose Tolerance. LBG – Lowest Blood Glucose. GLU – Glucose based meal. SUC – Sucrose based meal. ISO- Isomaltulose based meal.

Appendix 12. Young adult consent form.

Study Title: The effect of consuming a novel food item on cognition and mood.

Thank you for deciding to take part in our study about the benefits of consuming a novel food item. This letter is just to give you a little more information about what you will be asked to do during the experiment.

The food we eat can potentially affect the way we think and feel. The broad aim of this study is to determine if supplementing a new food item at breakfast can improve mental performance and blood flow.

The study will take place on one morning between 8.30 am and 1pm. You will need to attend for the duration of this time slot. You will be asked to fast for at least 12 hours (overnight) and refrain from drinking anything other than water. Upon arrival at the University laboratory you will be provided a small breakfast (Cornflakes with milk) and a choice of tea or coffee which you will be required to consume in its entirety. You will also be given a drink at 10am; again you will need to drink it all.

Throughout the morning you will be required to give a number of capillary blood samples. This involves a small prick on your finger and may cause minor discomfort. You will also complete a number of mental tasks that test your memory, attention and reaction times. You will fill out some questionnaires about how you are feeling as the morning progresses.

You will be paid by direct debit into your bank account. Payments are sent at the end of each month. Please note that due to cost and sample size we are unable to pay cash.

If you require any additional information regarding the study please contact Hayley Young at ----- or phone ----- . In addition if you know anyone else who may be interested in taking part in this study please tell them to contact us.

Please turn over and answer the following questions.

- | | | |
|---|-----|----|
| 1) I have read about the study and any questions have been answered | YES | NO |
| 2) I have enough information to decide whether to take part | YES | NO |
| 3) I understand that my participation is voluntary and that I am free to withdraw at any time | YES | NO |
| 4) There is no medical reason for me to avoid any foods | YES | NO |
| 5) I have never had an allergic or other adverse reaction to food | YES | NO |
| 6) I have normal or corrected to normal vision and hearing | YES | NO |
| 7) I do not have any digestive difficulties such as Crohn's disease | YES | NO |
| 8) Are you currently taking any medications? | YES | NO |

IF YES PLEASE SPECIFY _____

If you agree to take part in this study please provide the information below.

Participant's Signature _____

PRINT NAME _____

Address _____

POST CODE _____

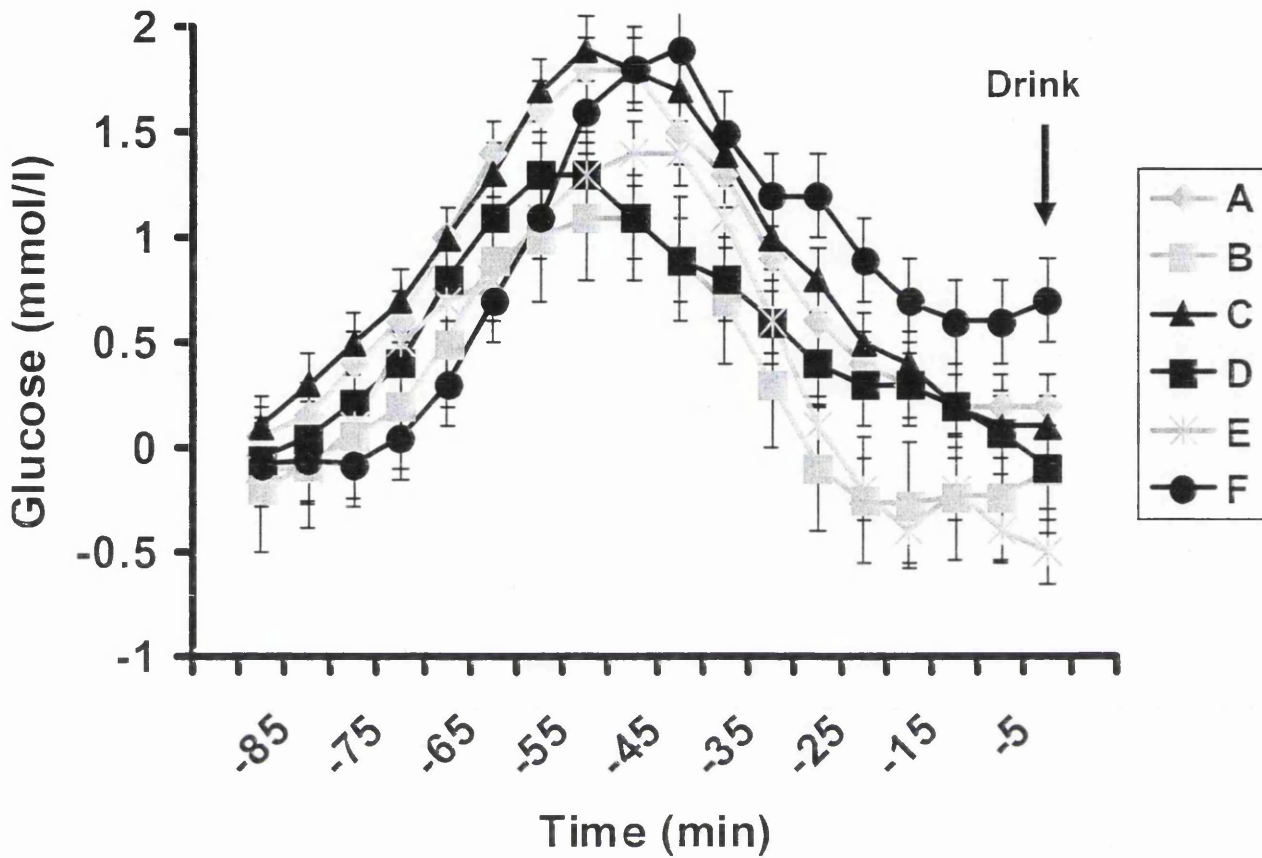
Date _____

Phone _____

Appendix 13. Higher order interaction involving gender.

DV	Higher order interactions (not otherwise reported in the text)
Depressed/Elated	n/a
Anxious/Composed	n/a
Agreeable/Hostile	n/a
Confident/ Unsure	n/a
Energetic/ tired	n/a
Clearheaded/ Confused	Time X Caffeine X Gender (F(1.3, 496.0) = 3.854 p<0.03). No significant follow up tests.
Episodic memory	Time X Gender X Vehicle X Caffeine (F(3.8,654.7) = 3.359, p<0.05). No significant follow up tests.
Working memory Reaction times	Time X Gender X Vehicle X Caffeine (F(3.9,659.3) = 2.795, p<0.02). No significant follow up tests.
Working memory Accuracy	n/a
Vigilance Reaction times	Minute of test X Gender X Vehicle (F(7.5,1194.5) = 2.450, p<0.02). Males were quicker than females after milk during the 4 th minute of the test. No other significant effects.
Vigilance Accuracy	ns
Focused attention Reaction times	n/a
Focused attention Errors	n/a
Decision times	n/a
Movement times	Time X Lamps X Gender (F(10.2, 790.0)= 2.178). Males were faster than females during the last testing session on the eight lamp task.

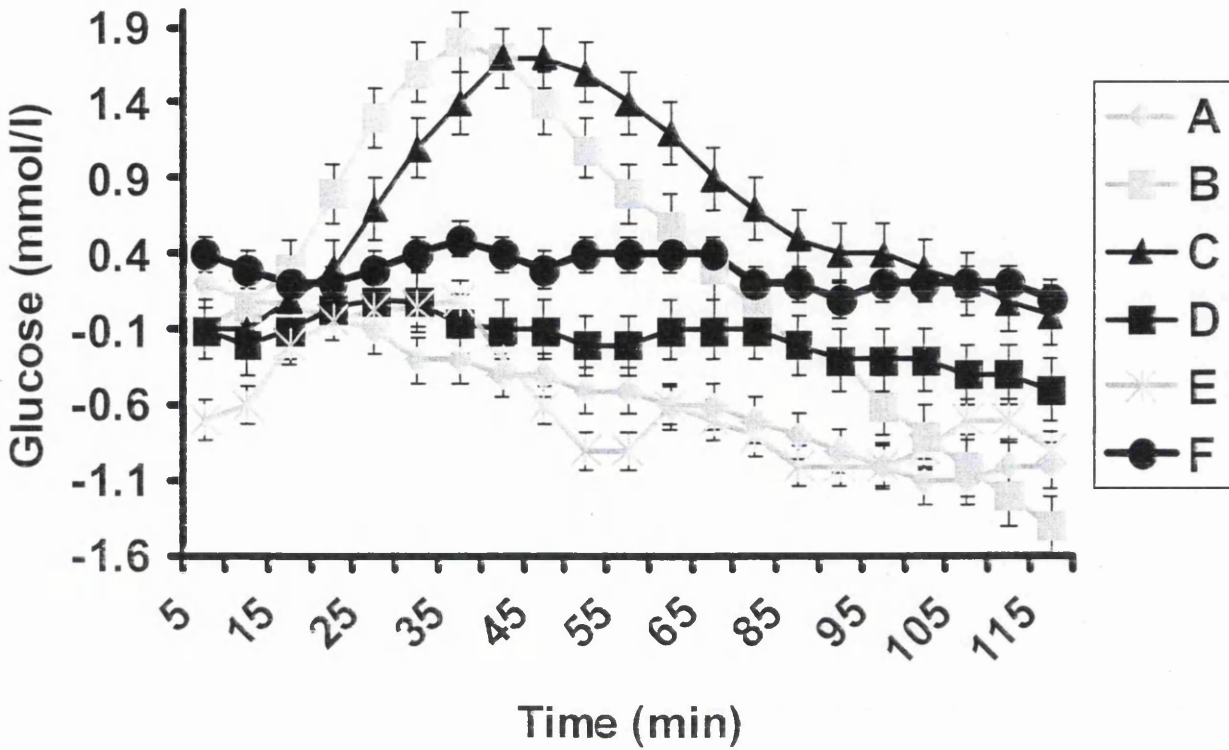
Appendix 14. The glycaemic response to breakfast.



Drink A – Water no caffeine. Drink B – Glucose no caffeine. Drink C – Glucose caffeine. Drink D – Milk caffeine. Drink E - Milk no caffeine. Drink F – Water caffeine.

The effects of breakfast on interstitial glucose levels. Data are average changes in interstitial glucose, as mmol/L, from the value immediately prior to consuming breakfast.

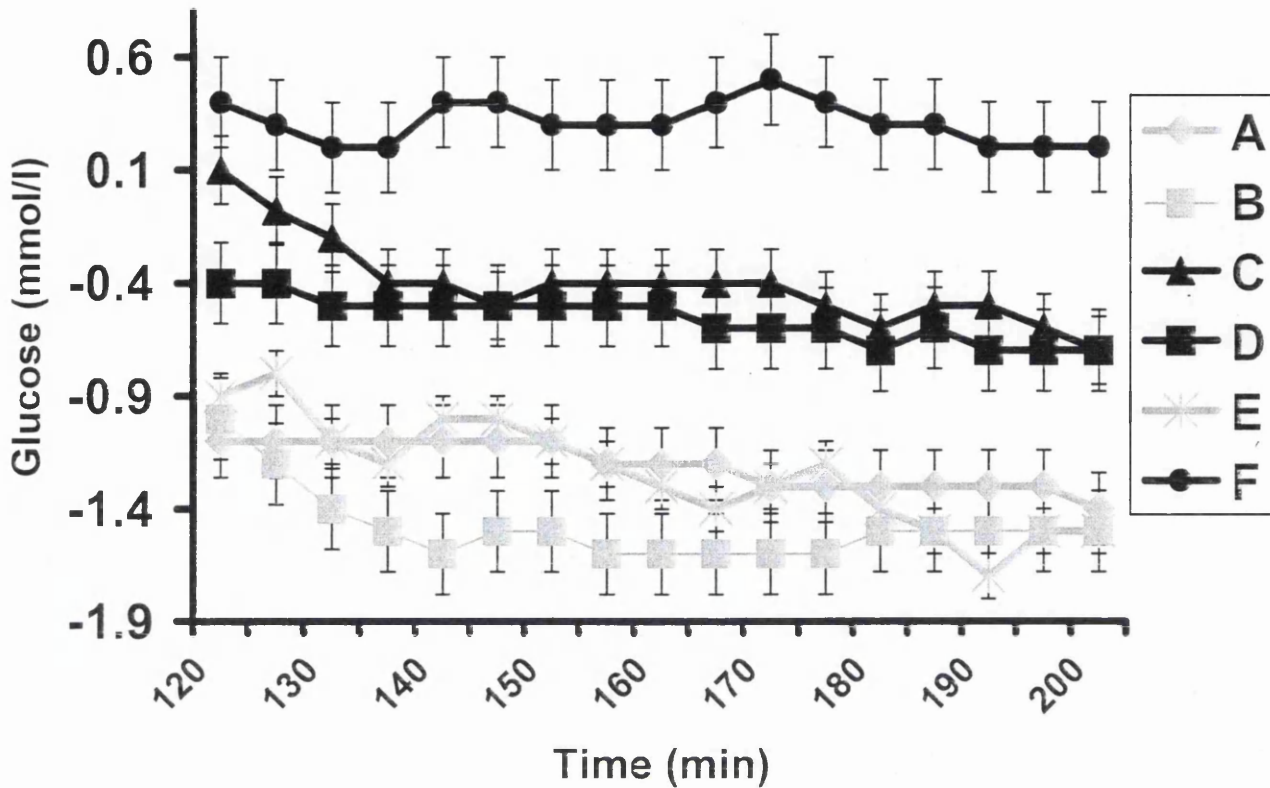
Appendix 15. The short term effects of type of drink on glycaemia.



Drink A – Water no caffeine. Drink B – Glucose no caffeine. Drink C – Glucose caffeine. Drink D – Milk caffeine. Drink E - Milk no caffeine. Drink F – Water caffeine.

The short term effects of type of drink on interstitial glucose levels. Data are average changes in interstitial glucose, as mmol/L, from the value immediately prior to consuming the drink.

Appendix 16. The longer term effects of type of drink on glycaemia.



Drink A – Water no caffeine. Drink B – Glucose no caffeine. Drink C – Glucose caffeine. Drink D – Milk caffeine. Drink E - Milk no caffeine. Drink F – Water caffeine.

The longer term effects of type of drink on interstitial glucose levels. Data are average changes in interstitial glucose, as mmol/L, from the value immediately prior to consuming the drink.

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